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ABSTRACTS COLLECTION

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P501. Value Representation, Working Memory, and Negative Symptoms in Early Psychosis

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Background: Negative symptoms of schizophrenia often involve difficulty orienting behavior toward long-term goals. These and other negative symptoms are typically stable over time and contribute to disability. However, no effective treatments for negative symptoms are available, partly due to a poor understanding of the psychological mechanisms involved. The NIMH Research Domain Criteria subconstruct of "value representation" has been proposed as a fundamental mechanism underlying negative symptoms. Value representation refers to the ability to generate, maintain, and use mental abstractions of reward value to guide decision-making. People with chronic schizophrenia show deficits in a range of behavioral tasks requiring intact value representation, including delay discounting (DD) paradigms, which assess the tendency to favor smaller, more temporally immediate rewards over larger, more distal rewards. Greater discounting rates are also associated with more severe negative symptoms and working memory impairments, consistent with the hypothesis that deficits in cognitive control impair the generation or maintenance of value representations and thereby reduce goal-directed activity. Despite these findings in chronic schizophrenia, very little is known about the extent to which DD impairments extend to the early course of illness, when disease mechanisms are rapidly evolving. Moreover, only one study to our knowledge has compared DD performance between people with early schizophrenia spectrum vs. affective psychosis, despite evidence that these two syndromes may represent distinct neurodevelopmental subtypes. Here, we examined DD in a transdiagnostic sample of people with early psychosis and tested for relations of DD with working memory and negative symptoms.

Methods: This study drew from the Human Connectome Project for Early Psychosis, representing what is to our knowledge the largest study to date of DD in early psychosis. The sample included 161 patients ages 18-36 with a psychotic disorder emergent in the past two to just under 5 years (115 schizophrenia spectrum, 47 affective) and 62 healthy controls. Participants completed measures of DD (Connectome Discounting Task), working memory (NIMH Toolbox List Sorting), and negative symptoms (Positive and Negative Syndrome Scale). In the DD task, participants chose between smaller ($200) and larger ($40,000) hypothetical monetary outcomes that were delivered trial-wise at one of five delay intervals (3 months to 10 years). DD values were calculated as the area under the curve of indifference points, with smaller DD values reflecting greater discounting. We hypothesized that (a) patients as a group would present with impaired DD, particularly at higher monetary values (given the added mental abstraction required), but that these impairments would be explained by the schizophrenia-spectrum subgroup. We also hypothesized that greater DD impairment would be associated with (b) poorer working memory performance and (c) greater negative symptom severity, particularly in patients with schizophrenia-spectrum disorders; and that (d) controlling for working memory would eliminate patient-control differences in DD. Finally, given that negative symptoms are more prominent in males, we expected that male patients would present with greater DD impairment than females patients.

Results: Patients as a whole presented with lower DD scores (greater discounting) than controls at higher ($t[221] = 2.34$, $p = .020$) but not lower ($t[221] = 0.599$, $p = .550$) monetary values. ANOVA with post hoc tests revealed schizophrenia-spectrum patients showed lower DD scores at higher monetary values than both controls ($t[173] = 3.181$, $p = .002$) and patients with affective psychosis ($t[160] = 2.502$, $p = .013$), whereas patients with affective psychosis did not differ from controls ($t[105] = 0.320$, $p = .750$). Reduced DD scores at higher monetary values were associated with poorer working memory ($r = .224$, $p = .019$) and greater negative symptom severity ($r = -.235$, $p = .014$) in schizophrenia-spectrum but not affective psychosis patients ($r = .164$, $p = .289$ and $r = .174$, $p = .258$, respectively). Patient-control differences in DD were eliminated when controlling for working memory performance ($F[1, 209] = 2.177$, $p = .142$). Among patients with schizophrenia spectrum disorders, males showed lower DD scores than females at higher monetary values ($t[113] = -2.115$, $p = .037$).

Conclusions: Similar to chronic schizophrenia, individuals in the early course of psychosis present with reduced DD performance. However, these impairments appear more specific to individuals with schizophrenia spectrum vs. affective psychosis. Impairments seen at higher vs. lower hypothetical monetary values are consistent with the hypothesis that schizophrenia involves a deficit in value representation, particularly at higher levels of abstraction. This interpretation is bolstered by observed

correlations between DD and working memory, which accounted for patient-control differences, and with negative symptoms. Greater DD impairment in males vs. females with early schizophrenia spectrum disorders further highlights the relevance of DD impairments to neurodevelopmental features of psychosis, which are more common in males. Future work in early psychosis would benefit from studies integrating DD tasks with measures of neural activity to point toward brain-behavior mechanisms of negative symptoms.

Keywords: Early Psychosis, Negative Symptoms, Delay Discounting, Cognitive Control, Reward

Disclosure: Nothing to disclose.

P502. Connectivity of a Pons-To-Cerebellum Sensory Prediction Circuit Correlates With Hallucination Severity in Schizophrenia

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Background: Sensory prediction occurs rapidly and outside of conscious awareness, allowing the brain to anticipate and parse incoming self-generated sensory information from externally produced signals. The pons is a central node in a broader cortico-ponto-cerebellar-thalamo-cortical circuit thought to support sensory prediction. For every action, an efference copy of the motor plan generates a corollary discharge of the expected sensory consequences, which is compared to the actual sensation; this process is driven by automatic cerebellar side-loops, where motor information is transmitted to the cerebellum via the pons. If supporting mechanisms go awry, sensations that should have been predicted, but were not, may acquire inappropriate salience and lead to aberrant sensory experiences and/or delusional schema. To date, no studies have directly tested whether pons connectivity directly relates to neurophysiological and clinical signatures of sensory prediction among people with psychosis.

The extent to which self-produced are expected and align with actual sensations can be studied using vocalization paradigms (e.g., Talk-Listen paradigm), where neural responses to self-generated sounds are compared to responses to external sources. The N1 component of the EEG-based event-related potential is suppressed during vocalizing relative to listening and is indicative of expectancy when self-generating auditory stimuli. N1 suppression is attenuated in psychosis and may reflect a deficiency in distinguishing self versus externally generated sensory feedback. One possibility is that deficient N1 suppression in psychosis arises from aberrant connectivity between the pons and other sensory prediction circuit nodes (e.g., hypoconnectivity with the cerebellum and/or thalamus). Aberrant pons connectivity may also correlate with relevant clinical features, i.e., hallucinations and delusions.

We tested these hypotheses using a combination of resting-state fMRI (rsfMRI) and EEG data in a large dataset of psychotic-spectrum participants (PSP with schizophrenia, schizoaffective, or bipolar disorder), first-degree relatives of individuals with psychosis (REL), people at clinical high-risk for psychosis (CHR-P), and unaffected comparison participants (UCP). This unique study design allowed us to test for variation along the illness trajectory and across the psychosis spectrum.

Methods: 137 PSP, 69 REL, 45 CHR-P, and 124 UCP underwent rsfMRI at the University of California, San Francisco or the University of Illinois, Chicago. Seeding from the pons, we tested for thalamic and cerebellar clusters showing PSP versus UCP differences and extracted mean connectivity values from significant clusters for all four groups. 129 PSP, 66 REL, 37 CHR-P, and 114 UCP also completed an EEG-based Talk-Listen paradigm. In the Talk condition, participants repeated short "ah" vocalizations in a self-paced manner. Speech sounds were transmitted back in real time to the participant through headphones. In the Listen condition, participants listened to recorded vocalizations from the Talk condition. N1 peak amplitudes were measured as the max negative voltage between 60-150 ms (site Cz). rsfMRI and EEG metrics were age- and site-corrected based on UCP data to account for normal brain maturation and study site differences. We tested if mean pons connectivity from significant clusters correlated with 1) N1 Talk-Listen difference scores among PSP relative to UCP, and 2) hallucination and delusion severity in PSP.

Results: PSP showed pons hypoconnectivity in two cerebellar clusters based on a voxel height threshold of $z > 3.29$ (corresponding to $p < .001$, two-tailed) and a corrected cluster-significance threshold of $p < .05$. Compared to UCP, REL, and CHR-P, PSP showed hypoconnectivity with the cerebellar vermis IX ($d = 0.59$). PSP and REL also showed hypoconnectivity with the right cerebellar VIIIa/VIIb lobules ($d = 0.56$) compared to UCP and CHR-P. Connectivity values extracted from these cerebellar clusters were used in the remaining analyses. There was an interaction between UCP and PSP for the cerebellar vermis IX cluster ($F = 5.45$, $p = .02$), driven by a positive association between connectivity and N1 auditory suppression for Talk vs Listen in UCP ($p = .02$). N1 suppression was not related to cerebellar vermis IX connectivity in PSP. Pons-cerebellar vermis IX connectivity also negatively correlated with hallucination (but not delusion) severity in PSP, specifically PSP with schizophrenia (and not schizoaffective or bipolar disorder) (t-ratio $= -3.09$, $p = .007$). Connectivity within the VIIIa/VIIb lobules was not significantly related to EEG or clinical measures.

Conclusions: Deficient pons-to-cerebellum connectivity was present in PSP and REL, but not CHR-P, suggesting a possible genetic liability. Greater connectivity between the pons and cerebellar vermis correlated with better N1 suppression in UCP, suggesting feedback between the pons and cerebellum may help distinguish self-generated versus external sounds in people without psychosis. While this N1 association was not found in PSP, greater pons-to-cerebellar vermis connectivity correlated with less severe hallucination in PSP with schizophrenia; i.e., greater synchrony of the same circuit corresponded with a clinical marker of sensory prediction success. Together, these findings highlight both shared features and clinical heterogeneity along the psychosis spectrum.

Keywords: Resting State Connectivity, EEG, Psychosis, N1, Efference Copy/Corollary Discharge

Disclosure: Nothing to disclose.

P503. Identification of Kat III as a Novel Pharmacological Target for the Treatment of Cognitive Impairment

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Background: Cognitive impairment is commonly observed in patients with psychiatric disorders and infectious diseases, and there is a lack of pharmacological treatment for this condition. Growing evidence suggests immune activation to be important for cognitive dysfunction. Indeed, immune activation increases the levels of brain kynurenic acid (KYNA), a neuroactive metabolite interfering with neurotransmission and impairing cognitive functioning. Kynurenine aminotransferases (KAT I-IV) convert kynurenine to KYNA. Under normal conditions, KAT II is

responsible for 70% of the production of KYNA. However, the role of KATs in KYNA production during immune activation is not clear.

Methods: Quantitative reverse transcription was used to analyze the mRNA expression levels and western blot to analyze the protein expression levels of KAT isoforms (I-IV). To obtain single-cell transcriptomic profiles, we used publicly available human postmortem data sources. KYNA levels were quantified using an ultra-performance liquid chromatography–tandem mass spectrometry system.

Results: We first observed an upregulation of KAT III expression in the mouse brain. Thus, KYNA levels in brain tissue were measured after repeated lipopolysaccharide (LPS) injections (2×0.83 mg/kg, i.p.) in wildtype (WT) and KAT II knockout (KO) mice. A main effect of treatment and treatment x genotype interaction was observed. No effect of genotype was found in the analysis (treatment: $F(1, 29) = 50.21$, $p < 0.0001$; Interaction: $F(1, 29) = 4.722$, $p = 0.038$; genotype: $F(1, 29) < 1$). Brain KYNA levels increased following repeated LPS injections in WT (mean ± SEM: WT saline+saline: 2.3 ± 0.7 nM ($n = 8$); WT LPS+LPS: 8.8 ± 1.3 nM ($n = 8$); **$p < 0.01$) and KAT II KO mice (mean ± SEM: KAT II KO saline+saline: 0.3 ± 0.1 nM ($n = 8$); KAT II KO LPS+LPS: 12.5 ± 2.0 nM ($n = 9$); ****$p < 0.0001$). Quantification of mRNA expression levels on all four KATs in mice revealed effects of genotype on KAT I ($F(1, 20) = 116.0$, $p < 0.0001$), KAT III ($F(1, 20) = 57.1$, $p < 0.0001$) and KAT IV ($F(1, 20) = 9.8$, $p = 0.0054$). KAT II mRNA was not detected in KAT II KO mice. Repeated LPS injections did not affect KAT I, KAT II or KAT IV expression levels of mRNA in brain. However, this treatment was associated with increased KAT III mRNA expression (Treatment: $F(1, 20) = 34.3$, $p < 0.0001$) in WT and KAT II KO mice at P 22 (WT LPS+LPS vs WT saline+saline: ***$p < 0.001$; KAT II KO LPS+LPS vs KAT II KO saline+saline: *$p < 0.05$).

Follow-up experiments confirmed that immune activation induces KAT III and increases KYNA production in human fibroblasts, epidermal cells, and monocytes. Interestingly, we detected increased expression of KAT III in post-mortem brain samples from COVID-19 infected patients. In ongoing effort to develop selective and potent KAT III inhibitors (ongoing drug discovery project together with Science for Life Laboratory, Stockholm, Sweden), we have data showing that a selective KAT III inhibitor effectively reversed the immune-induced KYNA in our human cell model.

Conclusions: Our study provides evidence that KAT II is not the primary enzyme responsible for KYNA production under conditions of immune activation. Instead, our study highlights a critical role of KAT III. These findings have significant implications for the development of therapeutic strategies aimed at addressing the cognitive impairments commonly observed in patients with psychiatric disorders and infectious diseases.

Keywords: Kynurenic Acid, Drug Discovery - New Approaches, COVID-19, Cognitive Impairment Associated with Schizophrenia, Cognitive Impairment

Disclosure: Nothing to disclose.

P504. Longitudinal Study of Intrinsic Motivation in Youth at Risk for Psychosis

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Background: Amotivation in psychosis risk is disabling, lacks proven treatments, portends a worse prognosis, yet is understudied. Most fMRI research on motivation impairment has examined responses to extrinsic reinforcers (e.g., money). However, intrinsic motivation (IM), related to internal desires like mastery or curiosity, may be even more impaired than extrinsic motivation (EM). We previously demonstrated that fMRI response in ventral striatum (VS), a core brain motivation region, is greater for correct than incorrect responses during cognitive tasks even in the absence of any feedback. This fMRI operationalization of IM is reduced in schizophrenia as well as youth with subclinical psychosis spectrum symptoms (PS). Here we describe preliminary results from a longitudinal study comparing IM and EM during a challenging cognitive task, in relation to trait self-reported IM and EM and clinical amotivation. We hypothesized that clinical amotivation and PS status would be more strongly associated with impairment in IM than EM. We expected intrinsically motivated performance to activate VS as individuals internally evaluate their performance relative to their expectations, and that VS activation would relate to IM both cross-sectionally and longitudinally.

Methods: Data collection for 2-year longitudinal follow-up (T2) was recently completed, achieving 67% retention of participants who completed the identical baseline (T1) procedures. Preliminary results here utilized the fMRI-analyzable T1 sample of 95 individuals with PS and 31 typically developing controls (TD) aged 16-26 (40% female, group-balanced); at T2 there were 64 PS and 21 TD. During fMRI, participants performed a visual fractal memory task, under three counterbalanced feedback conditions: 1) none 2) accuracy information 3) monetary. On each trial, participants identified which of two presented fractals was the one viewed previously during pre-scan encoding. Trials included three phases: choice, confidence rating, and feedback, separated by jittered delays. fMRI analysis focused on VS, together with secondary regional and whole-brain analyses. The primary measure of IM was our new brief self-report measure of trait-like/general IM; additional study measures include clinical amotivation and other negative symptoms (CAINS), positive symptoms (SIPS, PRIME), self-reported and behavioral (free-choice) measures of task IM, and a behavioral discounting battery. MRI data also included structural MPRAGE and resting state BOLD (ABCD sequences).

Results: At T1, PS showed elevated clinical amotivation (CAINS) ($t = 6.2$, $p < 0.001$) and impaired trait IM ($t = 2.6$, $p = 0.01$), but intact trait EM ($t = 0.14$, $p = .89$), CAINS amotivation correlated more strongly with self-reported trait IM (all: $r = -0.37$, $p < 0.001$; PS only $r = -0.29$, $p = 0.005$) than EM (all: $r = -0.09$, $p = 0.33$; PS only: $r = -0.10$, $p = 0.35$). fMRI revealed robust VS activation to the fractal stimulus (memory choice) phase, to parametric variation in across-trial confidence ratings during choices, to correct vs. incorrect feedback and to across-trial prediction errors (outcome-confidence) during feedbacks (p's < 0.01). VS activation during the choice phase across feedback conditions was dimensionally related to IM ($t = 3.85$, $p < 0.001$) but not EM ($t = 1.26$, $p = 0.21$) across all participants, controlling for group status. VS activation did not show significant categorical PS vs. TD differences. At T2, 67% of PS and 68% of TD returned, with no significant retention differences within based on clinical amotivation or trait IM (p's > 0.10).

Trait-like IM and EM correlated robustly across T1 and T2 (IM $r = 0.67$; EM $r = .67$), showing greater two-year stability than the CAINS clinical interview amotivation measure ($r = 0.55$) or the 3-item IM measure from the Quality of Life Scale ($r = 0.44$). Preliminary analysis of T2-only fMRI data showed the same pattern of VS activation relating to IM seen at T1, and 2-year change (T2 minus T1) in VS activation significantly related 2-year change in IM ($p < 0.05$, corrected).

Conclusions: Our findings support the hypothesis that intrinsic motivation is selectively impaired in PS and selectively related to clinical amotivation, relative to extrinsic motivation. The fMRI

results demonstrate that VS responses during a challenging cognitive task indeed reflect internally generated reinforcement signals related to accuracy and confidence, as well as to self-reported trait IM. Preliminary longitudinal analysis suggests that trait-like IM is fairly stable over two years, but also exhibits meaningful change that correlates with change in VS activation. Thus, VS encodes IM-related task responses both cross-sectionally and longitudinally. Our ultimate goal is to characterize neural mechanisms of amotivation and develop biomarkers for neurobehaviorally-defined amotivation dimensions or subtypes. These biomarkers will be tested for prognostic utility and as moderators or mediators for early interventions in at-risk youth.

Keywords: Psychosis-Risk, Intrinsic Motivation, Avolition, fMRI

Disclosure: Nothing to disclose.

P505. Weaker Surround Suppression in Schizophrenia is Consistent With Narrower Spatial Attention

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Background: In the visual illusion known as surround suppression, the salience of a central stimulus is reduced by the presence of a surrounding image. Previous studies from our group and others have shown that this illusion is weaker among people with schizophrenia. A recent study from our group (Pokorny et al., 2023) found weaker surround suppression in schizophrenia for center stimuli with orthogonal surrounds, but no difference for parallel surrounds. In that study, relative orientation served as the primary cue for segmenting (or grouping) center and surround. Cues for segmentation and grouping can influence the allocation of visual attention. As abnormal spatial attention is well documented in schizophrenia, one possibility is that there may have been differences in the allocation of spatial attention between groups. Recent studies have shown that narrower spatial attention yields weaker surround suppression in healthy adults.

Methods: In a series of experiments, we examined how task manipulations that influenced center-surround grouping affected the surround suppression illusion among people with schizophrenia and healthy controls. In Experiment 1, we measured surround suppression using a visual contrast discrimination task, in which grouping cues were strong (i.e., simultaneous center and surround onset, parallel orientation). Experiment 1 included data from 33 healthy controls and 31 people with schizophrenia (both sexes). In Experiment 2, we measured surround suppression of perceived contrast across conditions in which grouping cues varied (i.e., surround onset preceded center, parallel or orthogonal surrounds, presented to the same or to opposite eyes). Experiment 2, which is ongoing, included 27 healthy controls and 18 people with schizophrenia (both sexes). Finally, we used an established computational model based on divisive normalization (Reynolds and Heeger, 2009) to explore how broader vs. narrower spatial attention may affect the strength of surround suppression.

Results: In Experiment 1, we quantified surround suppression by measuring the ratio of contrast discrimination thresholds for parallel surrounds / no surrounds. Surprisingly, suppression was stronger among people with schizophrenia vs. controls (Wilcoxon rank sum, $Z = -1.96$, $p = 0.050$). We did not observe significant differences in surround suppression among people with bipolar disorder ($n = 20$), nor among biological relatives of people with psychosis ($n = 44$) vs. controls. In Experiment 2, we measured suppression of perceived contrast for parallel and orthogonal surrounds presented to the same or opposite eyes. Here, we found significantly weaker surround suppression among people with schizophrenia (ANOVA, main effect of group, $F(1,43) = 6.86$, $p = 0.012$; group x condition, $F(4,172) = 2.63$, $p = 0.036$), in agreement with previous findings in the literature. Our computational modeling results were consistent with the idea that differences in attention between groups could modulate the strength of surround suppression. In particular, our model showed that narrower spatial attention could evoke weaker surround suppression, whereas broader attention made suppression stronger.

Conclusions: Significantly weaker surround suppression in schizophrenia was observed only when center and surrounding stimuli were easily segmented (Experiment 2). When grouping cues were strong (Experiment 1), people with schizophrenia instead showed stronger suppression. Using a computational model, we found that this pattern of results was consistent with differences in the width of spatial attention across groups and experiments. In particular, people with schizophrenia may have particularly struggled to segment center and surrounding stimuli in Experiment 1, leading to broader spatial attention and stronger surround suppression. In Experiment 2, stronger segmentation cues may have allowed people with schizophrenia to focus their attention more narrowly on the center stimuli, yielding weaker suppression compared to controls.

Keywords: Psychosis, Visual Perception, Visual Attention, Context

Disclosure: Nothing to disclose.

P506. Medial Frontal GABA in Psychosis: Preliminary Results From an Ongoing Study

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Background: Multiple lines of evidence support the hypothesis that abnormalities of the gamma aminobutyric acidergic (GABAergic) system play a role in psychosis. Magnetic Resonance Spectroscopy (MRS) can quantify regional GABA concentrations non-invasively and has revealed differential patterns of regional medial frontal cortex (MFC) GABA alterations in patient groups with psychosis. In schizophrenia, reduced GABA concentrations have been observed in mid- and posterior MFC, whereas increased GABA in more rostral regions of MFC has been shown in bipolar disorder. In addition, lower GABA concentrations in MFC are associated with higher levels of negative affect and poor tolerance for stress - traits which are frequently present in individuals with psychosis. Here, we present a preliminary analysis from a large, multimodal dataset, investigating regional MFC GABA concentrations in schizophrenia and bipolar disorder, as well as exploring relationships between these regional GABA concentrations and levels of negative affect.

Methods: Participants included 25 patients with schizophrenia spectrum disorders (schizophrenia, schizoaffective disorder and psychotic disorder not otherwise specified; 15 women, 10 men), 21 bipolar I patients with a history of psychosis (13 women, 8 men), and 34 demographically matched healthy control participants (17 women, 17 men). All patient participants were on stable doses of psychotropic medication. To estimate GABA, MRS data was acquired using a MEGA-PRESS sequence, from three distinct voxels in MFC– the posterior medial frontal cortex, the rostral anterior cingulate cortex, and the dorsomedial prefrontal cortex. MRS data were preprocessed and modelled using the Gannet 3.3 toolbox, and GABA estimates were corrected for voxel tissue composition. Participants also completed the 9-item version of the

Psychological Stress Index (PSI9), a self-report measure of sensitivity to stress, on the same day as the MRS session.

Results: Interim analysis revealed there were no significant differences between groups in any of the three MRS voxels. In the healthy control participants, lower GABA concentrations in rostral anterior cingulate cortex exhibited the predicted association with higher PSI9 scores ($r34 = -.41$, $p = .03$). There were no significant relationships between PSI9 and GABA estimates in the posterior medial frontal cortex or dorsomedial prefrontal cortex, or in either of the patient groups in rostral anterior cingulate cortex.

Conclusions: Our findings are in line with previous evidence supporting a link between higher levels of negative affect and lower GABA concentrations in the medial frontal cortex, although we have so far failed to find group differences in GABA concentrations. Surprisingly, we did not find evidence of a relationship between negative affect, measured as stress sensitivity, in patients with psychosis. These results are, however, preliminary and data collection is ongoing.

Keywords: Magnetic Resonance Spectroscopy, GABA, GABA MRS, Negative Affect

Disclosure: Nothing to disclose.

P507. Neural Markers of Loneliness and Isolation in Psychotic Disorders and Associations With Trust and Social Distance

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Background: For the past several decades, public health experts have called attention to an "epidemic" of loneliness (perceived isolation) and social isolation (objectively few social contacts or interactions) in the general population that was exacerbated by the COVID-19 pandemic. Recent data have also shown that loneliness and isolation are particularly common in those suffering from a psychotic disorder, affecting ~80% of people with these conditions. Previous research has linked loneliness and isolation in the general population to poor cardiometabolic health and premature mortality, yet little is known about the psychological and neurobiological underpinnings of these associations and how they vary in different populations. However, some clues have emerged from studies showing that certain cognitive and behavioral biases are associated with loneliness, including a bias towards social mistrust and sensitivity to rejection, and a tendency to maintain a greater physical distance ("personal space") from others. In the current study, we employed a fMRI paradigm and analytic approach that exploited these biases, with the goal of identifying biases in neural responses associated with loneliness and isolation in people with and without psychotic disorders.

Methods: Functional and structural MRI data, alongside clinical assessments and self-report scales measuring loneliness (the UCLA Loneliness Scale), amount of social contact (the Social Network Index, SNI) and social withdrawal (the Time Alone Questionnaire (TAQ)), were acquired for 100 individuals, 37 with a diagnosis of either an affective ($N = 17$) or non-affective ($N = 20$) psychotic disorder (PD) and 63 age- and sex-matched healthy controls (HC). During acquisition of fMRI data, participants viewed images of human faces which either increased in size (approaching) or decreased in size (withdrawing) at the rate of change equivalent to a typical walking speed (the Looming task). Each fMRI scan included 16 task trials where race and gender of the faces were pseudorandomized. Following the scanning, participants rated the trustworthiness of each face they had viewed. Freesurfer's Functional Analysis Stream (FS-FAST) was used for post-processing of the fMRI data and implemented motion correction, slice timing correction and registration to the anatomical scan for region-of-interest analysis. Freesurfer's general linear model (GLM) tool was used to generate individualized contrast effect size (CES) maps for each participant by contrasting approaching versus withdrawing faces weighted by perceived face trustworthiness. Subsequently, voxel-wise GLM group-level analyses were conducted to: 1) identify regions activated by the task (approach vs. withdrawal contrast), 2) test for HC versus PD group differences covarying for potentially confounding clinical measures, and 3) quantify the association between the fMRI task response and scores on the UCLA loneliness scale, SNI and TAQ measures. The regression analyses were conducted in the whole group and in the HC and PD groups separately. A clusterwise correction for multiple comparisons of $p < .001$ was applied to all analyses.

Results: The PD group had significantly higher levels of loneliness, social isolation and social withdrawal than the HC group, as expected (all p Withdrawal activation in the hippocampus, striatum and hypothalamus. Social isolation was linked to variation in responses of the same areas, as well as the amygdala and parahippocampal gyrus regions. When the loneliness regression analysis accounted for levels of social isolation, responses of the striatum, hippocampus and thalamus were associated with loneliness. Of note, the pattern of findings in the HC and PD groups were similar to each other and to that of the whole group.

Conclusions: Loneliness and social isolation may be associated with a greater response to stimuli that signal social withdrawal (i.e., withdrawing faces) in subcortical brain regions involved in emotion, memory, and homeostatic regulation. This pattern of response was evident across the PD and HC groups, suggesting that it may represent a transdiagnostic neural marker of loneliness and isolation. Such a marker could inform intervention development or early detection and monitoring efforts. Ongoing work will test whether this putative marker is linked to some of the known systemic effects of loneliness and isolation (cardiometabolic and inflammatory dysregulation) and whether such effects play a role in the poor health outcomes and earlier mortality associated with psychotic disorders.

Keywords: Psychotic Disorders, Loneliness, Task fMRI

Disclosure: Nothing to disclose.

P508. Neural Activity During Cognitive Empathy is Associated With Social Functioning and Negative Symptoms in Schizophrenia: Preliminary Analyses

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Background: Empathy deficits are well established in schizophrenia, and contribute to poor social functioning. Empathy may be defined as the ability to understand and share the perspectives and mental states of others. Cognitive empathy is a deliberate, effortful process that involves taking the perspective of others, ranging from simple visual perspective taking (e.g., inferring which objects someone else sees) to making complex inferences about another's feelings, beliefs, and intentions. It is consistently impaired in schizophrenia. In healthy adults, cognitive empathy is associated with activation in several brain regions, including medial prefrontal cortex (mPFC) and posterior cingulate/precuneus (PCC). Functional implications associated with alterations in activity in these regions during cognitive empathy tasks are unknown.

Methods: In this task-based functional magnetic resonance imaging (fMRI), we examined neural activity during a mentalizing

task designed to isolate processing specific to cognitive empathy in a preliminary sample of 28 individuals with schizophrenia and 23 nonpsychiatric comparison individuals. In this task, participants are asked to take the perspective of a "director" that is either the same as or different from their own perspective, depending on where the director is located. Participants must use this information to correctly determine which object on a set of shelves should be moved at the director's request. The primary behavioral dependent variable is accuracy by condition, and the primary fMRI contrast of interest is trials in which the participant and director have the same perspective vs. trials where they have different perspectives. Beta weights extracted from regions of interest (ROIs) were analyzed with repeated measures ANOVA for within and between-group effects. Association between condition effects and measures of social functioning and negative symptoms were examined using bivariate correlation.

Results: Individuals with schizophrenia showed significantly lower accuracy across conditions compared to comparison participants ($t(49) = -5.63$, $p < .001$). There was a significant condition by group interaction ($F(1,49) = 7.06$, $p = .011$) such that accuracy did not vary by condition for the comparison group, but individuals with schizophrenia performed worse when taking a different perspective from their own. ROI analyses revealed a typical pattern of activation in both groups, with deactivation of mPFC and greater activation of PCC during the different versus same perspective condition ($F(1,49) = 12.49$, $p < .001$). However, individuals with schizophrenia had significantly less deactivation of mPFC than comparison participants ($F(1,49) = 9.10$, $p = .004$). In individuals with schizophrenia, activity in PCC during both task conditions was positively correlated with social functioning (RFS social functioning scale score; $r = .67$, $p = .001$ and $r = .68$, $p < .001$, respectively) and negatively correlated with negative symptoms (CAINS Expressive subscale score; $r = -.58$, $p = .008$ and $r = -.70$, $p < .001$).

Conclusions: Results from this study help to elucidate our understanding of cognitive empathy deficits in schizophrenia, including their neural correlates, and their functional implications. The extent to which individuals with schizophrenia tend to activate posterior versus anterior cortical midline regions during other-oriented processing is reflected in better social functioning and fewer negative symptoms. This pattern of activity may be compensatory for disrupted self-oriented processing and inadequate self-other boundary observed in the disorder.

Keywords: Empathy, Schizophrenia, Functional MRI (fMRI), Social Functioning, Negative Symptoms

Disclosure: Nothing to disclose.

P509. Advanced Brain Age in Early Illness Schizophrenia and Clinical High-Risk Conversion and Associations With Experiential Negative Symptoms

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Background: Brain aging is not uniform across individuals and is shaped by the interplay among genes, environment, lifestyle, and health. Advanced brain aging is associated with deleterious processes such as underlying health problems, chronic stress and worry, and psychiatric disorders. These insights have motivated research to characterize aging from a biological perspective, relative to chronological age, to better model the effects of disease and other maladaptive life processes on the brain. One example of these efforts is the use of machine learning models that use high-resolution and multidimensional structural magnetic resonance imaging (MRI) data to predict chronological age for a given individual, thereby deriving a proxy of a person's biological brain age. The difference between the predicted brain age from these models and chronological age is commonly known as the brain age gap. There is consistent evidence that biological aging processes may occur at an earlier or more rapid rate in schizophrenia, with longitudinal research showing the greatest acceleration rate in the early years of the disorder. In comparison, evidence for advanced brain aging effects in individuals at clinical high-risk for psychosis (CHR-P) are less established, and it's unclear whether advanced brain aging serves as a risk factor for escalation to a full psychotic disorder. Advanced brain aging is also related to a greater negative symptom burden. Because most studies to date assessed negative symptoms via global (and consequently, heterogenous) metrics, the question remains as to whether advanced brain aging corresponds with more specific facets of schizophrenia symptoms. We therefore sought to replicate prior findings of advanced brain age in early illness schizophrenia (ESZ), and to test whether advanced brain age represents a psychosis conversion biomarker in CHR-P, and how brain aging maps onto specific symptom facets, including individual negative symptom domains.

Methods: Using structural MRI, we compared brain aging among CHR-P ($n = 51$), ESZ ($n = 78$), and healthy control (HC; $n = 90$) participants. To examine associations with psychosis conversion, we compared brain aging in CHR-P individuals who converted to psychosis ($n = 10$) and CHR-P individuals who were clinically followed for 2 years without converting ($n = 23$). Further, in CHR-P and ESZ, we examined brain aging associations with specific negative and positive symptom subdomains. For negative symptoms, we separately examined experiential (avolition and anhedonia items) and expressive (affective flattening and alogia items) subdomains. For positive symptoms, we examined hallucinations, delusions, suspiciousness, grandiosity, bizarre behavior, and disorganized communication subdomains. The Centile BrainAGE algorithm, which was formulated and tested on over 40,000 Enhancing Neuro Imaging Genetics by Meta-Analysis (ENIGMA) participants, was used to compute the brain age gap (i.e., predicted brain age minus chronological age). This algorithm encompassed the age range of our study participants and showed good model fit in all three groups as depicted by low mean absolute error scores.

Results: ESZ showed a larger brain age gap (i.e., "older-looking" brains) relative to HC and CHR-P (ps < .010). Although CHR-P as a whole did not show advanced brain aging, CHR-P individuals who converted to psychosis ($n = 10$) showed a larger brain age gap ($p = .043$; Cohen's $d = 0.67$) relative to CHR-P who were followed for 2 years without converting ($n = 23$). Greater brain age gap in ESZ was correlated with longer duration of illness ($pFDR = .040$) and greater negative experiential symptoms ($pFDR = .008$), but not negative expressive symptoms ($pFDR = .726$). Greater brain age gap scores correlated with greater negative experiential symptoms even after accounting for expressive symptoms and duration of illness (ps < .002). Post-hoc tests of the two negative experiential symptom subdomains comprising the experiential facet showed that greater brain age gap was positively associated with both avolition ($p = .001$) and anhedonia ($p = .027$). Brain age gap did not correlate with positive symptoms or or CPZ dosage equivalents (psFDR > .726). In CHR-P, brain age gap was not correlated with clinical symptom severity (psFDR > .537).

Conclusions: These findings highlight the importance of future mechanistic studies that further explore the factors related to advanced aging in schizophrenia and its associations with negative experiential symptoms. Our brain age findings can guide future efforts to disentangle natural age-related processes from disease specific changes in the early stages of schizophrenia, and lead to advances in predicting which CHR-P individuals are more

likely to convert to a full psychotic disorder. Our work also informs research concerned with motivational deficits in schizophrenia, raising future inquiries as to whether interventions that reduce and/or prevent brain aging advancements could correlate with enhanced goal-directed behavior and functioning.

Keywords: Brain Age, Schizophrenia (SCZ), Clinical High-Risk of Psychosis, Magnetic Resonance Imaging, Negative Symptoms

Disclosure: Nothing to disclose.

P510. Mapping Heterogeneous Patterns of Brain Atrophy in Schizophrenia to a Common Brain Network

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Background: Schizophrenia presents significant heterogeneity in its neuroanatomical correlations across various neuroimaging studies, particularly in regard to brain atrophy patterns. This variability impedes the development of reliable biomarkers or targeted interventions. Amidst this heterogeneity, we hypothesized that atrophy coordinates reported in published studies of patients with schizophrenia would converge to a common brain network unique to the disorder.

Methods: To test our hypothesis, we utilized the human connectome as a wiring diagram and employed coordinate network mapping (CNM)—a method that identifies network-level connections between heterogeneous brain coordinates. While traditional neuroimaging meta-analytic approaches such as activation likelihood estimation (ALE) identify common brain regions across studies, CNM enables the mapping of brain disorders to connected brain networks. Our analysis incorporated data from 113 published studies, totaling more than 11,000 individuals. Our sample included patients with schizophrenia ($n = 3,756$), individuals at high risk for psychosis ($n = 1,507$), and healthy controls ($n = 6,007$).

Results: We identified a common brain network preferentially connected to atrophy coordinates in schizophrenia, which we refer to as the 'schizophrenia network'. After correcting for multiple comparisons ($p < 0.05$), the anterior cingulate cortex and the mid-insula, bilaterally, emerged as peak areas in the network. The schizophrenia network is distinct from atrophy patterns observed in high-risk individuals (including clinical and genetic high risk), normal aging ($n = 4,195$), neurodegenerative disorders ($n = 3,707$), and other psychiatric conditions ($n = 3,432$). Moreover, it remains stable with disease progression and across various clusters of psychotic symptoms. Interestingly, we found that patterns of brain atrophy in schizophrenia were negatively correlated with lesions associated with psychosis-related thought processes in an independent cohort of patients with penetrating head trauma ($p < 0.05$) ($n = 181$).

Conclusions: We identified a unique, stable, and unified schizophrenia network, addressing a significant portion of the heterogeneity observed in prior atrophy studies. The stability of this network across disease progression underscores its potential as a trait-like characteristic, and its uniqueness suggests it could be useful for development of biomarkers and brain stimulation targets in patients with schizophrenia. Our findings also challenge traditional understanding, revealing that brain atrophy in schizophrenia could represent a compensatory process. Future studies may investigate this network further, potentially leading to improved diagnostic and therapeutic strategies for schizophrenia.

Keywords: Schizophrenia (SCZ), Brain Networks, Neuroimaging

Disclosure: Nothing to disclose.

P511. Sex Differences in the Functional Network Correlates of Childhood Psychosis-Like Experiences

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Background: Sex differences exist in the prevalence, expression, and treatment of psychotic disorders. Males exhibit earlier onset and greater negative symptoms, while females display greater affective symptoms and greater social functioning. However, whether these differences exist in childhood psychosis-like experiences and map onto neurobiological correlates is not yet known.

Brain-based predictive models can successfully capture the neural correlates of cognition, personality, and psychopathology, and predict these behaviors at an individual level. Recently, sex-specific models–compared to sex-independent ones–have shown promise when studying behaviors and psychiatric illnesses with known sex differences. These models quantify brain-behavior relationships that are specific to each sex to yield individually tailored biomarkers of behavior.

Here, we leverage sex-specific brain-based predictive modeling approaches to establish the neural correlates of childhood psychosis-like experiences in a large-scale sample of children and adolescents.

Methods: Using resting-state functional MRI and behavioral data from the Adolescent Brain Cognitive Development dataset ($n = 5260$, ages 9-10, 2571 females), we characterize the resting-state functional connectivity correlates of childhood psychosis-like experiences in a sex-specific manner.

We assessed sex differences in the number and overall severity of symptoms (including hallucinations and delusions). We then trained sex-specific linear ridge regression models using k-fold cross-validation to predict number of symptoms and severity based on functional connectivity at an individual-level. Data were split into 100 training/test sets, and separate models were optimized and trained to predict number of symptoms and severity (measured using the Prodromal Questionnaire) on the training sets and evaluated for accuracy in the test set. Prediction accuracy is defined as the correlation between the true and predicted behavioral scores and averaged across all splits. We used exact tests for differences to evaluate whether prediction accuracies were better than chance, as determined by 1000 null permutations.

Feature weights were extracted from the models and Haufe-transformed to improve reliability and interpretability. We evaluated correlations between the Haufe-transformed feature weights corresponding to the models trained across sexes and measures.

All analyses presented in this abstract are new and have not yet been published.

Results: Males reported a greater number of overall symptoms than females ($t = 2.51$, $p = 0.01$), but there were no sex differences in severity ($t = 0.35$, $p = 0.72$).

Brain-based models accurately predicted the number of symptoms in males (prediction accuracy, $r = 0.100$, $p = 0.003$) and females ($r = 0.085$, $p = 0.008$), as well as the overall severity in males ($r = 0.114$, $p = 0.001$) and females ($r = 0.081$, $p = 0.007$).

Brain functional connectivity patterns associated with number of symptoms and severity were strongly correlated across measures ($r = 0.97$ for females, $r = 0.97$ for males), and moderately correlated across sexes ($r = 0.58$ for number of symptoms, $r = 0.57$ for severity).

Functional connections within/between limbic and somatomotor networks were associated with the expression of psychosis-like symptoms in males, whereas connections within/between limbic, temporal parietal, default, and attention networks were associated in females.

Conclusions: Sex differences in the expression of psychosis-like experiences begin to emerge in childhood. Both the presence and the severity of these symptoms can be reliably predicted based on individual functional connectivity. Moreover, these symptoms are associated with distinct functional networks in males and females.

In males, brain-based correlates are largely found within unimodal sensory networks whereas in females, they are dispersed throughout heteromodal association networks. Functional network maturation follows a unimodal to heteromodal gradient, and typically occurs 1-2 years earlier in females. The stronger relationships observed between heteromodal networks and the expression of symptoms observed in females may reflect the different network maturation trajectories that males and females exhibit during this developmental stage. Further research is needed to quantify whether these unique correlates across the sexes are tied to the differential expression of positive and negative symptoms.

Taken together, these results suggest that distinct functional network correlates underlie the expression of childhood psychosis-like experiences across the sexes. As such, the use of sex-specific brain-based prediction models may yield more accurate markers for early diagnosis and intervention for psychosis than sex-independent ones.

Keywords: Brain-Based Predictive Modeling, Resting State Functional Connectivity, Sex Differences, ABCD, Psychosis

Disclosure: Nothing to disclose.

P512. Disorganization Disrupts the Core: Localizing Clinical Dimensions of Speech and Language Disturbance Within Clause Structure Using Multi-Layered Semantic Graph Analysis

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Background: Natural language processing (NLP) has been shown to detect features that are meaningful in predicting psychosis and its disease dimensions, but the specific relationships between NLP features and different dimensions of speech disturbance are poorly understood. Three latent factors have been identified for speech and language disturbances: impaired expressivity, inefficiency, and incoherence. One way to capture signals related to these clinical dimensions of speech disturbance is by using mathematical features describing the size, connectedness, and organization of semantic speech graphs. In order to generate more sensitive and specific linguistic features, we sought to localize graph features to different semantic domains within the clause structure. Clauses can be divided into a core element which captures the primary action (who does what to whom), and peripheral elements which convey secondary information related to the time, location, and/or manner of the primary action. We hypothesize that disorganized speech dimensions (inefficiency and incoherence) are reflected by disturbances to the core of the clause structure whereas impaired expressivity is equally distributed over the core and periphery. The tool used in this study will be freely available at: http://huggingface.co/spaces/amirhnikzad/MLSG_01.

Methods: Speech samples were collected from a transdiagnostic cohort of participants with (n = 27) and without (n = 44) psychosis, in response to picture description and autobiographical prompts. Transcribed samples were divided into constituent sentences. Each sentence underwent automated semantic role labeling (SRL) to annotate predicates, active/passive arguments, and non-argument expressions related to setting (time and location), and manner. Subsequently, each sample was dissected into five semantic layers corresponding to: 1) flow of predicates: connect each predicate to the next; 2) predication: connect predicates to their active/passive arguments; 3) action: connect active arguments to passive arguments; 4) setting: connect spatiotemporal expressions to predicates; and 5) manner: connect expressions of manner (e.g., general adverbs) to predicates. Each semantic layer was represented as a graph where nodes represented linguistic entities (predicates, arguments, and non-argument expressions) and edges represented semantic relations. The graph features describing size (number of edges and largest connected component – LCC), connectedness (density, average weighted degree – AWD, number of connected components – NCC, and largest strongly connected component LSCC), and organization (clustering coefficient – CC, number, and size of connected components as compared with random graphs – NCCZ, LCCZ, and LSCCZ) of each semantic layer were computed task-wise and averaged per participant.

For clinical characterization of speech and language disturbance, each participant was rated on the Scale for Assessment of Thought Language and Communication (TLC) and two items from the Scale for Assessment of Negative Symptoms (SANS; decreased vocal inflection and increased latency). Impaired expressivity, inefficiency, and incoherence factor scores were computed based on previously published methods.

The relationships between NLP features and dimensions of speech disturbance were separately evaluated for the semantic core and periphery. The effects were quantified using Spearman's rank correlation coefficient.

Results: Impaired expressivity showed multiple strong and significant correlations with graph features of all semantic layers (absolute rho = 0.2-0.6). There were also equal strengths of the relationships to graph features describing size, connectedness, and organization.

Inefficiency and incoherence showed more specificity toward layers representing the core of the sentence (flow, predication, and action). Incoherence was significantly correlated with LSCCZ and NCC in the flow layer (absolute rho = 0.2-0.3), CC, NCC, and LSCC in the predication layer (absolute rho = 0.21-0.27), and density, NCC, and LCCZ in the action layer (absolute rho = 0.2-0.3). Inefficiency was significantly correlated with NCC in the flow layer (rho = −0.21), LSCC in the predication layer (absolute rho: 0.2-0.27), and AWD, LCC, LSCC, NCCZ, LCCZ, and LSCCZ in the action layer (absolute rho = 0.2-0.3).

Conclusions: Consistent with our hypothesis, impaired expressivity and disorganized speech dimensions (incoherence and inefficiency) showed different patterns in the core vs. periphery of clause structure. Disorganized speech was more evident in the core: information related to events and their respective participants. Impaired expressivity was associated with a generalized shrinkage in all semantic layers. Because disorganization has different clinical implications compared to impaired expressivity, it is important to be able to identify these dimensions separately using NLP. Our findings enable us to incorporate features selectively based on their semantic localization, thereby allowing us to target clinical speech disturbances more specifically and more robustly.

Keywords: Natural Language Processing (NLP), Formal Thought Disorder, Semantic Speech Graph, Disorganization, Network Analysis

Disclosure: Nothing to disclose.

P513. GPT Reveals Selective Impairments in Global Vs. Local Context Use in Speech Among Treatment-Naïve Patients With Positive Thought Disorder

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Background: The advent of large language models is revolutionizing psychosis research, offering excellent discriminatory capabilities to distinguish between patients and control participants. However, they have not yet been used to quantify or understand the most prominent language atypicality in schizophrenia—disorganized language production, i.e., positive thought disorder. Psychopathologists have long characterized positive thought disorder as relying on relationships between individual words (local context) at the expense of broader discourse coherence (global context). Until now, however, there has been no way to objectively and automatically characterize the relationship between words and their prior context in natural speech. Instead, to assess thought disorder, clinicians and researchers are reliant on subjective, time-consuming rating scales. Here, we use GPT-3 to quantify the influence of global vs. local context on each word in speech samples produced by a large sample of treatment-naïve, first-episode psychosis patients. This allowed us to determine (a) the extent to which each word's surprisal relies on global vs. local context in patients vs. controls, and (b) whether the degree of selective dependence on local vs. global context specifically predicts the severity of positive thought disorder.

Methods: Seventy first-episode psychosis patients (all unmedicated and treatment-naïve) and 36 demographically-matched control participants described three pictures, each for approximately one minute. After speech transcription, we used GPT-3 to extract the probability of each word while manipulating the amount of context the model had access to. The surprisal (i.e., negative log probability) of each word, based on these different context lengths, served as the dependent measure in a series of linear mixed-effects models with which we tested our primary hypotheses. Thought disorder was assessed using the Thought and Language Index (TLI). Symptoms more generally were assessed using the PANSS. Finally, domain-general cognitive function was assessed using Semantic Fluency, Digit-Symbol Substitution, and the Trail-Making Test.

Results: We began by comparing the surprisal of each word using all available context to its surprisal with no context (estimated by replacing prior words with random words from an unrelated speech sample). This revealed a significant interaction between Context and Group (Est. = .322, $p = .001$), indicating that patients were less able than controls to use the available prior context to reduce the surprisal of each word they produced. We then asked the key question of whether this was driven by a selective impairment in using global vs. local context by titrating the window of context GPT had access to (from 1 to 50 prior words). We found a significant interaction between Log Context Length and Group (Est. = .086, $p < .001$), such that, as context length increased, the differences in surprisal between patients and controls became increasingly larger, indicating a disproportionate deficit in the use of global context in schizophrenia. This effect could not be explained by atypical domain-general cognitive function in patients. Most importantly, within the patient group, graded insensitivity to global-vs.-local context predicted the severity of positive thought disorder (assessed via the Disorganization subscore of the TLI). This effect was specific: There was no evidence of a relationship with overall symptom severity (PANSS total) or negative thought disorder (TLI Impoverishment subscore).

Conclusions: We show, for the first time, that global-vs.-local surprisal selectively predicts positive thought disorder in first-episode schizophrenia. This has several important implications. First, from a clinical perspective, we provide a measure that could be developed into a sensitive linguistic biomarker for fast, automated, and objective quantification of language disorganization. Such a biomarker could facilitate early detection of illness, symptom monitoring, prediction of outcome, and possibly the trajectory of thought disorder over time. It could also potentially provide a sensitive measure for detecting more subtle, subclinical atypicalities in communication that might impair psychosocial functioning in schizophrenia. Second, from a neurocognitive perspective, these findings directly bridge clinical characterizations of thought disorder in natural speech with neurocognitive evidence for selective deficits in the processing of global vs. local information in language comprehension, as well as in other perceptual and cognitive domains. Third, from a neurocomputational perspective, these findings are consistent with hierarchical generative models of psychosis. These theories posit that uncertainty over global representations, represented over longer time-scales at the highest levels of the cortical hierarchy, results in weaker predictions being propagated down to lower cortical levels, representing individual words, leading to reduced suppression of word-level surprisal (prediction error). To directly test this hypothesis, we need to move beyond GPT, which lacks the feedback connections that drive healthy language processing in the brain. We are therefore developing a model that is based on a more biologically plausible predictive coding architecture, which will allow us to explicitly simulate the effects of perturbed feedback on global vs. local surprisal.

Keywords: Formal Thought Disorder, Computational Neuroscience, Predictive Coding, Natural Language Processing (NLP), First Episode Psychosis

Disclosure: Nothing to disclose.

P514. Linguistic Risk Score for Schizophrenia: Applying a Polygenic Risk Approach to Synthesize Multivariate Features From Computational Speech and Language Analysis

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Background: Computational speech analysis offers powerful methods to objectively quantify linguistic signals which can reflect the mental state of the speaker. A wide range of features can be generated to describe linguistic content: e.g., parts of speech, phrase structure, semantic coherence, graph metrics, tempo, and lexical properties, among others. These features can be analyzed in different ways, e.g., with moving windows of varying sizes, verbatim vs. filtered content, etc., and using language collected from different tasks, e.g., picture description, open-ended narratives, fluency tasks, etc. Taken together, studies employing computational speech and language analysis can easily accumulate thousands of features. Understanding and accounting for this large feature space is a prominent challenge in this field. Here, we take an analogous approach to the calculation of polygenic risk scores to first identify candidate features associated with schizophrenia diagnosis, and then calculate a single weighted sum to represent the individual's "Linguistic Risk Score" (LRS). We hypothesized that the LRS would demonstrate intermediate effects in non-psychotic psychiatric disorders, and that within individuals with schizophrenia spectrum disorders

(SSD), the LRS would demonstrate both state and trait-like characteristics.

Methods: Three samples were included. Sample 1 was the training sample and included 52 healthy control (HC) and 85 SSD participants, with no significant differences in age, gender, race, or ethnicity between groups. Sample 2 was an independent cross-diagnostic test sample, including 21 randomly set-aside SSD participants, 81 participants with unipolar depression, 40 with bipolar disorder, and 22 with mood disorders with psychotic features. Sample 3 included an overlapping sample of 62 participants with SSD with up to 4 timepoints of longitudinal follow-up. Psychosis symptoms were assessed dimensionally with the Brief Psychiatric Rating Scale (BPRS).

To calculate the LRS from 1227 computational speech and language features, we first selected features which were significantly different between groups in Sample 1 and calculated a weighting factor based on the effect size. Because many features were non-parametric, the group comparison was conducted using the Mann-Whitney U test, and the effect size was calculated using Cliff's Delta. Next, for each feature in each subject, a normalized rank score ranging from −1 to 1 was computed relative to the HC training sample, such that the test subject received a −1 if they scored lower than all HC subjects, 0 if they scored at the median, +0.50 if they scored at the 75%, and +1 if they scored higher than all HC subjects, etc. The normalized rank score was multiplied by the weighting factor for each feature, then summed across all features for each participant.

Group comparisons were made using ANOVA with pairwise effects calculated with t-tests (p values corrected using FDR). Between-subject dimensional relationships to psychosis symptom dimensions were calculated with linear regressions. To assess within-individual relationships between psychosis symptoms and LRS, we examined linear mixed models with random slopes and random intercepts by participant.

Results: LRS was calculated for p-value thresholds of 0.01, 0.05 and 0.10. In each case, we first verified that the HC LRS was centered at 0 and the SSD training sample produced a significantly elevated LRS. Comparing the HC sample to the independent cross-diagnostic test samples, we found elevated LRS for all of the psychiatric groups at all of the p-value thresholds, with marginally larger effect sizes for $p < 0.01$. Here, LRS in all groups was significantly elevated relative to HC with $p < 0.001$ (Cohen's d: Unipolar = 1.45, Bipolar = 1.75, Mood with psychosis = 2.18, SSD = 2.33). LRS in the psychosis groups were significantly elevated relative to unipolar depression (Mood with psychosis: $p = 0.02$, d = 0.61; SSD: $p = 0.03$, d = 0.61). There was no difference in LRS between the SSD and mood with psychosis groups, and trend level differences between these and the bipolar disorder group. Cross-sectionally, higher BPRS total score was associated with higher LRS between subjects (Std. Beta = 0.28, $p = 0.03$). Longitudinally, accounting for random effects in slope did not improve the prediction of LRS based on BPRS total score over the random intercept model. Here, we found significant values for the overall intercept (i.e., LRS when there are no active psychosis symptoms; Beta = 4.06, $p < 0.001$), the fixed effect of BPRS score (i.e., average relationship between LRS and BPRS; Beta = 0.08, $p < 0.001$), and the random effects for subject (95% CI of standard deviation: 0.96-2.29).

Conclusions: We successfully calculated a LRS to summarize SSD-related effects across 1227 computational speech and language features at p-value thresholds of 0.01, 0.05 and 0.10. The LRS demonstrated expected cross-diagnostic group differences, with the highest scores in SSD and mood disorders with psychotic features, and intermediate scores in unipolar depression. For individuals with SSD, LRS appears to demonstrate both state- and trait-like effects: LRS is elevated at baseline (i.e., even when psychosis-free), and further increases with greater psychosis severity.

Keywords: Natural Language Processing (NLP), Schizophrenia (SCZ), Polygenic Risk Score, Language, Computational Methods

Disclosures: Winterlight Labs: Consultant, Contracted Research (Self). North Shore Therapeutics: Board Member, Stock/Equity (Self). Psyrin: Advisory Board, Stock/Equity (Self).

P515. The Toronto Adolescent and Youth Cohort Study: Early Findings Related to Psychosis Spectrum Symptoms, Functioning, and Suicidality in Youth Seeking Mental Health Care

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Background: Psychosis spectrum symptoms (PSS) occur in a sizeable percentage of youth, and are associated with poorer cognitive performance, functioning, and suicidality (i.e, suicidal thoughts and behaviors). PSS may occur more frequently in youth already experiencing other mental illness, but the antecedents are not well known. The Toronto Adolescent and Youth (TAY) Cohort study aims to characterize developmental trajectories in youth with mental illness and understand associations with PSS, functioning, and suicidality.

Methods: The TAY Cohort study is a longitudinal cohort study that aims to assess 3,000 youth with 'light' phenotyping and of these, 1,500 with 'deep phenotyping' (11-24 years of age) presenting to tertiary care. The present analysis describes the extensive diagnostic and clinical characterization of psychopathology, substance use, functioning, suicidality, and health service utilization in these youths, with follow-up every 6 months over 5 years, along with early baseline data related to PSS, functioning, general psychopathology, and suicidality.

Results: 417 participants were enrolled between May 2021-February 2023. Participants met diagnostic criteria for an average of 3.5 psychiatric diagnoses, most frequently anxiety and depressive disorders. 49.2% of participants met a pre-established threshold for PSS, and exhibited higher rates of functional impairment, internalizing and externalizing symptoms, and suicidality compared with non-PSS participants.

Conclusions: Initial findings from the TAY Cohort study demonstrate the feasibility of extensive clinical phenotyping in youth seeking mental health care. PSS prevalence is much higher than in community-based studies. Our early data support the critical need to better understand longitudinal trajectories of clinical youth cohorts in relation to psychosis risk, functioning, and suicidality.

Keywords: Psychosis Spectrum Symptoms, Ultra High-Risk Youth, Clinical High-Risk of Psychosis

Disclosure: AbbVie: Advisory Board (Self).

P516. Presence and Severity of Incomplete Hippocampal Inversion in Psychosis Spectrum and 22q11.2 Deletion Syndrome

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Background: Abnormalities of the hippocampus are common in neurodevelopmental and neuropsychiatric disorders. The human hippocampus begins developing early in gestation thus measuring specific structural changes of the hippocampus may provide insight into the origins of some neurodevelopmental and neuropsychiatric symptoms. Between gestational weeks 20-30 a morphologic inversion of the dentate gyrus and cornu ammonis occurs around the hippocampal sulcus. Failure to complete this inversion results in the incomplete hippocampal inversion (IHI), which is characterized by a round, verticalized, medially positioned hippocampal body in the coronal plane and a deep collateral sulcus. IHI tends to be higher in the left hemisphere (~17%) than right (~6%), has higher prevalence in some neurodevelopmental disorders but has not been systematically assessed in psychosis spectrum youth nor in patients at a genetic high-risk for psychopathology such as 22q11.2 deletion syndrome (22q11DS). 22q11DS a genetic deletion that is known to increase individuals' risk for schizophrenia, depression, anxiety, and OCD. Therefore, investigating IHI 22qDS and psychosis risk may provide a valuable chance to uncover the underlying neurobiological correlates of certain mental health disorders.

Methods: Using T1-weighted images form 3T MRI the incidence of IHI in 22q11DS, psychosis spectrum (PS) illness other psychopathology (OP) and typically developing (TD) youth individuals were examined. Expanding upon published criteria (Cury et al., 2015) two raters assessed the prevalence of IHI using a semi-automate algorithm in 1,665 individuals [488 TD, 457 PS, 637 OP; 83 22q11DS]. Follow-up analysis of hippocampal subfield volumes were performed using Freesurfer.

Results: As expected the prevalence of IHI was higher in the left (22%) than right (10%) hemisphere across all diagnostic groups ($p < 0.0001$). The prevalence of IHI in the left hemisphere was significantly higher (Chi-sq(3) = 75.48, $p < 0.001$) in 22q11DS (59%) as compared to TD (21%), PS (19%), or OP (17%). A similar pattern was observed in the right hemisphere (Chi-sq(3) = 17.04, $p < 0.001$): 22q11DS (23%), TD (8%), PS (10%), and OP (11%). 22q11DS show lower volume in most, but all bilateral subfields of the hippocampus as compared to PS, OP and TD (ps < 0.01). As compared to TD, PS participants showed lower volumes in the left hippocampal head ($p < 0.01$) including smaller subiculum, CA1, and molecular layer. There were no differences in the left granule cell layer between PS and TD nor any difference in the right hippocampus.

Conclusions: These preliminary results indicate significant morphological changes of the hippocampus in patients with 22q11DS and PS and OP, particularly with in the hippocampal head. Future work will link morphology and volumetric measures to clinical and neurobiological phenotypes which will help identify those features most directly linked to psychopathology. Also, to the extent that these developmental markers are evident before sub-psychotic symptoms are evident, they may allow more reliable identification of psychosis risk.

Keywords: Hippocampus, Psychosis-Risk, 22q11 Deletion Syndrome, MRI

Disclosure: Nothing to disclose.

P517. Synergistic Impact of Microglia-Mediated Adverse Effect of Adolescent THC Exposure and 16p11.2 Duplication on Prefrontal Cortex Maturation and Social Memory

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Background: Considering recent marijuana legalization in the US, the deleterious effect of cannabis use during adolescence, a critical period for prefrontal cortex (PFC) maturation, has gained further attention as a risk factor for psychiatric disorders. Importantly, most cannabis users do not develop psychiatric symptoms, suggesting that cannabis exposure may be an environmental risk factor in individuals genetically predisposed to psychiatric disorders. Cannabinoid receptor type 1 (Cnr1) is expressed not only in neurons and astrocytes, but also in microglia, which shape neural connections during adolescence. However, the role of microglia in mediating the adverse effect of cannabis remains unexplored. In this study, we investigate the impact of adolescent delta-9-tetrahydrocannabinol (THC) exposure on microglia of which disturbance may be exacerbated by a genetic risk factor for psychiatric disorders, leading to aberrant PFC maturation and producing adult pathophysiology. In particular, we focus on the interplay between THC exposure and 16p11.2 duplication (16p11dup), a major CNV risk for psychiatric disorders.

Methods: 16p11dup and control mice (both sex) were chronically exposed to THC during adolescence or adulthood by single daily subcutaneous injections, followed by molecular, histochemical, biochemical, electrophysiological, and behavioral assays to determine convergent effect of THC and 16p11dup on microglial function, resulting in disturbance of medial PFC (mPFC) neuronal function and cognition in adulthood.

Results: Adolescent THC exposure induced mPFC-specific microglial molecular and morphological changes via Cnr1-mediated mechanisms ($p = 0.0001$, $t = 10.97$, $df = 10$). We also found that these microglia changes were exacerbated by 16p11dup, leading to neuronal subtype-specific deficits in intrinsic excitability of mPFC pyramidal neurons and resultant social memory deficits (THC x 16p11dup interaction [$F1,30 = 5.665$, $p = 0.0239$]). By performing microglia-specific RNA-seq experiments, we identified specific genes which may mediate these phenotypes.

Conclusions: We identified the role of microglial Cnr1 for mediating the adverse effect of THC on adolescent mPFC maturation and social memory in 16p11dup mice. We are currently investigating molecular mechanisms of how adolescent THC exposure impairs microglia-mediated mPFC maturation in a neuronal subtype-specific manner. Our findings highlight the importance of microglial Cnr1 to produce the adverse effect of cannabis exposure in genetically vulnerable individuals.

Keywords: Microglia, Adolescent Cannabis, Copy Number Variation

Disclosure: Nothing to disclose.

P518. Maldevelopment of Primate Pulvinar-Prefrontal Cortex Connectivity Reproduces the Functional, Behavioral and Cellular Changes Observed in Schizophrenia

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Background: Schizophrenia (Sz) is characterized by the cellular and cognitive impairment of the prefrontal cortex (PFC) and dysfunction of the thalamus. A thalamic nucleus most heavily implicated is the medial pulvinar (PM), which has robust bidirectional connectivity with the PFC. Previously, we revealed how another pulvinar nucleus, the medial inferior pulvinar (PIm), is crucial for the normal development of visual cortical areas and visuomotor behavior. Therefore, here we proposed that the PM is critical for the normal development of the PFC, and that perturbation of the PM and its connectivity with the PFC in early life will lead to the well-characterized cellular and cognitive phenotype associated with Sz.

Methods: To test our hypothesis, we bilaterally lesioned (NMDA injection) the PM of infant marmoset monkeys (Callithrix jacchus) at postnatal day 14 using an MRI-guided stereotaxic surgery ($n = 8$ lesion, $n = 7$ sham control, both sexes), and then longitudinally examined PFC anatomy, connectivity and function into adulthood (>18 months age (m.a.)). We primarily selected non-invasive methods akin to those used to assess human patients with Sz to maximize clinical translatability. First, marmosets underwent diffusion MRI to confirm the loss of frontal thalamocortical connectivity and characterize local changes in PFC cytoarchitecture. Resting-state epidural EEG was recorded from the PFC using internalized radiotelemetry implants to examine the function of local PFC circuits. Next, animals underwent regimented behavioral training using a novel, home-cage integrated touchscreen platform and were assessed in cognitive tasks to challenge PFC function. Finally, cerebral tissues were examined to confirm the extent of the PM lesion and changes in cortical cytoarchitecture.

Results: Prior to adolescence (< 6 m.a.), diffusion MRI tractography confirmed reduced PM-PFC connectivity in PM-lesioned animals compared to sham controls. During adolescence (6-18 m.a.), anatomical refinement of local PFC circuits was observed through changes in cortical fractional anisotropy in sham control ($p < 0.01$) but not PM-lesioned animals ($p = 0.69$). In adulthood (> 18 m.a.), resting-state EEG revealed that this difference coincided with a selective reduction in relative gamma power (30-80 Hz) in the PFC following early life lesions (-3.2%; $p < 0.05$). Behaviorally, lesioned animals exhibited impairment in PFC-mediated cognitive functions, including reduced cognitive flexibility and a diminished capacity to maintain working memory over extended delay periods > 2s. Post-mortem immunohistochemical staining of the PFC revealed a 54.5% reduction in parvalbumin-expressing neurons, known generators of cortical gamma oscillations, in the thalamo-recipient layer 3 ($p < 0.0001$).

Conclusions: Together, these findings provide a framework in which thalamocortical input originating from the medial pulvinar may be critical for the postnatal development of the PFC and cognitive functions in primates. The replication of several clinical components of Sz (cellular, functional, and behavioral) following early-life PM lesions suggests that perturbation of PM may be a component of the pathogenesis of Sz.

Keywords: Pulvinar, Marmoset, Parvalbumin Neurons, Developmental Model, Gamma Oscillations

Disclosure: Nothing to disclose.

P519. Genetic, Pregnancy, and Negative Early-Life Risks Shape Children's Brain (Dis)similarity to Schizophrenia

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Background: Genetic, per-and-perinatal, and early-life life adversity factors may act as risks for schizophrenia spectrum disorder (SSD). These factors act insidiously early-to-adolescent development altering the brain developmental trajectory and leading to the formation of characteristic brain deficit patterns prior to onset of symptoms. We hypothesized that the effects of these risks may increase brain similarity to adult SSD deficit patterns in prepubescent children.

Methods: We used data collected by the Adolescent Brain Cognitive Development (ABCD) Study ($N = 8940$, age $= 9.9 \pm 0.1$ years, 4307/4633 female/male), including 727 (age $= 9.9 \pm 0.1$ years, 351/376 female/male) children with family history of SSD, to evaluate unfavorable cerebral effects of ancestral SSD history, pre/perinatal environment, and negative early-life environment. We used a regional vulnerability index (RVI) to measure the alignment of a child's cerebral patterns with the adult SSD pattern derived from a large meta-analysis of case-control differences.

Results: In children with a family history of SSD, the regional vulnerability index captured significantly more variance in ancestral history than traditional whole-brain and regional brain measurements. In children with and without family history of SSD, the regional vulnerability index also captured more variance associated with negative pre/perinatal environment and early-life experiences than traditional brain measurements. Furthermore, higher RVI-SSD values were significantly and negatively associated with performance in cognitive domains that are specifically linked to SSD including the speed of information processing and working memory.

Conclusions: We used a cohort of normally developing children to show that familial, pre/perinatal, and early developmental risks can alter brain patterns in the direction observed in adult patients with SSD. Approximately 1% of participants are expected to develop SSD, nonetheless, the effects of these risk factors are present across the entire cohort. Individual similarity to adult SSD patterns may provide an early biomarker of the effects of genetic and developmental risks on the brain prior to psychotic or prodromal symptom onset.

Keywords: Schizophrenia (SCZ), MR Imaging, Big Data, ENIGMA Working Group

Disclosure: Nothing to disclose.

P520. The Influence of Multiple Early Risk Factors on Age of Onset in Psychosis: A Danish Register Study

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Background: While multiple early risk factors for schizophrenia have been identified, the individual effects are relatively small. The relative and additive influence of multiple risk factors acting in combination have not been thoroughly explored. Moreover, how these early risk factors influence age of illness onset and whether there are differential influences across sexes is currently unclear.

Methods: This is a population-based case-control study using register data from Denmark including all individuals receiving a diagnosis of a schizophrenia spectrum disorder between 1973 and 2018 ($N = 29{,}142$). We also included a healthy control sample matched 5:1 patient on age, sex, and parental socioeconomic status ($N = 136{,}387$). The register data included: parental history of psychiatric disorder (as a proxy for genetic vulnerability), low birth weight, premature birth, winter/spring birth, urbanicity of

birthplace, second-generation immigrants, advanced paternal age, and Apgar scores.

Results: Using logistic regression incorporating all risk factor variables in one model revealed parental history of psychiatric disorder (OR = 2.3), advanced paternal age (OR = 1.3), and low birth weight (OR = 1.3) as significant risk factors after adjusting for all other risk factors. Surprisingly, on the other hand, being a second-generation immigrant (OR = 0.7) and urbanicity decreased the risk (OR = 0.9) in this population. We observed no significant interactions between any of the included risk factors. Rerunning the model separately for females and males, showed no significant differences regarding the influences of the included risk factors across sexes. These findings were supported by a machine learning model (decision tree), where parental history of psychiatric disorder, paternal age and birth weight contributed most to the classification of patients vs. healthy controls (ACC test = 0.7, AUC test = 0.6, $p < .001$).

Approximately 20% of the patients could be characterized as early-onset cases (diagnosis < 18 years). Within the patient sample, female sex (OR = 1.8) and parental history of psychiatric disorder (OR = 1.6) were the only significant risk factors for having an onset during childhood or adolescence.

Conclusions: Several early factors contribute independently of each other to increase the risk of developing a schizophrenia spectrum disorder, suggesting that the additive effects lead to symptom onset once a certain threshold has been reached. Routine assessments of the most influential risk factors, i.e., parental history of psychiatric disorder, low birth weight and high paternal age could readily be incorporated into clinical practise facilitating individual risk stratification.

Keywords: Psychosis-Risk, Risk Factors, Age of Onset, Schizophrenia Spectrum Illness, Epidemiology

Disclosure: Nothing to disclose.

P521. Timing and Computational Mechanisms of Delusion and Hallucination Emergence

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Background: Positive psychotic symptoms–hallucinations and delusions–are frequently studied together. However, recent evidence suggests that these symptoms may be driven by distinct and potentially conflicting cognitive and computational mechanisms: delusion propensity has been tied to an increased tendency to learn inappropriately about the world, and hallucinations have been consistently linked to an overweighting of perceptual priors. Several theories have been proposed to reconcile their co-occurrence in individuals with psychosis, despite these seemingly opposite mechanisms. However, no one has yet explored the possibility that the mechanisms driving these symptoms may be causally linked, with the bottom-up noise driving delusion formation also engendering a compensatory overweighting of priors and hallucinogenesis. By examining the timing of delusion and hallucination emergence, we wished to gain a better understanding of the independence of these mechanisms.

Methods: We analyzed data from two large samples of individuals at clinical high risk for psychosis (NAPLS-2, $N = 720$; NAPLS-3, $N = 700$) and one sample of individuals experiencing their first episode of psychosis (McGill's Prevention and Early Intervention for Psychosis, PEPP, $N = 695$). Data were derived prospectively and retrospectively, using the Structured Interview for Psychosis-Risk Syndromes (SIPS), Circumstances of Onset of Symptoms and Relapse Schedule (CORS), and the Topography of Psychotic Episode (TOPE) to determine timing and severity at symptom onset, ascertainment, and scheduled follow-up intervals in each respective study.

Results: Across all three groups, delusional thoughts tended to emerge before hallucinations in the majority of individuals (57.0%, 57.9%, and 61.7%, respectively), roughly 4 times more frequently than hallucination-first emergence in the same groups. Delusions were also found to be more stable over time, while hallucinations were more volatile up until the point of conversion to psychosis, after which stability for both symptoms were roughly equal. Lastly, Bayesian causal modeling demonstrated a potential causal link between severity of delusional ideation and onset of hallucinations among those who were observed prospectively during hallucinogenesis.

Conclusions: Together, results support a new understanding of positive symptom emergence in which hallucinations emerge after, and perhaps as a compensatory response to, the mechanisms driving delusion formation. This pattern is consistent with a secondary account of hallucinogenesis, and may help to reconcile seemingly opposed computational theories of hallucination and delusion susceptibility.

Keywords: Prodromal Psychosis, Computational Psychiatry, Hallucinations, Delusions

Disclosure: Nothing to disclose.

P522. Genetic Architecture of Schizophrenia in African Ancestry Individuals

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Background: Schizophrenia and related psychoses occur in all human populations, but are diagnosed most frequently among Black and African ancestry individuals. Environmental exposures and adversities, disparities in access to care, and historical trends of over- and racialized diagnosis contribute to this discrepancy. Inherited genetic factors also strongly influence primary susceptibility, but do not explain differences in prevalences across populations.

Methods: Building on our recent work validating electronic health records (EHR) based diagnoses in the Million Veteran Program (MVP), we undertook a genome-wide association study (GWAS) of schizophrenia in 6,570 cases and 21,043 controls in the discovery phase. We applied genome-based restricted maximum likelihood (GREML) to obtain direct estimates of SNP-based heritability. Towards attaining sample size parity, we combined data with the All of Us (AoU), COGS, GPC, MGS, and PAARTNERS studies, yielding a combined "freeze" of 15,000 cases and 56,642 controls of African ancestry. We combined our novel African ancestry GWAS results with findings from a recent Psychiatric Genomics Consortium (PGC3) study, and re-estimated the number of credible causal SNPs at each locus.

Results: Meta-analyzing MVP, AoU, COGS, GPC, MGS, and PAARTNERS, we identified a single genome-wide significant locus on chromosome 18 upstream of PMAIP1. This is remarkable given that at the same sample size, 22 independent loci had been identified for European ancestry populations.

Strikingly, we did not observe any evidence for association of the MHC locus on chromosome 6p21, which we confirmed by imputation of C4 structural variants. We obtained GCTA heritability

estimates of 0.23 (P < 10-8) for schizophrenia in African ancestry populations, recapitulating widely-cited results for European ancestries; and found that most of this explained variance was attributable to common SNPs with frequency greater than 10%.

Across 270 PGC3 loci, 65% showed the same direction of allelic effect in African American veterans (P = 1e-7), compared to 90% in European Americans (P = 9e-48); furthermore, when considering only European or African ancestry tracts (i.e., haplotypes) in African American veterans, 69% (P = 3.4e-10) and 58% (P = 0.007) of index SNPs showed a consistent direction of allelic effect. Expectedly, multi-ancestry meta-analysis of MVP with published PGC3 results yielded more than 50 new loci, increasing the count of replicated susceptibility loci to more than 320. More intriguingly, fine-mapping of PGC3 loci saw the total number of credible SNPs reduced by 13% following meta-analysis with African ancestry based results, and 20% when only African tracts were analyzed.

Finally, we attempted to address the observation of increased prevalence of schizophrenia in African American populations by stratifying the MVP cohort by military service period, highlighting that in the Vietnam War era, both African and European Americans were diagnosed at higher rates, with no evidence of genetic enrichment, and that following 1990s, these rates have fallen substantially but are still elevated in African versus European Americans.

Conclusions: Our expanded analyses of schizophrenia in African ancestry populations highlight both the challenges and opportunities of enhanced diversity in neuropsychiatric genetics research. We explore the implications of ancestry-based disparities in representation, leveraging multi-ancestry polygenic risk score (PRS) and phenome-wide association studies (PheWAS) to examine evidence of broad pleiotropy of schizophrenia risk alleles, and benchmark the relevance of current instruments for risk stratification, and predicting hospitalization.

Keywords: Psychosis, African American, Genome-Wide Association Studies, Polygenetic Risk Score, Race Disparities

Disclosure: Nothing to disclose.

P523. Phenotypic Associations of Complement Component 4 (C4) Structural Variation in a Large-Scale Healthcare System Derived Biobank

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Background: Structural variation at the complement component 4 (C4) locus within the chromosome 6 major histocompatibility (MHC) region has been reported to be associated with schizophrenia, with risk varying by isotype (C4A and C4B) and copy number, and the presence or absence of a retroviral HERV insertion, denoted as long (L), or short (S). Previously, of the four most common C4 haplotypes, schizophrenia risk was found to be highest for 'AL-AL' followed by 'AL-BL', then 'AL-BS' and 'BS' (relative risks ranging 1-1.27) and proportionate with increased expression of C4A in post-mortem brain. Complement component 4 is also known to be associated with other disorders, including autoimmune diseases, such as lupus (reported 7-fold variation in risk attributable to C4 variation) and Sjogren's syndrome (reported 16-fold variation in risk attributable to C4 variation). The current analyses leveraged a healthcare-system derived biobank, with genotype data linked to electronic health record data, to broadly assess for associations of C4 structural variation, both diagnostic outcomes and common serum lab values, within a subset of biobank participants of European ancestry. Further, the C4 structural variant was imputed in the larger multi-ancestry biobank cohort, enabling future multi-ancestry association analyses.

Methods: As previously reported, BioMe biobank participants recruited from the Mount Sinai healthcare system, were genotyped on the Illumina Global Screening Array (GSA), and subsequent to genotype QC/filtering, genotype data for n = 31,705 samples were analyzed (mean age 56.2 years, 56% female, and diverse genetically determined ancestry, 38% European, 32% African, 21% Hispanic, 4% East Asian, 5% Other). For 11,704 individuals of European ancestry, C4 structural variation was imputed from flanking single nucleotide polymorphisms, using a tool derived from a subset of HapMap reference samples. REGENIE was then used to test 434 phecodes, hierarchically-clustered diagnostic codes, for association with C4 haplotype and C4 copy number variation. Further, each of 36 common serum lab measures was tested for association with C4 structural variation. Of note, the C4 association analyses were not conditioned on HLA type. C4 structural variation was also imputed for the multi-ancestry cohort using an experimental tool for multi-ancestry imputation, 'Osprey', derived from the 1000 genomes 30x coverage reference dataset.

Results: Within the European subset of 11,704 individuals, top C4 allele frequencies yielded by imputation were AL-BL (0.54), AL-BS (0.26), AL-AL (0.08), and BS (0.07). Increased C4A copy number was found to be significantly associated with decreased risk of Type 1 Diabetes (Beta = −1.06, FDR = 6.8x10-8), but no other significant phenotypic associations were identified. Among common serum tests, C4A copy number was associated with decreased serum glucose (Beta = −0.05, FDR = 0.04) and increased platelet count (Beta = 0.05, FDR = 0,05). The BS haplotype was found to be significantly associated with decreased total protein count (Beta = −0.09, FDR = 0.05) and decreased white blood cell count (Beta = −0.10, FDR = 0.05). C4 imputation in diverse ancestries yielded divergent C4 allele frequencies. For example, for the African ancestry subset, C4 allele frequencies were, AL-BS (0.64), AL-BL (0.08), AL-AS (0.07), BS (0.04) and for the Hispanic ancestry subset, AL-BS (0.40), AL-BL (0.30), AL-AL (0.05), BS (0.03).

Conclusions: Within a subset of biobank participants of European ancestry, some associations of C4 structural variation were identified. C4A copy number was associated with decreased risk of Type 1 Diabetes, an autoimmune disorder. Increased C4A copy number was also associated with decreased serum glucose and increased platelet count, while the BS haplotype was associated with decreased total protein and decreased white blood cell count. Overall, the associations did not account for other HLA alleles known to be in strong linkage disequilibrium with C4 variation, so future analyses will condition on HLA type or pursue other fine-mapping approaches to discern the specific causal effect of C4 variation. No associations of C4 structural variation with schizophrenia were identified, and some disorders of autoimmune etiology were excluded from the current analyses due to relatively low prevalence (Lupus and Sjogren's syndrome). Future analyses will validate C4 imputation calls in a subset of whole-genome sequenced biobank participants. Future analyses will also increase scale for more well-powered analyses, and evaluate the associations of C4 structural variation in a multi-ancestry context, given the apparent divergent allele frequencies, by ancestral group.

Keywords: Complement Component 4, Genetic Association Analyses, Schizophrenia, Biobank

Disclosure: Nothing to disclose.

P524. Proteomic Mendelian Randomization Reveals Novel Molecular Targets for Schizophrenia

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Background: Genome-wide association studies (GWASs) have successfully identified hundreds of genetic loci linked to susceptibility for schizophrenia (SCZ); however, the causal genes and underlying pathophysiological mechanisms at these loci are not well elucidated. Mendelian randomization (MR) analysis has emerged as a powerful tool to investigate potential causal relationships between molecular traits and disease phenotypes in GWAS data. In this study, we utilized Two-Sample MR to integrate large-scale plasma protein quantitative trait loci (pQTL) data from the UK Biobank Pharma Proteomics Project (UKB-PPP) and deCODE genetics with the most robust schizophrenia GWAS available.

Methods: We leveraged the largest schizophrenia GWAS conducted by the SCZ Working Group of the Psychiatric Genomics Consortium (PGC3), which included 39,910 SCZ cases and 60,558 controls of European ancestry, as the outcome. For the exposure, genetic variants associated (in cis) with 2800 plasma proteins identified in the large-scale UKB-PPP (N~34,000) and/or deCODE genetics (n~35,000) databases, were used. The potentially causal effects of the plasma proteins on schizophrenia were analyzed using the TwoSampleMR R package, accounting for heterogeneity across SNPs. Horizontal pleiotropy was tested to ensure validity of Mendelian randomization assumptions.

Results: We identified 75 plasma protein-to-disease associations that remained significant after applying the Benjamini-Hochberg correction. Of these, 47 have never been reported in prior protein quantitative trait (pQTL) studies of schizophrenia. In addition to replicating several genes previously reported in pQTL studies of SCZ (e.g., ITIH3, MAD1L1), our study identified several novel genes revealing new clues to SCZ pathophysiology. For example, ENPP5 and NRP1 are both involved in neurodevelopment of the cerebellum, and numerous genes implicating inflammatory processes linked to TNF-alpha were identified. Notably, lower RABEP1 in the blood was associated with risk for schizophrenia. RABEP1 encodes an enzyme called Rab GTPase-binding effector protein 1, a part of the rabaptin family involved in regeneration of injured axons, representing an attractive target for potential pharmacologic manipulation.

Conclusions: These findings shed light potential involvement of several novel genes in schizophrenia and potential role of genes involved in inflammatory and neurodevelopmental pathways in the disorder. Identification of novel gene RABEP1 further supports the significance of our study, as it not only provides a potential molecular basis for the disorder but also proposes a conceivable pharmaceutical intervention. While our methodology, focused on the plasma proteome, may exclude essential proteins exclusively expressed in the brain, it is important to note that a significant number of shared pQTLs exist across different tissue types. Moreover, our approach offers a remarkable increase in statistical power, surpassing existing brain eQTL and pQTL datasets by an order of magnitude. Finally, identification of circulating plasma proteins associated with schizophrenia may contribute to development of blood-based biomarkers of illness.

Keywords: Schizophrenia (SCZ), Proteomics, Mendelian Randomization, GWAS

Disclosure: Nothing to disclose.

P525. Estimating the Genome-Wide Contribution of Rare Coding Variation to Schizophrenia and Bipolar Disorder

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Background: A decade of GWAS in schizophrenia and bipolar disorder has revealed substantial genetic contributions to these complex psychiatric disorders. However, these associations include only common genetic variants, and typically explain less than the heritability predicted by family studies, raising the possibility of a substantial contribution from rare variants. While recent progress in exome sequencing has led to the identification of individual genes associated with both schizophrenia and bipolar disorder via rare variants, it remains unclear how much heritability rare variants explain in aggregate, owing to issues of statistical power. This limits our understanding of the genetic architecture of schizophrenia and bipolar disorder in many ways: it leads to ambiguity about the theoretical utility of rare variants in clinical screening, obscures the patterning of rare variant heritability across different gene categories, prevents estimation of the rare variant genetic correlation between traits, and leaves unclear how much signal remains to be found by increasing sample size.

Methods: We developed Burden Heritability Regression (BHR) to estimate heritability due to the burden of rare coding variation. BHR leverages the burden approach, wherein variants with similar functional consequences are aggregated into gene-wise test statistics, to powerfully estimate components of rare coding heritability. Furthermore, BHR leverages a regression based estimator to eliminate bias from most sources of population stratification, taking advantage of the fact that rare damaging variants are not expected to have correlated population stratification. BHR provides unbiased estimates of heritability in simulations, and can be extended to estimate rare variant heritability enrichments and genetic correlations.

Results: We first applied BHR to 22 complex traits and common diseases in the UK Biobank, to gain a sense of the landscape of rare variant genetic architecture across many traits. We found that the burden of rare coding variants, aggregated across loss-of-function and missense variants, explains 1.3% of phenotypic variance, and is concentrated in genes that are intolerant to loss-of-function variation. Turning to schizophrenia and bipolar disorder, we then applied BHR to publicly available summary statistics from the SCHEMA (Singh et al, 2022) and BipEx (Palmer et al, 2022) consortia. We found that the burden of rare coding variation explains approximately 2% of liability-scale phenotypic variance for both schizophrenia and bipolar disorder. The nine exome-wide significant genes for schizophrenia found by the SCHEMA consortium explain only 7% of the burden heritability of schizophrenia. The burden heritability of schizophrenia appears to be uniquely concentrated in genes intolerant to loss-of-function (enrichment for schizophrenia: 9.6x, enrichment for bipolar and median UKB trait: 4.5x); we estimate that the top quintile of loss-of-function intolerant genes explain 70% of the burden heritability of schizophrenia. The rare burden genetic correlation between schizophrenia and bipolar disorder was ~0.45 (SE: 0.2).

Conclusions: These results shed new light on the genetic architecture of schizophrenia and bipolar disorder, with several key implications. Firstly, the low burden heritability of schizophrenia and bipolar disorder suggests that rare variants may not contribute meaningfully to population risk stratification or solve the "missing heritability" problem. This result should bound expectations for rare variant polygenic risk scores for these conditions. While non-coding variants could theoretically explain this gap, previous observations (Gazal et al, 2018) suggest that heritability becomes increasingly concentrated in coding regions as frequency decreases, and that the contribution of rare non-

coding variants to heritability may be even smaller. Secondly, while rare variants may not explain substantial heritability, they can powerfully reveal core biological pathways and therapeutic targets, and increasing the sample size of exome-wide association studies will yield many new associations; current significant genes explain less than 10% of the genome-wide signal. Lastly, patterns of pleiotropy appear to be shared between common and rare variant associations for these conditions, suggesting that, as in common variants, many rare associations will be shared between diagnoses, and pooled tests can be used to augment power. Our results provide insight into the rare variant genetic architecture of neuropsychiatric disease, motivating both deeper study of schizophrenia and bipolar disorder, and application of BHR to other conditions.

Keywords: Rare Genetic Variants, Schizophrenia (SCZ), Bipolar Disorder (BD), Whole Exome Sequencing, Genetic Association Study

Disclosure: Nothing to disclose.

P526. Sex Differences in Gene Expression Within Striatal Subregions in Psychosis

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Background: Psychosis is a defining feature of schizophrenia and highly prevalent in bipolar disorder. Prior work from our group identified diurnal alterations in gene expression across the human striatum in subjects with psychosis. Moreover, recent studies have reported sex differences in both symptomology and the transcriptome in cortical regions among subjects with schizophrenia. In this study, we investigated sex differences in the transcriptome within striatal subregions in subjects with psychosis and comparison subjects. In a separate set of analyses, we also examined gene expression patterns across striatal subregions within disease groups.

Methods: RNA-seq was performed on nucleus accumbens (NAc), caudate, and putamen samples from subjects with psychosis ($n = 36$) or unaffected subjects ($n = 59$). For analysis of sex differences, we created a sex-matched cohort (psychosis: $n = 10$/sex; unaffected: $n = 11$/sex) and evaluated sex and psychosis effects within each brain region using an ANOVA of expression data. Transcripts were considered differentially expressed if $p < 0.01$ and a fold change of 1.2 (20% expression change). In a separate set of analyses, we performed differential expression analyses between striatal regions within disease groups using the full cohort of subjects. For these analyses, transcripts were considered differentially expressed if $q < 0.05$ and a fold change of 1.5 (50% expression change). Ingenuity Pathway Analyses and Metascape were used for pathway and biological process enrichment, respectively.

Results: Significant effects of sex and psychosis were observed across striatal regions. Notably, immune/inflammation-related transcripts were significantly enriched in the caudate and putamen of unaffected male subjects compared to unaffected female subjects. This sex difference was not observed in subjects with psychosis. However, there was a gain of a sex difference in myelination-related transcripts in psychosis subjects, with increased expression in the caudate and putamen of male compared to female subjects. In female subjects with psychosis (relative to unaffected female subjects), angiogenesis- and immune/inflammation-related transcripts were upregulated across all striatal regions, while mitochondrial-related transcripts were downregulated in the NAc. In between-region comparisons within disease groups, both psychosis and unaffected subjects showed similar patterns of expression, with the NAc being transcriptionally unique. Furthermore, transcriptional gradients in expression across striatal subregions were observed.

Conclusions: We found significant effects of sex and psychosis across the dorsal and ventral striatum, which may provide insight into sex differences in psychosis. We also identified unique expression patterns across striatal subregions, suggesting regional and functional specialization that can be explored in future studies.

Keywords: Psychosis, Schizophrenia, Postmortem, Striatum, RNA-Seq

Disclosure: Nothing to disclose.

P527. SNX19 in Human Autopsy Brains and hiPSC-Derived Model

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Background: Our recent postmortem brain studies identified that SNX19 is a schizophrenia risk gene. Single-molecule in situ hybridization experiments found that SNX19 is highly expressed in neurons, particularly excitatory neurons, compared to glia in human postmortem brains. SNX19 is a key player in endolysosomal and autophagy pathways, which have been extensively reported in neuronal dysfunction and neuropsychiatric diseases. Although genetic and cellular evidence suggests SNX19 contributes to neuropathology, the underlying mechanisms remain unknown. Here, we propose to study the mechanism in postmortem brain tissue at single cell level and model SNX19 in human induced pluripotent stem cell (hiPSCs) derived brain organoids.

Methods: We collected human postmortem brain dorsolateral prefrontal cortex (DLPFC) single nucleus RNA-seq (snRNA-seq) from ROSMAP ($N = 48$). We obtained two hiPSCs from HipSci.

Results: We performed snRNA-seq and obtained 63,781 cells with high-quality data. We identified and annotated the six major cell types of the human brain including excitatory neurons, inhibitory neurons, microglia, astrocytes, oligodendrocytes, and oligodendrocyte progenitor cells. We identified that SNX19 is expressed in 7,082 excitatory neurons. We compared levels of SNX19 gene expression across cell types and found that SNX19 is significantly associated with cognitive impairment in excitatory neurons in human postmortem brain.

Cerebral organoid technology has made it possible to model human neurophysiology and disease with increasing accuracy in human-derived tissue cultures. We performed advanced CRISPR gene editing in hiPSCs to knockout SNX19. We then differentiated them into 2D neurons and 3D cerebral organoids to evaluate the SNX19 impact. Our preliminary data has shown that SNX19 knockout can increase synaptic markers' expression in hiPSC-derived neurons. We observed morphological changes in SNX19 knockout organoids and replicated the synaptic markers' change in the SNX19 knockout brain organoids.

Conclusions: Our study suggests that SNX19 functions specifically in excitatory neurons and is associated with cognitive impairment. Our 2D and 3D hiPSC-derived models indicate SNX19 has an impact on neuronal function.

Keywords: Schizophrenia, iPSCs, Organoids

Disclosure: Nothing to disclose.

P528. Whole Genome Sequencing in Early-Onset Psychosis: Preliminary Diagnostic Results and Their Impact on Parental Empowerment

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Background: Early-onset psychosis (EOP) is defined as any psychiatric diagnosis with pronounced psychotic symptoms with onset before age 18, is associated with lower premorbid psychosocial function, more hospitalizations, poorer cognitive functioning, and worse overall prognosis than adult-onset illness. There have been recent reports of an elevated rate of recurrent copy number variants in EOP compared to both unaffected matched controls and matched subjects with autism spectrum disorder. However, there is still a limited understanding of the full spectrum of genetic variation that underlies EOP. Furthermore, it remains unclear how genetic test results impact parental empowerment, defined as the perceived ability to understand and seek new information related to the genomic sequencing, manage emotions related to the diagnostic process and outcomes, and utilize genomic sequencing information to the betterment of the individual/child and family. Therefore, we initiated a prospective, observational study utilizing whole genome sequencing in youth with EOP. Parents/guardians were asked to complete the Genome Empowerment Scale (GEmS)

Methods: We performed CLIA-grade, diagnostic WGS on 11 consecutive youth of both sexes, ages 11-17 who were admitted to the Rady Children's Hospital Inpatient Psychiatry Service with EOP. Probands were sequenced first followed by parental samples (when available) to determine inheritance stance of variants of interest. Results of WGS were returned to parents/guardians and then they were asked to complete the GEmS, which consists of four scales: Meaning of a diagnosis, emotional management of the process, seeking information and support, and implications and planning.

Results: Eleven youth with EOP were enrolled with a mean age of 15.5 +/- 2.2 years. 63.6% of subjects were female and 72.7% identified as Hispanic. Mean PANSS positive symptoms scale scores were 25.2 +/- 8.1, negative symptom scale scores were 23.2 +/- 11.2, and general psychopathology scale scores were 55.4 +/- 19.6. Six subjects had proband and both parental samples available for WGS, two subjects had proband and maternal samples available and three subjects had only proband samples available. No subjects had pathogenic or likely pathogenic variants on WGS, 4 patients had variants of unknown significance (VUS) identified on WGS (all single nucleotide variants (SNVs)), and 7 patients had negative results. The VUS occurred in genes associated with established neurodevelopmental disorders and were involved in chromatin modification, synapse formation, and neural progenitor proliferation. For the GEmS, meaning of a diagnosis subscale scores were 42.4 +/- 14.0, emotional management of the process subscale scores were 25 +/-6.5, seeking information and support subscale score 28.7 +/- 7.2, and implications and planning subscale scores 23.7 +/- 6.2.

Conclusions: Despite sample size limitations at this preliminary stage of analysis, we already demonstrate that WGS used in EOP can identify genetic variants involved in critical neurodevelopmental pathways and potentially expand the clinical phenotype of known genetic disorders to include EOP. All variants identified to date are SNVs, not recurrent CNVs, as previously reported in EOP. This suggests that through WGS, we may be able to identify disease variants overlooked when using microarray alone. Furthermore, the results of the GEmS suggest that receiving a genetic diagnosis for a child with EOP provides the most empowerment to parents around the meaning of a diagnosis.

Keywords: Genetic Testing, Early Onset Psychosis, Childhood-Onset Schizophrenia, Whole Genome Sequence

Disclosure: Nothing to disclose.

P529. Rare Copy Number Variants and Phenotypic Variability in Schizophrenia Spectrum Disorders

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Background: One of the greatest barriers to clarifying the core biological processes that underlie schizophrenia and ultimately improving patient outcomes is the clinical and genetic heterogeneity of the disorder. Determining whether severe phenotypes in schizophrenia such as borderline intellectual functioning or child-onset psychosis can be explained by distinct genetic profiles remains a key question, with important implications for personalized medicine.

Methods: Rare copy number variants (CNVs) were called from genotyping array data in a cohort of 1,514 individuals (F = 42.7%, mean age = 25.96 +/- 13.49) with diverse genetic ancestry, including 645 patients with schizophrenia spectrum disorders (SCZ), 253 relatives of SCZ patients, and 616 healthy controls. Logistic mixed models adjusted for ancestry and genetic relatedness were used to investigate differences in known neuropsychiatric CNV rate among SCZ cases vs non-cases, and to test associations between borderline intellectual functioning and child-onset psychosis and presence of known neuropsychiatric CNVs or burden of deletion of individual genes in 18 distinct neurodevelopment gene-sets. Neurodevelopmental gene-sets were defined previously via weighted gene co-expression network analysis of BrainSpan transcriptomic data from the developing human brain (Forsyth et al., 2020).

Results: Rates of known SCZ- and broader neurodevelopmental disorder (NDD-) associated CNVs were elevated in SCZ cases compared to non-cases (OR = 6.70 and 3.51, respectively). SCZ- and NDD-associated CNVs were found at non-significantly higher rates in child-onset psychosis (i.e., onset before 13 years of age) compared to later-onset psychosis and were associated with significantly higher likelihood of borderline intellectual functioning in SCZ (OR = 6.76 and 4.92, respectively). There were no associations between deletions of genes in any neurodevelopmental gene-set and child-onset psychosis. However, deletions of genes involved in regulating gene expression during fetal development were associated with increased likelihood of borderline intellectual functioning in SCZ (OR = 2.14). This relationship replicated in SCZ relatives and controls (OR = 2.79). Preliminary analysis incorporating age-normalized MRI-based neuroanatomic metrics indicated that deletion of fetal gene-regulatory genes was also associated with increased cortical thickness across SCZ patients, relatives, and controls.

Conclusions: Results suggest that poor cognitive functioning in SCZ is associated with the presence of known neuropsychiatric CNVs. Deletions of genes involved in orchestrating the large-scale changes in gene expression that drive early brain development are also associated with poor cognitive function and altered brain structure across SCZ, relatives, and healthy individuals. Associations between neurodevelopmental gene-sets and severe

phenotypes in SCZ offers opportunities to prioritize high-impact genes outside known risk loci.

Keywords: Childhood-Onset Schizophrenia, Copy Number Variants, Neurodevelopment, Cognitive Functioning, Structural MRI

Disclosure: Nothing to disclose.

P530. Targeted Association Testing to Identify Genetic Risk Variants for Antipsychotic Induced Weight Gain (AIWG) in Children and Older Adults

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Background: Relative risk for antipsychotic induced weight gain (AIWG) is thought to be highly genetic, but our understanding of the genetic contributors and mechanisms remains limited. Discovery of genetic variation influencing AIWG has the potential to identify important pathways relevant to weight and cardiometabolic outcomes, and alternative treatments. As part of the NIMH-funded Obesity Related to Antipsychotic Liability and Exposure (ORAcLE) consortium, existing anthropometric and genotype data from similarly designed randomized clinical trials (RCTs) conducted in antipsychotic-naïve children with severe irritability/aggression, and in older adults with treatment resistant depression were analyzed for SNP x treatment interactions using 5 SNPs previously shown to exhibit large effects on treatment-related change in body weight, and linked to genes involved pathways relevant to obesity biology, eating behavior, and satiety. Brain Derived Neurotrophic Factor (BDNF), 5-Hydroxytryptamine Receptor 2C (5-HTR2C), Dopamine Receptor D2 (DRD2), Guanine Nucleotide Binding Protein, subunit Beta 3 (GNB3), and Melanocortin 4 Receptor (MC4R).

Methods: RCT data from two 10-12 week studies was included in the analysis: the NIMH-funded Metabolic Effects of Antipsychotics in Children (MEAC) study,2 which randomized antipsychotic-naïve children ages 6-18 with severe irritability or aggressive behavior with one or more DSM diagnoses were randomized to treatment with aripiprazole, olanzapine, or risperidone, and the two-phase PCORI-funded Optimizing Outcomes of Treatment-Resistant Depression in Older Adults (OPTIMUM) study,3 which randomized older adults (60+ yrs) to augmentation with aripiprazole or bupropion, or switch to bupropion alone in Phase 1; participants who did not respond to Phase 1 treatment, or who had already failed trials of the medications in Phase 1, were randomized to augmentation with lithium or switch to nortriptyline in Phase 2. Data was pooled and participants categorized based on Y/N antipsychotic treatment. Using a linear mixed effects model, interactions between selected SNPs (continuous) and treatment assignment on % change in body weight from baseline. A repeated measures mixed effects model was used to evaluate for time x SNP x treatment interactions associated with change in BMI and body weight, respectively. Percent change in height from baseline was included as a continuous covariate in all models to account for weight changes associated with age-related growth or loss of height.

Results: In the pooled dataset, 655 individuals with SNPs of interest were identified, 273 of whom were randomized to antipsychotic treatment, and 382 of whom were randomized to non-antipsychotic; 18.0% were children (n = 118), 61.5% female (n = 403), 13.6% Black (n = 89), 1.4% Asian (n = 9) and 5% (range: 5.03% to 5.34%) increase from baseline body weight compared to small reductions in weight in the group treated with non-antipsychotic medications (range: −0.43% to −0.40%).

Conclusions: AIWG is a major barrier to effective treatment of psychiatric disorders, addressable with precision medicine. In the present study, about half the population were treated with antidepressant medications or lithium, and included both children and older adults, allowing for comparisons relevant to real-world clinical decision-making. Moreover, multiple clinically relevant weight outcomes were available for the whole sample. Although all SNPs evaluated were associated with clinically relevant weight gain (>5% increase from baseline), the GNB3 x treatment interaction was significantly associated with AIWG across weight outcomes. Of note, the C825T SNP in the GNB3 gene has been associated with essential hypertension and obesity in addition to increased risk for AIWG. The T allele is associated with treatment response to serotonin reuptake inhibitors (SRIs) in depression, may be involved in the pathophysiology of schizophrenia via accelerated programmed cell death, and has been implicated in antipsychotic treatment response. These preliminary results are subject to important limitations, including the lack of a non-antipsychotic treated pediatric sample, and most of the antipsychotic exposure was with aripiprazole. However, these results illustrate how existing RCT data can be used for targeted association testing in populations at increased risk for adverse treatment outcomes.

Keywords: Pharmacogenetic Response, Antipsychotic, Obesity

Disclosures: Alkermes, Carelon: Advisory Board (Self). Novo Nordisk: Consultant (Self). Usona Institute: Other Financial or Material Support (Self). COMPASS Pathways: Contracted Research (Self). LB Pharmaceuticals: Grant (Self).

P531. How Diverse are Large Multi-Site Schizophrenia Studies? Modelling Race, Ethnicity and Gender Parity in COGS2

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Background: Multi-center studies are increasingly necessary to advance psychiatric neuroscience and evaluate novel interventions for patients with schizophrenia (SZ), but successfully translating results from such studies to the clinic remains challenging. Recruitment disparities in race, ethnicity and gender are increasingly being investigated to understand how they contribute to this translational barrier. Here, we report on race, ethnicity and gender diversity in the Consortium on the Genetics of Schizophrenia 2 (COGS2) study, and quantify the extent of additive sampling necessary to achieve racial, ethnicity, and gender parity with the cities in which COGS2 was carried out.

Methods: The analysis cohort included 841 healthy control subjects (HCS) and 1060 SZ subjects between ages 18 and 65 from four of the five COGS2 sites: Los Angeles, New York, Philadelphia, and Seattle. We generated a Diversity Index using methodology from National Equity Atlas and previously published entropy index calculation to characterize the diversity within this sample. We categorized subjects into 24 categories based on combinations of race (Caucasian Americans, African-American, American Indian/Alaska Native, Asian, Pacific Islander/Native Hawaiian, and Multiracial/Other), ethnicity (Hispanic/Latino or non-Hispanic/Latino), and gender (Male/Female) from demographic information assessed at study entry and generated Diversity Index values for both HCS and SZ subjects for each city. These proportions and

Diversity Indexes were compared to age-matched American Community Survey (ACS) census data for each of the above cities from 2010-2014. We carried out a simulation algorithm which sequentially expanded the COGS2 cohort by 50% by randomly resampling from the already recruited cohort, excluding subjects who were already oversampled in COGS2 based ACS data. The algorithm was allowed to resample until all 24 category proportions were within 2.5% of ACS data, and simulations were repeated 1,000 times.

Results: Analyses revealed multiple groups were over- or underrepresented in both HCS and SZ cohorts, compared to ACS demographic data. Compared to the HCS cohort, the SZ cohort had overrepresentation of African-American non-Hispanic/Latino females and African-American Hispanic/Latino males, and underrepresentation of Asian-American and Caucasian non-Hispanic females. On average the HCS cohort required 22.7 additive resamples (standard deviation, s.d. = 6.27) to approximate ACS race, ethnicity and gender proportions of the cities in which they were conducted, while SZ required 46.8 additive resamples (s.d. = 11.0). For the SZ cohort to approximate the recruited HCS race, ethnicity and gender proportions, 17.6 additive resamples were required (s.d = 6.69).

Conclusions: Data highlights the extent to which both HCS and SZ subjects differ in demographic composition both between each other and compared to the city populations in which they are recruited. While such discrepancies have been observed in many large multi-site SZ trials, our results suggest relying on usual recruitment and ascertainment strategies would be inadequate for achieving demographic parity: COGS2 would need to be expanded by a factor of >10 for HCS and > 20 for SZ to achieve representative racial, ethnic, and gender diversity. Ongoing analyses will investigate whether primary outcomes in COGS2 would be altered in simulated cohorts which are adequately diverse. Our findings emphasize the need for nuanced and targeted approaches for recruitment in large SZ multi-site studies.

Keywords: Schizophrenia (SCZ), Diversity, Race, Ethnicity, Gender

Disclosure: Nothing to disclose.

P532. Pleiotropic Meta-Analysis with Cognitive Endophenotypes Differentiates Neurodevelopmental, Synaptic, and Apoptotic Pathways in Schizophrenia

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Background: We have previously investigated the biological mechanisms underlying the complex and paradoxical relationship between cognitive ability, educational attainment and (Lam et al. 2019, AJHG, PMID: 31374203). By leveraging the genetic pleiotropy inherent in these partially overlapping phenotypes, we identified two subsets of SNPs with distinct characteristics: 1) those with "concordant" alleles, following the expected association pattern across the three phenotypes: lower cognitive ability, lower educational attainment, and increased susceptibility to schizophrenia and 2) those with "discordant" alleles, exhibiting a counterintuitive pattern of associations, with higher educational attainment, higher cognitive ability but greater schizophrenia. Gene set analyses revealed that concordant SNPs were enriched in neurodevelopmental genes, while discordant SNPs were linked to synapse-related pathways. Building upon these previous findings, the present study aimed to reassess the relationship between schizophrenia, cognitive ability, and educational attainment by utilizing more robust GWASs and employing an expanded analytic framework.

Methods: Pleiotropic meta-analysis was conducted using Pleiotropic Locus Exploration and Interpretation using Optimal test (PLEIO) to identify specific loci harboring concordant and discordant SNPs. MAGMA was employed to map those SNPs to genesand conduct competitive gene-set analysis. Furthermore, we investigated the expression patterns of these distinct gene groups across multiple developmental stages using the BrainSpan dataset.

Results: Through PLEIO analysis, we identified 788 independent loci associated with cognitive ability, education, and/or schizophrenia at a threshold of $p < 5 \times 10\text{-}8$. Among these, 344 loci specifically harbored concordant SNPs, while 268 encompassed discordant SNPs. Additionally, our study revealed 155 loci that contained both concordant and discordant SNPs. Consistent with our previous analysis, the concordant SNPs were linked to genes involved in neurodevelopmental pathways, such as neurogenesis and forebrain neuron generation. In contrast, the discordant SNPs were mapped to genes associated with synaptic pathways, including synaptic-density pathways and postsynaptic cytoskeleton. Notably, the CHD8 pathway, known for its role in both early brain development and synapse formation, showed significant associations with both concordant and discordant genes. Furthermore, genes from the common loci were associated with more general pathways, such as translation initiation. Genes involved in neuronal apoptosis, as well as GABA neuron differentiation, were associated with exclusively with schizophrenia but not the cognitive endophenotypes. Finally, we observed that concordant genes exhibited higher expression levels during earlier developmental stages, while discordant genes showed increased expression during later stages of life in the BrainSpan dataset, consistent with our findings in pathway analysis.

Conclusions: These results expand on our previous study of cognitive endophenotypic pleiotropy in schizophrenia, more than doubling the number of associated loci, and greatly expanding the number of significant gene sets identified. These findings highlight the temporal dynamics of gene expression in schizophrenia, and suggest distinct roles for concordant and discordant genes in different stages of etiology of the disorder.

Keywords: GWAS, Pleiotropy Analysis, Schizophrenia (SCZ), Cognitive Function

Disclosure: Nothing to disclose.

P533. Greater Choline and Myo-Inositol in Treatment-Resistant Vs. Responsive Schizophrenia: A 1H-Magnetic Resonance Spectroscopy Meta-Analysis

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Background: The neurobiology of treatment-resistant schizophrenia (TRS) is poorly understood. Accordingly, proton magnetic resonance spectroscopy studies of TRS have shown mixed findings for levels of glutamate, choline, myo-inositol, and other metabolites, and consensus regarding spectroscopic profiles in the condition is lacking.

Methods: In this meta-analysis, we examined the metabolites N-acetyl aspartate, choline, myo-inositol, creatine, glutamate, and glutamate+glutamine (glx) in the medial prefrontal cortex (mPFC) and dorsal striatum in people with TRS vs. non-TRS (nTRS) as well as TRS vs. healthy controls (HCs) and TRS vs. ultra TRS (i.e., TRS with clozapine resistance).

Results: A MEDLINE search revealed 9 articles including 239 people with TRS (pooled TRS and ultra TRS), 59 with ultra TRS, 175 with nTRS, and 153 HCs that met meta-analytic criteria. Significant effects included higher mPFC choline (g = .36, 95% CI = .10 to .61,

Z = 2.76, $p = .006$) and myo-inositol (g = .46, 95% CI = .15 to .77, Z = 2.89, $p = .004$) in TRS compared to nTRS as well as in TRS vs. HCs (choline: g = .63, 95% CI = .38 to .89, Z = 4.87, p < .001; myo-inositol: g = .99, 95% CI = .61 to 1.37, Z = 5.07, p < .001), but no differences in other metabolites or regions. All datasets reporting choline and myo-inositol showed qualitatively higher mPFC levels in TRS vs. nTRS.

Conclusions: The observed metabolite profile in TRS (higher choline and myo-inositol) suggests activation of inflammatory processes in the brain. A similar profile is seen in healthy aging, which is known to involve increased neuroinflammation and glial activation. The current findings suggest that similar inflammatory processes may distinguish people with TRS from nTRS. As the overall number of datasets was low, however, results should be considered preliminary and highlight the need for additional research in this area.

Keywords: Functional Magnetic Resonance Spectroscopy, Meta-Analysis, Treatment-Resistant Schizophrenia, Choline, Myo-Inositol

Disclosure: Nothing to disclose.

P534. Resting State Hypoconnectivity Between Medio-Dorsal Thalamus and Prefrontal Cortex is Associated With Glu/GABA in Clinical High Risk for Psychosis vs. Healthy Control Subjects: A 7T fMRI and MRSI Study

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Background: The clinical high risk (CHR) state includes individuals uniquely enriched for risk of developing psychosis and schizophrenia (SCZ). Although robust, increasing evidence point to reduced resting state functional connectivity in a thalamocortical circuit implicating the mediodorsal (MD) thalamus and the dorsolateral prefrontal cortex (DLPFC) in chronic and early course patients with SCZ, it is less clear whether this reduction is also present in CHR relative to HC individuals and what are the molecular mechanisms associated with MD-DLPFC functional connectivity in CHR and HC. To begin addressing these knowledge gaps, in this study we collected 7 Tesla (T) resting state (rs)-fMRI and magnetic resonance spectroscopy imaging (MRSI) scans in CHR and HC individuals.

Methods: Thirty-two HC and thirty-one CHR individuals underwent 7T rs-fMRI and MRSI scans. A 7T Siemens Magnetom scanner was used to acquire structural and rs-fMRI and magnetic resonance spectroscopic imaging (MRSI) data; rs-fMRI data (matrix size = 98 × 98 × 48, volumes = 220) were acquired using a multi-band accelerated echo planar imaging (EPI) sequence with a voxel size of 2 mm x 2 mm x 2 mm. and preprocessed with SPM12 and Further, MRSI were acquired using a slice selective J-refocused coherence transfer sequence (TE/TR = 34/1500 ms) in a slice angulated along the DLPFC plane (matrix size = 24x24 over a FOV of 216 mm x 216 mm, and slice thickness = 10 mm with 0.9 ×0.9 ×1.0 cm nominal resolution). After preprocessing and data quality check, 30 HC (16 female, age = 21.27 ± 4.76) and 23 CHR (13 female, age = 20.63 ± 3.13) were retained for further analyses; rs-fMRI connectivity analysis was performed in CONN toolbox while whole brain gray matter, whole thalamus, left and right thalamus, and 14 subsections of the thalamus considered as seed. For MRSI data, spectral analysis was performed using LCModel and further estimate γ-aminobutyric acid (GABA) and glutamate (Glu).

Results: We first considered whole brain gray matter analysis and found that the thalamus was the region showing the largest reduction in function connectivity in CHR vs. HC (peak cluster (x = −6, y = −4, z = +4), size = 378 voxels, p-FDR < 0.001). We then selected the whole thalamus as a seed and found that the DLPFC was the brain region showed the largest functional connectivity reduction with the thalamus (peak cluster (x = −2, y = +24, z = +58), size = 867 voxels, p-FDR < 0.001, and cluster (x = 0, y = +48, z = −8), size = 256 voxels, p-FDR = 0.006). We also performed seeding analyses using 7 anatomically identified sub-sections of the thalamus and found that the thalamic-DLPFC hypoconnectivity was most pronounced for MD (peak cluster (x = −2, y = +56, z = +30), size = 3897 voxels, p-FDR < 0.001). Finally, we extracted significant connectivity values for all subjects from MD and DLPFC and performed correlation analyses with MD and DLPFC GABA, Glutamate, and Glu/GABA while controlling for the effect of group. This analysis revealed a significant negative association between rs-fMRI DLPFC and DLPFC Glu/GABA across groups ($p = 0.01$, r = −0.42).

Conclusions: Our findings suggest that MD-DLPFC hypoconnectivity could be a putative neurobiological biomarker for the at-risk state. Additionally, our results highlight the importance of acquiring both fMRI and MRS data to characterize the interaction between molecular and neural mechanisms implicated in the risk of developing psychosis and schizophrenia.

Keywords: Medio-Dorsal Thalamus, Dorsolateral Prefrontal Cortex (DLPFC), Clinical High Risk for Psychosis, 7 Tesla fMRI, 7T MRS

Disclosure: Nothing to disclose.

P535. Replication of a Neuroimaging Biomarker for Striatal Dysfunction in Psychosis

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Background: Biomarker development has been compared with drug development, in the sense that it comprises a series of contingent consecutive steps: target identification, internal validation, external validation, and ultimately demonstration of clinical utility. The functional striatal abnormalities (FSA), is among the most advanced neuroimaging biomarkers in schizophrenia, trained to discriminate diagnosis, with post-hoc analyses indicating prognostic properties (i.e., prediction of treatment response). Before proceeding to demonstrate the clinical utility of this biomarker, it should be demonstrated that it is generalizable, reliable, and that it is able to maintain performance within the constraints of routine clinical conditions (i.e., relatively short scans).

Methods: We calculated FSA scores using the original scripts in scans from individuals with psychosis (n = 101) from healthy controls (n = 51) from the Human Connectome Project for Early Psychosis. In the initial set of analyses, we used area under the curve (AUC) in receiver operator characteristic curves to test the accuracy of the diagnostic predictions. In a second set of analyses, since various runs of resting state fMRI were obtained in this dataset, also measured the test-retest (run 1 vs 2) and phase encoding direction (i.e., AP vs PA) reliability with intraclass correlation coefficients (ICC). Additionally, we concatenated both runs in each phase encoding direction to obtain a longer scan that we subsequently segmented in increasing lengths, from which we calculated FSA scores, to test the changes in predictive accuracy and reliability with increasing scan length. Finally, we tested the

prognostic capability of the FSA by calculating the correlation between baseline scores and symptom improvement over 12 weeks of antipsychotic treatment in a separate cohort (n = 97). Since the FSA is a relatively novel analytic construct, we also calculated from these scans the Yeo networks intrinsic connectivity, which were used across analyses as a reference.

Results: The FSA had good/excellent diagnostic discrimination (AUC = 75.4%, 95%CI = 67.0%-83.3%; in non-affective psychosis AUC = 80.5%, 95%CI = 72.1-88.0%, and in affective psychosis AUC = 58.7%, 95%CI = 44.2-72.0%). Test-retest reliability ranged between ICC = 0.48 (95%CI = 0.35-0.59) and ICC = 0.22 (95%CI = 0.06-0.36), which was comparable to that of networks intrinsic connectivity. Phase encoding direction reliability for the FSA was ICC = 0.51 (95%CI = 0.42-0.59), generally lower than for networks intrinsic connectivity. By increasing scan length from 2 to 10 minutes, diagnostic classification of the FSA increased from AUC = 71.7% (95%CI = 63.1%-80.3%) to 75.4% (95%CI = 67.0%-83.3%) and phase encoding direction reliability from ICC = 0.29 (95%CI = 0.14-0.43) to ICC = 0.51 (95%CI = 0.42-0.59). FSA scores did not correlate with symptom improvement.

Conclusions: The FSA was developed as a diagnostic biomarker, trained to discriminate functional connectivity features between cases with schizophrenia and controls. Our results reassure that the FSA is a generalizable diagnostic biomarker. In addition, these results suggest that the reliability of the FSA is comparable to that of better-known functional connectivity measures, although was slightly more susceptible to phase encoding direction artifacts. Generally, even very short scans made already diagnostic predictions significantly over chance. However, the post-hoc finding originally reported of the FSA as a prognostic biomarker, potentially a much more impactful use, since it could inform treatment decisions, did not replicate. This is not surprising, since the model was developed to discriminate between cases and controls, rather than response vs non-response in schizophrenia. These results encourage to continue working on biomarker development, with a focus on treatment response to develop generalizable biomarkers.

Keywords: Biomarker, Early Psychosis, Neuroimaging

Disclosures: TEVA, Janssen, Karuna: Advisory Board (Self). Alkermes: Grant (Self). Saladax, Neurocrine: Contracted Research (Self).

P536. A Transdiagnostic Multi-Dimensional Approach to Examining Cognitive and Psychosocial Functioning, White Matter Structure, Brain Volumes, and Protein Markers in the Human Connectome Project for Early Psychosis (HCP-EP)

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Background: Research in recent years suggested the importance of transdiagnostic phenotyping when characterizing and treating mental disorders. Specifically, psychotic illnesses show high symptomatic overlap across diagnoses and common underlying alterations in brain networks. The Human Connectome Project for Early Psychosis (HCP-EP) was designed in the Research Domain Criteria (RDoc) spirit to study domains of psychopathology across the early phase of several psychotic disorders and integrate this with the HCP approach to acquire high-quality imaging data. The present study investigates cognitive and psychosocial functioning, white matter structure, brain volumes, and protein markers across the psychosis spectrum.

Methods: The HCP-EP study was carried out across four recruiting and three scanning sites. It recruited individuals within five years of the onset of a DSM-5 diagnosis of psychosis (schizophrenia, schizophreniform disorder, schizoaffective disorder, psychosis not otherwise specified, delusional disorder, brief psychotic disorder, major depression with psychosis, or bipolar disorder with psychosis). Individuals underwent extensive testing, including NIH Toolbox, a structural and diffusion-weighted magnetic resonance imaging (MRI) scan, and a blood draw.

Here, we analyzed 203 individuals with preprocessed and harmonized diffusion-weighted MRI data, of which 202 had a structural MRI, 182 had complete cognitive data, and 82 had protein markers analyzed by a commercial platform (O-link). First, we grouped individuals based on diagnosis (non-affective psychosis = 136, affective psychosis = 67). Then we run stepwise cluster analyses with internal validation. Clusters were based on 2) seven cognitive tests (Picture Vocabulary, Flanker Inhibitory, List Sorting Working Memory, Dimensional Change Card Sort, Pattern Comparison Processing Speed, Picture Sequence Memory, Oral Reading Recognition), and 3) clusters derived from protein markers previously associated with cell aging, inflammation, and cardiometabolic health (IGFBP2, TIMP1, GDF 15, MMP 1, MMP2, CTSD, CST3, CTSZ, ALCAM, IGFBP7).

For groups based on 1) diagnosis, 2) cognition, and 3) protein markers, we compared general functioning (GAF), NIH Toolbox measures of Perceived Stress, Life Satisfaction, Meaning and Purpose, and Self-Efficacy, voxel-wise cellular and extracellular white matter structure (fractional anisotropy and free water), and gray matter volumes (hippocampus, superior frontal, rostral middle frontal, medial orbital frontal, pars opercularis, middle temporal, and fusiform gyrus). Imaging analyses were corrected for age, age2, sex, and motion for diffusion-weighted analyses and age, age2, sex, site, and total intracranial volume for volume analyses.

Results: We derived two clusters with good stability and prediction strength based on cognitive measures: cluster 1 with lower (n = 82) and cluster 2 with higher performance (n = 100) and two protein-based clusters. The protein clusters were driven by a higher TIMP1, MMP1, and ALCAM expression in cluster 2 (n = 24) compared to cluster 1 (n = 38). There were more individuals with affective psychosis in cognitive cluster 2 ($X2(1) = 5.70$, $V = 0.19$, $p = 0.017$), but no association was found between diagnosis or cognitive clusters and protein-based clusters.

The two diagnostic groups demonstrated group differences in the GAF Social Functioning Scale ($T(198) = -3.40$, $d = 0.51$, $p < 0.01$). Individuals with non-affective psychosis also presented with higher fractional anisotropy and lower free water than individuals with affective psychosis ($p < .005$).

Groups based on cognitive clusters demonstrated lower Meaning and Purpose ($T(176) = 3.38$, $d = 0.51$, $p < 0.01$), a trend toward more Social Functioning ($T(179) = -2.06$, $d = 0.31$, $p = 0.041$) and Self Efficacy ($T(180) = -2.23$, $d = 0.33$, $p = 0.027$), and higher fusiform gyrus volumes ($F(1, 176) = 16.69$, $p < .001$) in cluster 2.

Protein-based clusters showed no differences in psychosocial functioning but demonstrated more extracellular free water in cluster 2 ($p < .005$).

Conclusions: Our findings demonstrated the complex interplay between diagnosis, cognition, and biological-based phenotypes for the pathophysiology of psychosis. Notably, we found that individuals with early psychosis can robustly be grouped based on their cognitive functioning and protein markers. While diagnoses are related to social functioning, cognition seems more indicative of self-reported measures of Efficacy, Meaning, and Purpose, all

highly relevant for the quality of life but traditionally neglected in psychosis research. While we found few overall differences in brain structure, lower volume of the fusiform gyrus, a structure previously implicated in bipolar disorder, was related to poorer cognition. On the other hand, extracellular white matter abnormalities were related to a higher expression of proteins associated with cell aging, inflammation, and cardiometabolic health. Interestingly the three main proteins TIMP1, MMP1, and ALCAM are involved in extracellular matrix remodeling and blood-brain barrier regulation, which, together with the imaging findings, support the notion of a diagnostic independent inflammatory subtype of psychosis. Future studies should integrate the measures introduced in the present analyses to validate findings and examine their potential for treatment stratification and monitoring.

Keywords: Early Psychosis, Research Domain Criteria (RDoC), Multimodal Neuroimaging, Proteomics, Psychosocial Outcomes

Disclosure: Nothing to disclose.

P537. Generalizability and Out-Of-Sample Predictive Ability of Relationships Between Neuromelanin-Sensitive MRI and Psychosis in Antipsychotic-Free Individuals

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Background: Increased dopamine synthesis and release in the striatum relates to more severe positive (psychotic) symptoms. This association is relatively specific to positive symptoms versus other symptom domains and holds across different diagnostic categories. Recent work has positioned neuromelanin-sensitive MRI (NM-MRI) as a candidate biomarker for psychotic disorders. NM is a product of dopamine metabolism that accumulates in midbrain dopamine neurons. Importantly, previous work supports the use of NM-MRI as a marker of presynaptic dopamine function. To advance NM-MRI as a candidate biomarker, NM-MRI-psychosis associations must to be replicated and demonstrations of out-of-sample predictive accuracy are needed.

Here, we aim to replicate two previous findings (Cassidy et al., 2019) of selective associations between NM-MRI contrast in a ventral subregion of the substantia nigra (SN) and: (1) syndromal psychosis severity in patients with schizophrenia; and (2) subsyndromal psychosis severity in individuals at clinical high-risk for psychosis (CHR). We further aim to characterize the relationship of NM-MRI contrast with other factors relevant to dopamine function. Lastly, we conduct a proof-of-concept analysis of the out-of-sample predictive accuracy of an NM-MRI-based marker of dimensional psychosis severity.

Methods: The main samples consisted of 42 antipsychotic-free patients with schizophrenia and 53 antipsychotic-free CHR individuals. An external validation sample consisted of 16 antipsychotic-naïve patients with schizophrenia. The main clinical assessments were the PANSS and SIPS for schizophrenia and CHR, respectively. NM-MRI contrast maps were calculated in each voxel within an SN/ventral tegmental area (SN/VTA) mask as previously described (Wengler et al., 2020). A priori ROI-based analyses extracted mean NM-MRI contrast values from an unbiased mask comprising SN/VTA voxels with a positive association to psychosis severity in both schizophrenia and CHR in independent samples (ROIpsychosis; from Cassidy et al.). Our samples of $n = 42$ (schizophrenia) and $n = 53$ (CHR) resulted in power values of 0.83 and 0.99, respectively. Out-of-sample prediction of psychosis severity across the combined schizophrenia and CHR groups ($n = 95$) was assessed using support vector regression (SVR) based on multivoxel patterns of NM-MRI contrast across SN/VTA voxels to predict a composite psychosis score (combining z-scored, adjusted PANSS-PT and SIPS-PT).

Results: Results from Cassidy et al. replicated in the schizophrenia sample: psychotic symptom severity (PANSS-PT) positively related to NM-MRI contrast in the ROIpsychosis ($t = 2.24$, $p = 0.031$). Post-hoc analyses explored the contribution of other variables previously linked to dopamine; we found, in addition to PANSS-PT effects ($t = 3.65$, $p = 0.001$) simultaneous effects of sex ($t = 2.63$, $p = 0.013$), SES ($t = 3.55$, $p = 0.001$), and illness duration ($t = -2.28$, $p = 0.029$) in an extended model including all variables. We found no effects of past drug use, smoker status, first-episode status, antipsychotic-naïve status, duration of the antipsychotic-free period, body mass index, or fluid intelligence in reduced or extended models (all $p > 0.18$). Results from Cassidy et al. failed to replicate in the CHR sample: there was no significant association between subsyndromal psychosis severity (SIPS-PT) and NM-MRI contrast in the ROIpsychosis ($t = -0.55$, $p = 0.68$). To confirm the robustness and specificity of the association between syndromal positive symptoms and NM-MRI contrast in the ROIpsychosis, we evaluated fine-grained symptom measures in the schizophrenia sample. Both hallucinations and delusions drove the PANSS-PT effect; this was apparent in associations between NM-MRI contrast and hallucination subscale scores on the PSYRATS and SAPS ($r = 0.33$, $p = 0.044$; and $r = 0.37$, $p = 0.025$, respectively) and delusion subscale scores on the same scales ($r = 0.43$, $p = 0.007$; and $r = 0.39$, $p = 0.018$, respectively), which were comparable between the two symptoms (PSYRATS: Steiger's $z = -0.7$, $p = 0.484$; SAPS: Steiger's $z = -0.14$, $p = 0.886$). No effects were observed for other symptom domains on the SAPS (all $p > 0.18$) or negative-symptom subscale scores on the SANS (all $p > 0.08$). Voxelwise analyses showed a significant positive association between NM-MRI contrast and psychosis severity in schizophrenia (293 out of 2060 voxels, $p = 0.049$); significant voxels substantially overlapped with the ROIpsychosis (overla$p =$ 60.4%, $p = 0.001$).The 10-fold cross-validated prediction accuracy of the composite psychosis score was above chance in held-out test data (mean $r = 0.31$, $p = 0.014$; mean $RMSE = 1.001$, $p = 0.005$). Furthermore, predicted composite psychosis scores correlated with the mean NM-MRI contrast in the ROIpsychosis ($r = 0.30$, $p = 0.003$) but not with the mean contrast in the whole SN/VTA ($r = -0.03$, $p = 0.76$). Crucially, prediction accuracy for the final model in an external validation sample was also above chance ($r = 0.42$, $p = 0.046$; $RMSE = 0.882$, $p = 0.047$), providing initial support for external generalizability.

Conclusions: In summary, we replicated the association between NM-MRI contrast and psychosis severity in antipsychotic-free patients with schizophrenia but not in CHR individuals. Furthermore, cross-validated machine-learning analyses provided a proof-of-concept demonstration that multivoxel NM-MRI patterns can be used to predict psychosis severity in new data, suggesting potential for developing clinically useful tools.

Keywords: Psychosis, Neuromelanin-Sensitive MRI, Machine Learning, Replication, Generalizability

Disclosure: Nothing to disclose.

P538. Prefrontal Gamma-Aminobutyric Acid Levels in Never-Medicated Individuals With Chronic Schizophrenia

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Background: Schizophrenia is a chronic and debilitating psychotic disorder in which current pharmacological treatments (based on antagonists or partial agonists of dopamine receptors) have limited effects on negative and cognitive symptoms and a restricted efficacy in around a third of the patients over time, impacting symptom remission, recovery, and quality of life.

Abnormalities in Gamma-Aminobutyric Acid (GABA) have been described in schizophrenia; these alterations have been linked to cognitive deficits observed in the illness. Proton magnetic resonance spectroscopy studies have described medial-prefrontal GABA elevations in unmedicated patients at early stages of the disorder, while studies in chronic patients are scarce, reporting different results in medicated patients (increases, decreases, or lack of differences in this metabolite). Here, we examined GABA levels in a group of long-term, never-medicated, schizophrenia patients.

Methods: We recruited, from November 2016 to January 2023, 21 individuals with chronic schizophrenia, defined by a duration of untreated psychosis > 5 years. All patients included were antipsychotic-naïve and without comorbid disorders or current substance abuse. We also recruited 22 age- and sex-matched healthy controls. All participants were recruited at the Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico. The study was approved by the Ethics and Scientific Committees of this Institute and all participants provided written informed consent. GABA levels were obtained by proton magnetic resonance spectroscopy at 3T in a $2.5 \times 2.5 \times 2.5$ cm voxel centered in the medial prefrontal cortex bilaterally, using the Mescher-Garwood point resolved spectroscopy method (TR = 2000 ms, TE = 68 ms, 2048 points, 256 averages [128 edit-on and 128 edit-off], with water suppression and 16 averages without water suppression). All spectra were quantified using Gannet, referenced to water signal, and corrected to account for the tissue composition of the voxel. GABA values with a model error > 10% were rejected from all analyses. Cognition was evaluated with the MATRICS Consensus Cognitive Battery. Independent-sample t tests were used for comparisons of clinical and cognitive variables within groups. Frequency data was analyzed using $\chi 2$ test. Spearman rank correlations were used to examine potential associations between GABA levels, clinical variables, and cognitive domains. Statistical significance was set at $P < .05$ for all analyses, and P values for correlational analyses were corrected for multiple comparisons through Bonferroni method.

Results: Patients had a mean (SD) duration of untreated psychosis of 816 (493) weeks. Spectroscopy data from 7 participants (4 schizophrenia patients and 3 controls) were rejected from all analyses due to poor quality. Increased GABA levels were found in chronic schizophrenia patients compared to controls (4.6 (1.3) vs 3.7 (.49) institutional units; $t = -2.6$, $P = .02$; Cohen's $d = .91$). Also, a negative correlation between GABA levels and working memory scores across all study subjects was found ($r = -.37$, $P = .04$); However, this correlation did not survive correction for multiple comparisons. No correlation was found between GABA levels and the duration of untreated psychosis ($r = -.33$, $P = .19$), nor between GABA levels and other clinical or cognitive measures.

Conclusions: The main limitation of the study is the use of a spectroscopy sequence that does not account for macromolecule contamination of the GABA signal.

Future multi-center, longitudinal studies in unmedicated, chronic schizophrenia individuals are needed to confirm these results and determine whether these GABA elevations could help to stratify, not only the clinical outcomes of patients, but also serve as markers of treatment response and future treatment development targeting the GABAergic system.

Keywords: Schizophrenia (SCZ), Drug-Naive, GABA MRS

Disclosure: Nothing to disclose.

P539. Gene Dosage Effects on Subcortical Nuclei Volumes and Longitudinal Development in Individuals With 22q11.2 Copy Number Variants

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Background: Copy Number Variants (CNVs) at the 22q11.2 locus impact neurodevelopment and strongly increase risk for neuropsychiatric disorders. Compared to the general population, individuals with 22q11.2 deletion syndrome (22qDel) have increased rates of schizophrenia, autism, ADHD, and anxiety disorders. 22q11.2 duplication (22qDup) also increases risk for neurodevelopmental disorders including autism and ADHD, but may exert a protective effect against schizophrenia, as evidenced by large scale genetic studies. Cortical thickness has been shown to negatively correlate with gene dosage at this locus (22qDel > control > 22qDup), while the opposite direction of effect has been observed for cortical surface area. A previous cross-sectional analysis of subcortical morphometry in 22q11.2 CNVs indicated localized shape differences in subcortical structures including the thalamus, amygdala, and hippocampus. However, these subcortical differences have not yet been systematically mapped to specific nuclei within these larger anatomical structures, nor has their developmental trajectory been investigated.

Methods: In a longitudinal sample of T1-weighted structural MRI from individuals with 22qDel (n = 96 baseline, 53% female), 22qDup (n = 37 baseline, 46% female), and typically developing (TD) controls (n = 80 baseline, 51% female) ages 5.5-49.5 years, volumes were estimated for the whole left and right thalamus, amygdala, and hippocampus, as well as 54 thalamic, hippocampal, and amygdalar subregions per hemisphere. This was accomplished using the segment subregions tool in the FreeSurfer software package, which uses subcortical atlases derived from histology and ultra-high-resolution ex vivo MRI, warped to individual MRI images using Bayesian inference. Visual quality checks were performed on all images, as well as statistical exclusion of outliers. Data from two separate scanners were harmonized using the longitudinal ComBat approach. Individuals were assigned a numeric gene dosage based on CNV status (22qDel = 1 copy of the 22q11.2 locus genes; TD = 2; 22Dup = 3). Cross-sectional gene dosage effects were tested with a linear mixed model predicting regional volume from gene dosage, controlling for age, age2, sex, total intracranial volume, and scanner, with a random intercept for subject ID to account for follow up visits. This model was first tested separately for the left and right whole thalamus, hippocampus and amygdala, then each subregion was tested independently and the resulting p-values were corrected for multiple comparisons using False Discovery Rate (FDR). Linear group by age interactions were also tested. Non-linear age-related volume trajectories were then computed for each region.

Results: Significant gene dosage effects were observed for the left and right whole hippocampus (beta = 0.43, $p = 0.0000037$; beta = 0.47, $p = 0.0000003$) but not the whole thalamus or amygdala. Of the 108 subregions tested, 54 showed significant gene dosage effects after FDR correction, including bi-directional effects across different thalamic subregions such as the left and right mediodorsal medial nuclei (beta = −0.35, q = 0.00034; beta = −0.36, q = 0.00016), and the left and right lateral geniculates (beta = 0.21, q = 0.04091; beta = 0.46, q = 0.00001). In the amygdala, positive gene dosage effects were found in the bilateral basal nuclei (beta = 0.29, q = 0.00609; beta = 0.27,

q = 0.00322), accessory basal nuclei (beta = 0.23, q = 0.04091; beta = 0.20, q = 0.04163), and paralaminar nuclei (beta = 0.25, q = 0.01559; beta = 0.23, q = 0.02766). The majority of hippocampal subregions also showed positive gene dosage effects. No significant linear age by group interactions were observed, but analysis of non-linear developmental trajectories showed more significant age-related changes in hippocampal subregion volumes in controls compared to both CNV groups, with the opposite observed for several thalamic nuclei.

Conclusions: The dosage of genomic material at the 22q11.2 locus, approximated by CNV status, is predictive of specific subregion volumes in the hippocampus and thalamus and amygdala. Except for the body of the presubiculum, all significant hippocampal effects were in the same direction (22qDel < control < 22qDup) which is reflected in the large positive gene dosage effect for the whole hippocampus. Whole thalamus volumes were not related to gene dosage, but strong bi-directional effects were observed across various thalamic subregions, with positive effects preferentially in associative nuclei and negative effects in sensory nuclei. Similarly, whole amygdala volumes were not related to gene dosage; however, positive gene dosage effects were observed for several subregions. While linear age by gene dosage effects were not observed, CNV status was found to more subtly influence non-linear developmental trajectories. These novel findings provide new insight into the effects of 22q11.2 genes on brain development and will encourage further research into genetic mechanisms and cell-type specific effects.

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Keywords: 22q11.2 CNV, MRI, Thalamus, Hippocampus, Amygdala

Disclosure: Nothing to disclose.

P540. Variable Saturation of PET Imaging of Alpha7 Nicotinic Receptors by Antipsychotics in Primate Brain

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Background: We currently use PET to test the occupation of alpha 7 (a7) and a4 b2 nicotinic acetylcholine receptors (nAChR) in human brain by novel radioligands. To establish inclusion and exclusion criteria, we determined the effects of three commonly used antipsychotic medications in Papio Anubis olive baboon brain. We tested the hypothesis that binding of the three commonly used medications would not significantly affect the quantification in vivo of the a7 nicotinic receptors. There are previous reports of effects on a4 b2 receptors but the possible or potential effects on a7 nicotinic receptors in human or non-human primate brain are unknown.

Methods: We completed 9 studies in 4 baboons before and after chronic dosing with each of the antipsychotic medications olanzapine, aripiprazole, and risperidone. The animals received human-equivalent therapeutic doses for multiple days, required to reach steady state after adjustment for weight and metabolism of Papio Anubis olive baboons weighing 25-35 kg. We completed PET imaging with tracer [18F]ASEM at baseline and following chronic dosing with the three drugs administered to the baboons as monotherapy mixed with food. We obtained HRRT PET images with i.v. administration of approximately 12 mCi [18F]ASEM at baseline and following chronic dosing with the antipsychotics during the 90 minute dynamic PET imaging sessions. We obtained the baseline and subsequent PET images separated by multiple weeks with time of dosing fixed for each drug. We obtained plasma levels of the antipsychotics during dosing by mass spectrometry. We determined total volumes of distribution (VT) at steady-state by standard published methods (1). We calculated binding potentials (BPND) of the drug at the a7 nAchR as the outcome measure for the occupancy of the antipsychotics at the receptors. We measured occupancy by multiple published methods, with the final results obtained by the method that addressed non-specific tracer binding (e.g., protein binding in brain or blood), using the Extended Inhibition Plot (2).

Results: We used measures of the brain uptake of the tracer to calculate total volumes of distribution (VT) from the uptake of the tracer relative to concentrations in plasma. Values of VT at baseline varied from 15 to 30 ml/g. With competition from administered antipsychotics, we determined declines of total volumes of distribution consistent with saturation of receptors that varied from 24% (olanzapine) to 0.7% (risperidone) and 0.2% (aripiprazole). We determined the volume of distribution of non-displaceable binding during inhibition (VNDi) to be close to 5 ml/g, as determined independently at baseline (VNDb). We used standard reference region analysis to establish the baseline average binding potential of the tracer to be 3.8 + 0.3 in striatum in the absence of competitors. The estimate of maximum binding capacity (Bmax) consistent with this binding potential was 1.3 pmol/ml.

Conclusions: The doses of aripiprazole and risperidone had little effect on the volume of distributions (VT) of [18F]ASEM at the a7 nAChR. However, the dose of olanzapine revealed significant evidence of specific binding, to be further investigated in patients with schizophrenia on and off medication.

References:

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Keywords: PET Imaging, Schizophrenia, Alpha 7 nAchR, Antipsychotics, Primates

Disclosure: Nothing to disclose.

P541. Utilizing Widefield Calcium Imaging to Evaluate Activity and Network Connectivity in Wildtype and Schizophrenia Genetic Risk Model Mice

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Background: SETD1A loss-of-function mutations confer large increases in schizophrenia (SCZ) risk and have exceptional statistical support. Our group previously characterized aspects of SETD1A+/- mice, recapitulating several key SCZ phenotypes, including lower dendritic spine density and axon branching, and cognitive deficits. Decreased functional connectivity and altered network efficiency were reported in 3D cultures of neurospheres harvested from SETD1A+/- mice compared to wildtype (WT). We predict SETD1A+/- impairs sensory-evoked activity in and intrinsic/resting-state functional connectivity (rsFC) between murine cortical brain regions implicated in SCZ and test this using a promising new non-invasive in vivo imaging method: widefield calcium imaging.

Methods: Mutants are Thy1-GCaMP6f+/- transgenic C57Bl/6J mice and controls are their sex-matched WT littermates. Thy1

drives neuronal expression of the calcium indicator GCaMP6. GCaMP6 emission is collected during imaging and transformed into the primary outcome measure (ΔF/F) in image processing. At interim analysis, data from n = 5 mice (3F, 2M) per genotype were analyzed (eventual n = 10-12 mice/group). Both sexes are included but I do not expect sex differences based on prior work. Experiments are powered accordingly. I custom-built a widefield calcium imaging rig to monitor cortical dynamics during rest and sensory tasks across 4 sessions. Green (530nm) and blue (470nm) LEDs are strobed sequentially at 60Hz to detect and separate hemodynamic and calcium signals, respectively. The data are detrended, hemodynamics spatially regressed from calcium activity, transformed into ΔF/F and undecomposed data segmented into brain regions using LocaNMF, a method developed by my collaborator. I use students t-tests to evaluate group differences in baseline corrected and averaged activity peaks at the session level, and t-tests, correcting for multiple comparisons to evaluate differences in z-transformed Pearson's pairwise correlations for rsFC at the mouse level.

Results: The resting-state data were organized into locomoting ("sensory-evoked") versus non-locomoting epochs followed by peak analysis. This revealed significantly lower mean ΔF/F peak amplitude in left auditory area ($t = 3.519$, $df = 21$, $p = 0.002$) and marginally lower in left primary motor area ($t = 2.676$, $df = 25$, $p = 0.01$) of SETD1A+/- mice compared to WT during locomoting epochs. Similarly, mean area under the curve was significantly lower in left auditory of SETD1A+/- mice ($t = 3.09$, $df = 23$, $p = 0.005$), but not peak latency. In contrast, peak amplitude and area under the curve was unchanged in left primary visual area of SCZ risk model mice ($t = 1.329$, $df = 32$, $p = 0.1929$). Note: degrees of freedom differ here due to imaging artifacts during some sessions. At the network level, analysis of rsFC during >15m of non-locomoting continuous activity per mouse did not reveal significant genotype effects after correction for multiple comparisons.

Conclusions: At interim analysis peak analysis of calcium activity in primary sensory areas suggest strength but not timing of sensory-motor signals in auditory and motor cortices are aberrant in SETD1A+/- mice. These findings seem to support the proposition of altered corollary discharge in SCZ, and may relate to the generation of auditory hallucinations, but targeted follow up research must be conducted to pursue this intentionally and in depth. Consistent with prior work, deficits are not profound in/absent in primary visual cortex of SETD1A+/- mice. Interim analysis of pairwise correlations between cortical regions during non-locomoting continuous activity did not reveal significant genotype effects, which is inconsistent with our hypothesis as well as predictions based on findings of lower local and global connectivity in SETD1A+/- neurospheres. However, this rsFC finding is not surprising given the underpowered nature of the interim analysis; I will have sufficient power to detect genotype differences in pairwise correlations once I image and analyze remaining mice in my planned upcoming experiments. Future work includes analysis of auditory tone and whisker stimulation-evoked signals, rescues using LSD1 inhibitors previously shown to mitigate cognitive dysfunctions in SETD1A+/- mice, as well as additional experiments utilizing promotors other than Thy1 to drive calcium indicator expression in interneurons. This work aims to increase understanding of network mechanisms and highlight widefield calcium imaging as an important translatable tool for studying network mechanisms underlying cortical impairments in risk models of neuropsychiatric disorders.

Keywords: Resting State Functional Connectivity, Schizophrenia (SCZ), Motor Evoked Potentials

Disclosure: Nothing to disclose.

P542. Associations Between Neuromelanin Accumulation in Patients With Schizophrenia and Treatment Responsiveness: A Cross-Sectional NM-MRI Study

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Background: Neuromelanin (NM) is a product of monoamine metabolism, including dopamine. NM-sensitive magnetic resonance imaging (NM-MRI) sequences allow in vivo quantification of NM levels in the substantia nigra (SN). NM-MRI signal is believed to serve as a biomarker for SN dopamine neuron integrity and, consequently, striatal dopaminergic functioning. Higher striatal dopamine synthesis is associated with first-line antipsychotic responsive (FLR) patients with schizophrenia, while normal striatal dopamine synthesis is linked to treatment-resistant schizophrenia (TRS). Clozapine is currently the only antipsychotic approved for TRS. However, no studies have yet investigated the relationship between SN accumulations, as measured by NM-MRI, and clozapine response in patients with TRS.

Methods: This study enrolled patients with TRS who either did not respond to clozapine (ultra-resistant schizophrenia [URS]) or responded to clozapine (non-URS), FLR, and healthy controls (HCs). TRS was defined as resistance to at least two first-line antipsychotics and ongoing clozapine treatment at the time of the study. SN-NM were measured using 3T-MRI. The contrast ratio (CR) was calculated as the relative signal intensity difference between SN and crus-cerebri. SN-CR were compared between groups, controlling for age and sex. The associations between SN-CR and symptom severities were also explored within the patient groups.

Results: A total of 78 participants (URS: n = 16; non-URS: n = 16; FLR: n = 20; HCs: n = 26) completed the study. We found the overall group differences in SN-CR values ($F(3,72) = 3.80$, $\eta 2 = 0.21$, $p = 0.001$). Specifically, patients with URS (Cohen's $d = 0.86$, $p = 0.049$) and FLR (Cohen's $d = 1.27$, $p < .0001$) exhibited higher SN-CR values compared to HCs. SN-CR values showed no associations with any of the symptom severity scores within each group or the entire patient group.

Conclusions: Our study is the first to assess a proxy measure of mesostriatal dopamine function in patients with schizophrenia, stratified according to their treatment responsiveness, using NM-MRI. We report high SN-NM contrast in patients with URS and FLR compared to HCs, and similar to HCs in non-URS. Longitudinal studies are required to establish if SN-NM levels are a suitable biomarker for predicting treatment response in schizophrenia.

Keywords: Neuromelanin-Sensitive MRI, Treatment-Resistant Schizophrenia, Dopamine

Disclosure: Nothing to disclose.

P543. Monoclonal Humanized One-Armed Antibody Blocking Therapy for Anti-NMDA Receptor Autoantibody-Mediated CNS Disorders

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Background: Prominent psychiatric manifestations including visual or auditory hallucinations, acute schizoaffective episodes, depression, mania and addictive behaviors are observed among patients with anti-NMDA receptor encephalitis (ANRE), a severe neurological disorder caused by autoantibodies (autoAbs) against NMDA receptors. Previous studies showed a fraction of patients with psychosis, schizophrenia, bipolar disorder and depression had anti-NMDA receptor autoAbs which may play a role in disorder etiology and pathophysiology. The current standard of care for ANRE is general immunosuppressive approaches with slow onset, insufficient efficacy and infection risk. We are developing a novel mechanism-based therapeutic for ANRE patients hoping to treat patients with other CNS disorders caused by anti-NMDA receptor autoAbs.

Methods: Pathogenic anti-NMDA receptor autoAbs bind to limited epitopes in the N-terminal domain (NTD) of the NMDA receptor NR1 subunit, crosslink NMDA receptors with their two-arms, and induce receptor internalization leading to hypofunction of NMDA receptor. We engineered a non-pathogenic recombinant one-armed humanized IgG (ART5803) which binds to the NTD epitope of the NMDA receptor NR1 subunit. We tested ART5803 efficacy against pathogenic autoAbs from ANRE patients on binding, internalization and function of NMDA receptor expressed in HEK293 cells in vitro. Additionally, we established a marmoset model where patient-derived pathogenic autoAbs were continuously ICV infused to induce and maintain mental/motor abnormalities for a month to test in vivo efficacy of ART5803. This abnormal behavior was scored using a marmoset version of the abnormal behavior evaluation scale (Abnormal Rating Scale; ARS) using mental disorder items (attention, motivation, fear/anxiety) and movement disorder items (voluntary movement speed, motor coordination, presence/absence of jumping, stereotyped behavior, limb abnormalities) as evaluation indexes. To assess in vivo efficacy of ART5803, marmosets were continuously ICV infused with ART5803 ($N = 9$) vs control anti-KLH antibody ($N = 3$) or by peripheral IP injections of ART5803 ($N = 8$) vs vehicle ($N = 7$) for two weeks. Statistical significance was determined by Wilcoxon matched-pairs signed rank test compared with ARS at pretreatment and aftertreatment by ART5803 vs control/vehicle.

Results: X-ray crystallography, hydrogen deuterium exchange mass spectrometry and surface plasmon resonance revealed that ART5803 competes with an ANRE patient derived autoAb at shared NTD epitopes located in the human NMDA receptor NR1 subunit with a high binding affinity (KD = 0.69 nM). ART5803 blocked NMDA receptor internalization (NR1/NR2B expressed in HEK293 cells) induced by various ANRE patient-derived autoAbs. ART5803 restored NMDA receptor function (Ca2+ influx) in HEK293 cells suppressed by the patient-derived autoAb. ART5803 did not show any agonist or antagonist activities on NMDA receptor. In vivo efficacy of ART5803 was assessed in a marmoset model. Continuous ICV infusion of ANRE-patient derived pathogenic autoAbs evoked robust mental/motor abnormalities in marmosets. Simultaneous ART5803 ICV continuous infusion reversed these behavioral abnormalities within 2 weeks ($P < 0.01$) and ART5803 IP administration reversed abnormalities within 1 week ($P < 0.01$).

Conclusions: These data indicate a therapeutic potential for ART5803 as a faster acting, more efficacious, and safer treatment option for patients suffering from ANRE and other anti-NMDA receptor autoAb-mediated CNS disorders. Further studies are warranted to assess prevalence and significance of anti-NMDA receptor autoAbs in general neuropsychiatric disorders, such as psychosis, schizophrenia, bipolar disorder and depression. To this end, development of sensitive and selective standardized screening methods to detect autoAbs against NMDA receptor is currently underway at our laboratory.

Keywords: Autoantibody, NMDA Receptor, Autoimmune Encephalitis, Psychosis, Schizophrenia (SCZ)

Disclosure: Arialys Therapeutics, Inc.: Employee (Self).

P544. The Mediating Role of Inflammation on Associations Between Markers of Metabolic Dysfunction and Deficits in Motivation and Pleasure in Patients With Schizophrenia

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Background: Bidirectional relationships between inflammation and metabolic dysfunction may contribute to the pathophysiology of psychiatric illness, including patients with schizophrenia. Obesity and other metabolic disturbances drive systemic inflammation via activation of macrophages in adipocytes, and in turn, increased inflammation contributes to further metabolic dysfunction, such as insulin resistance. In patients with schizophrenia, inflammatory markers such as C-reactive protein (CRP) have been shown to be associated with deficits in motivation and pleasure, but it is unknown whether markers of metabolic dysfunction may also contribute to these debilitating symptoms that are difficult to treat. Herein, we hypothesize that markers of metabolic dysfunction would also be associated with deficits in motivation and pleasure, but not negative symptoms related to deficits in expressivity. Moreover, we hypothesize that the association between metabolic dysfunction and deficits in motivation and pleasure would be mediated by increased inflammation, as measured by CRP.

Methods: 57 medically healthy patients with schizophrenia were recruited from Grady Memorial Hospital in Atlanta, Georgia. Negative symptoms were assessed using the Brief Negative Symptom Scale (BNSS), from which individual items can be examined and domain scores can be calculated for the two domains of Deficits in Motivation and Pleasure (MAP) and Deficits in Expressivity (EXP). Fasting blood was collected between 8AM −10AM and stored at −80 °C for later batched analysis. Plasma markers related to glucose metabolism, including glucose, insulin, resistin, adiponectin, and leptin were measured and a composite was calculated from the sum of Z-scores of all markers to examine the shared contribution of all circulating metabolic markers to negative symptoms. High sensitivity CRP was measured using an immunoturbidimetric assay. Associations between symptoms and metabolic markers were assessed using correlation (Spearman) and in linear regression models including age and sex. Finally, mediation models were used to examine the mediating role of CRP on observed relationships between metabolic markers and negative symptoms. Bootstrapping was used to examine the indirect effects. All analyses were conducted using SPSS version 27, including the PROCESS version 4.2 macro for the mediation analyses. We assessed significance at a $p < 0.05$, uncorrected.

Results: There was a significant correlation between the metabolic composite score and the MAP Domain ($r = 0.277$) as well as the anhedonia subscale ($r = 0.273$, both $p < 0.05$). Only resistin showed a significant relationship with avolition ($r = 0.3$, $p = 0.02$). Trend level associations (all $p < 0.1$) were found between resistin and MAP and total BNSS score, leptin and total BNSS and anhedonia, as well as the composite score and avolition. No relationships were found with the EXP domain score or individual items. In regression models, there was a significant association between the composite score and MAP (beta = 0.292, $p = 0.041$), as well as resistin and avolition (beta = 0.277, $p = 0.039$). In the mediation analyses, the direct effect of the metabolic composite score on MAP was beta = 0.6987, SE = 0.4232, $p = 0.1045$ (95% CI, −0.1497, 1.5471). The direct effect of CRP on MAP was beta =

1.8288, SE = 0.8516, $p = 0.0363$ (95% CI, 0.1213, 3.5362). The indirect effect of the metabolic composite score on MAP was beta = 0.2937, bootstrapped SE = 0.1945, and bootstrapped 95% CI (0.0024, 0.7499).

Conclusions: These results provide preliminary evidence that metabolic dysfunction contributes to negative symptoms related to deficits in motivation and pleasure, and not deficits in expressivity, similar to what has previously been shown with CRP. The results of the mediation analyses suggest that the impact of metabolic dysfunction on these negative symptoms are mediated by the influence of inflammation. Of the metabolic markers that contributed to the composite score, resistin and leptin were most strongly associated with these negative symptoms. Resistin has been shown to induce production of inflammatory cytokines and could serve as a link between disturbances in metabolism and inflammatory processes that may impact the brain and subsequent behavior. Leptin is a protein made by adipose cells and controls appetite and satiety in order to regulate energy balance. Relevant to negative symptoms, it may function as a way to limit motivated behaviors in order to conserve energy stores. Future studies will need to elucidate the relative contributions of metabolic dysfunction and inflammation, which may provide insights into novel treatment approaches to address deficits in motivation and pleasure. Furthermore, this work has the potential to support precision medicine approaches to identify those patients who may benefit from targeting these metabolic and inflammatory pathways.

Keywords: Schizophrenia (SCZ), Negative Symptoms, Inflammation, C-Reactive Protein, Immunometabolism

Disclosure: Nothing to disclose.

P545. Longitudinal Inter-Relationship of C-Reactive Protein Levels and Body Mass Index in Schizophrenia

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Background: People with schizophrenia (PwS) suffer from premature morbidity and mortality, particularly from cardiovascular disease, but there are limited interventions to address these disparities. Cross-sectional studies find elevated inflammatory markers, such as c-reactive protein (CRP), among PwS compared to non-psychiatric comparison (NC) groups. In the general population, CRP elevation is associated with obesity, and older people also have higher inflammation, known as "inflammaging". However, the pattern of inflammatory marker changes over time and how these relate to changes in body mass index (BMI) within PwS is unclear. Understanding these inter-relationships could help to target interventions aimed at improving health and extending life in PwS.

Methods: We addressed this gap with longitudinal, observational data from PwS (n = 171) and NC (n = 153) aged 25-60 at baseline with 2-6 follow-ups about 1-2 years apart. At each visit, high senstivity CRP levels from fasting peripheral blood samples and body mass index (BMI) were measured. Hs-CRP levels were processed with a commercially available (MSD, Rockville, MD) enzyme-linked immunosorbent assay (ELISA). We used separate linear mixed models to examine trends over time in hsCRP and BMI and possible differences between groups. Continuous time structural equation modeling (CTSEM), an ideal method for examining predictive inter-relationships among longitudinal measures with variable assessment intervals, was then used to examine cross-lagged effects (hsCRP --> BMI and BMI --> hsCRP) and possible moderation of these by group, while controlling for sex, race/ethnicity, and education.

Results: CRP and BMI values over the whole study were significantly greater in PwS (p's BMI (7.8 [7.5-8.1]); the effect of one's current hsCRP level predicting next year's BMI was significantly stronger than the other lagged association ($p = .007$). Further, we found that hsCRP --> BMI relationships, but not BMI --> hsCRP relationships, were stronger in PwS than NC (moderator coefficient: 1.2 [0.6-1.8]).

Conclusions: Our findings extend beyond previous studies of cross-sectional relationships of obesity and inflammation in PwS to show intertwined influences of hsCRP and BMI using longitudinal data and a novel analytic method. That current levels of peripheral inflammation are particularly predictive of later BMI, and especially so in PwS, suggests that anti-inflammatory interventions could have a positive effect on obesity among those with psychosis. Additional analyses examining the moderating role of physical activity and diet in the observed lagged associations are needed. In addition, clinical trials would be important to confirm these observational findings.

Keywords: Schizophrenia, Inflammation, Obesity, C-Reactive Protein, Biology of Aging

Disclosure: Nothing to disclose.

P546. Regulatory T Cells Correlate Negatively With Serum Kynurenine in Schizophrenia Persons With Positive Serum Anti-Gliadin Antibodies (AGA)

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Background: One third of patients with Schizophrenia (SZ) have an elevation of anti-gliadin IgG antibodies (AGA-IgG). Clinically, this AGA-IgG positive sub-group has worse negative symptoms and consistently displays a pro-inflammatory cytokine profile. Additionally, this group has been shown to have elevated serum kynurenine (KYN), a precursor of the NMDAR antagonist kynurenic acid (KYNA). In SZ, elevations in serum IL-6, IFN-γ, and TNF-α can upregulate the synthesis of KYN, which is then shunted preferentially towards KYNA production and contributes towards cognitive dysfunction and negative symptomology in SZ. Along with neuroactive functions, KYN and KYNA function as potent immunosuppressive agents, as KYN upregulates synthesis and activation of Regulatory T cells (Tregs). As we have recently shown Tregs to be increased in proportion and to be correlated with less negative symptoms in AGA-IgG positive SZ persons, we sought to further identify the relationship between serum KYN, KYNA, and Tregs in this sub-group.

Methods: In total, 14 Healthy Controls (HC) and 23 persons with a diagnosis of a schizophrenia spectrum illness were included. We measured Negative symptoms utilizing the Scale for the Negative Assessment of Symptoms (SANS). We collected Treg (defined in this study as CD3+CD4+CD25+Foxp3+) measurements from peripheral blood mononuclear cells via flow cytometry. AGA-IgG levels were measured via ELISA, with positive titer defined as greater than or equal to 20U. KYN and KNYA were assayed from serum utilizing high performance liquid chromatography. We utilized Spearman Rank Correlation Coefficients as data was found to be non-normally distributed.

Results: In SZ persons with positive AGA-IgG titers, there was a negative correlation between Tregs and KYN but not in SZ patients with negative AGA-IgG titers ($rs = -0.72$ and $p < 0.05$* vs.

rs = 0.94, p > 0.05). There were no statistically significant correlations between serum KYNA and Tregs in the positive AGA-IgG (rs = −0.13, p > 0.05) or negative AGA-IgG titers (rs = 0.17, p > 0.05). In the AGA-IgG positive SZ group, we again found Tregs to correlate negatively with SANS total (rs = −0.61, p < 0.05*), SANS anhedonia (rs = −0.52, p < 0.05*), and SANS blunting (rs = −0.65, p < 0.05*).

Conclusions: To our knowledge, this is the first study investigating KYN, KYNA, negative symptoms, and Tregs in an AGA-IgG positive SZ sub-group. Our results suggest that Tregs may be protective in AGA-IgG positive SZ with regards to negative symptomology by sequestering KYN via the aryl-hydrocarbon receptor, increasing Treg suppressive function and preventing the generation of KYNA. Though the sample size in this study is small, these exploratory findings suggest a neuroprotective role of Tregs in AGA-IgG positive SZ.

Keywords: Neuropsychiatric Disorders [Schizophrenia, Parkinson's Disease, Major Depressive Disorder], Schizophrenia Subtypes, Kynurenine Pathway, Anti-Gliadin Antibodies (AGA), Immune Markers, Cytokines, Synapses, Schizophrenia, Autism

Disclosure: Nothing to disclose.

P547. A Combined Marker of Inflammation in Persons With Serious Psychiatric Disorders

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Background: Numerous studies have documented the critical role of the immune system in the etiology and pathogenesis of serious psychiatric disorders. While the immune system of the CNS is probably the most relevant to brain pathology, it is highly interactive with the peripheral immune system which can be interrogated by the measurement of markers in blood samples. However, environmental factors which are common in individuals with psychiatric disorders, such as tobacco smoking and obesity, can also increase the levels of these markers. These factors may confound attempts to link immune activation to psychiatric disorders. It is thus important to develop methods for the measurement of immune activation which are independent of immune-activating environmental factors. This might be accomplished by the measurement of multiple markers and the identification of disease-specific patterns of activation.

Methods: The sample was drawn from psychiatric participants and participants in a non-psychiatric comparison group at Sheppard Pratt in Baltimore, MD in the period between January 2008 and February 2023. Participants were adults and of both sexes.

We measured markers of inflammation in 2764 serum or plasma samples obtained from 1443 individuals. The study population included 467 individuals with schizophrenia, 433 with bipolar disorder, 197 with major depressive disorder, and 346 comparison individuals without a current or past psychiatric disorder. The blood samples were analyzed by solid phase immunoassays to determine the levels of a series of markers identified in our previous studies. These include matrix metalloproteinase-9 (MMP-9), C-reactive protein, and Pentraxin-3 as well as antibodies to the infectious agents Epstein Barr Virus, cytomegalovirus, measles virus, Toxoplasma gondii, Saccharomyces cerevisiae and the food-derived antigens, wheat gliadin and bovine casein. Levels of these markers were standardized, and principal components analyses (PCA) were employed to group the markers into factors. Mixed effects models were constructed correlating these PCA factors with the diagnostic groups. Included in the models were the environmental factors of tobacco smoking and obesity (Body Mass Index>-30) as well as demographic variables including age, sex, and race. Significance levels were adjusted for multiple comparisons.

Results: We identified a PCA factor which was highly associated with the diagnosis of a psychiatric disorder as compared to no current or past psychiatric disorder (chi2 = 36.08, df = 3, p < 8 10-7). This factor had no significant association with tobacco smoking or obesity. Major contributors to this factor were MMP-9, Pentraxin-3, and antibodies to the Epstein Barr virus. Another factor, driven largely by C-reactive protein and antibodies to gliadin and measles virus had specificity for schizophrenia (chi2 = 21.4, df = 3, p < .00007) and a third, driven largely by MMP-9 and Pentraxin-3 had specificity for major depressive disorder (chi2 = 20.0, df = 3, p < .002). On the other hand, 5 additional PCA factors showed high levels of association with tobacco smoking or obesity and little association with clinical diagnostic group.

Conclusions: Blood samples are a readily accessible source for the measurement of immune markers in individuals with psychiatric disorders. The measurement of multiple markers of inflammation can be used to distinguish the inflammatory effects of external factors such as tobacco smoking and obesity from patterns which may reflect underlying disease processes. These factors should be explored as potential biomarkers for psychiatric diagnosis and clinical monitoring.

Keywords: Immune Biomarkers, Schizophrenia (SCZ), Major Depression Disorder

Disclosure: Nothing to disclose.

P548. Ultrastructural Markers of Synaptic Function in Postmortem Human Brain

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Background: The morphological substrate of neural communication is the synapse. Investigations of individually-resolved synapses in human brain can provide unprecedented insight into the basis of normal brain function, as well as dysfunction in neuropsychiatric disorders. Analysis at this level of resolution requires a volume electron microscopic (VEM) approach to directly visualize synaptic structures within postmortem human brain tissue. Indeed, the relative level of synaptic activity, and functioning of synaptic and sub-synaptic components, is directly related to quantifiable ultrastructural features. Thus, synaptic function in human brain can be interrogated at individual synapses with an ultrastructural analysis.

Despite the well-characterized relationship between synaptic function and ultrastructure, and interpretative power of ultrastructural analyses of individual synapses, multiple technical and logistical barriers have prevented the large-scale application of VEM to postmortem human brain tissue. The application of focused ion beam-scanning electron microscopy (FIB-SEM) to postmortem brain tissue promises to make new strides in studying synaptic structures in human brain. FIB-SEM results in a high-resolution stack of images, allowing volumes of brain tissue to be quantitatively analyzed in 3-dimensions (3D). With this method, synapses can be studied within the context of the surrounding neuropil. We have optimized an approach for the acquisition, fixation, preparation, and imaging of postmortem human brain tissue for FIB-SEM imaging and analysis.

Methods: A sample of dorsolateral prefrontal cortex (DLPFC, Brodmann Area 46) from a 62-year-old male decedent with no clinical brain-related or neuropathological disorders was obtained

during an autopsy conducted at the Allegheny County Office of the Medical Examiner (Pittsburgh, PA) after obtaining consent from next-of-kin. The DLPFC sample was submerged in 4% paraformaldehyde/0.2% glutaraldehyde fixative for 48 hours, rinsed, and sectioned at 50um. Sections underwent high-contrast, heavy metal staining followed by stabilizing resin embedding. A sub-sample of DLPFC layer 3 was excised and imaged via FIB-SEM (ThermoFisher Helios 5CX) with a 5x5x5nm voxel size. Synaptic ultrastructural features, such as volumetric sizes of axonal boutons, synaptic vesicles, presynaptic active zones, postsynaptic dendritic spines and postsynaptic densities (PSDs) were quantified (Amira software, ThermoFisher) in 25 randomly-selected Type 1 glutamatergic synapses in the reconstructed layer 3 volume of neuropil. Mean, standard deviation, and Pearson correlation of measures were completed.

Results: All cortical neuropil components were readily identifiable in 3D using established ultrastructural criteria, including Type 1 glutamatergic synapses. Analyses show the expected strong correlation between presynaptic active zone size and PSD volume ($r = 0.92$), a moderate correlation between postsynaptic dendritic spine and PSD volumes ($r = 0.40$), and a mean synaptic vesicle diameter of 40.4nm and volume of $5.3e4 \pm 2.1e4$ nm3.

Conclusions: Our preliminary studies demonstrate DLPFC L3 samples of excellent fixation and staining for 3D imaging by FIB-SEM. Glutamate synaptic structures were clearly identified in the volume of L3 neuropil. Quantitative analysis of sub-synaptic measures showed mean values consistent with prior ultrastructural studies, including correlations between pre- and postsynaptic measures. Overall, these studies support the compatibility of postmortem human brain tissue research with FIB-SEM. Future analyses of ultrastructural measures of core pre- and postsynaptic processes at individual synapses in postmortem human brain will directly inform the basis of synaptic dysfunction in psychiatric disorders.

Keywords: Electron Microscopy, Prefrontal Cortex, Glutamatergic Synapses, Postmortem Human Brain Tissue

Disclosure: Nothing to disclose.

P549. Regional Specificity of Morphometric Similarity Network Alterations in Youth at Clinical High Risk for Psychosis

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Background: Given increasing recognition that psychosis onset is a late stage of a neurodevelopmental illness, and the importance of early intervention, efforts are focused on understanding the pathophysiology of early stages of psychosis. Here we characterize morphometric similarity networks (MSNs), which measure within-subject structural similarity between cortical areas, using baseline structural brain imaging measures in subjects at clinical high-risk (CHR) who converted (CHRc) or did not convert (CHRnc) to psychosis, relative to unaffected comparison subjects. We further investigate those subjects who did not convert but remained symptomatic (CHRncs) and those whose symptoms remitted (CHRncr). We incorporate postmortem transcriptomic data from the Allen Human Brain Atlas (AHBA) to relate transcriptomic and MSN cortical spatial patterns.

Methods: MRI data were available for 757 subjects from the NAPLS2 cohort, including 71 CHRc, 467 CHRnc, and 219 unaffected comparison subjects (Age (Mean, SD): 19.2 +/- 4.4 years old; Sex: M = 58%, F = 42%). For CHRnc subgroup analysis, data were available for 220 CHRncs and 87 CHRncr subjects. In one analysis to increase the sample size of symptomatic CHR subjects, the 71 CHRc and 220 CHRncs were combined to form the CHRcs group (converters and symptomatic nonconverters). MSNs, which represent the sum of inter-regional associations, were constructed from 7 structural MRI measures including volume, cortical thickness, surface area, and 4 curvature indices. Microarray data from the AHBA were related to MSNs using partial least squares regression (PLS) and gene ontology (GO) pathway analysis was used to annotate correlated gene groups. We used a modified granular version of the Desikan Killiany cortical surface parcellation, consisting of 308 regions of interest.

Results: Difference maps between CHR vs. unaffected comparison subjects showed significant increases in MSN degree in 17 posterior visual areas and decreases in 14 anterior frontal and temporal areas in CHR youth (all FDR $p < 0.05$). CHRcs showed significant increases in 3 visual areas and decreases in 4 frontotemporal areas, relative to unaffected comparison subjects (all FDR $p < 0.05$). No significant differences were observed between CHRnc vs. CHRc or between CHRcs vs. CHRncr. For the PLS regression analysis, we modeled effect sizes across cortical regions to examine regional differences in MSNs between CHR vs. unaffected comparison subjects and relate them to regional gene expression from the AHBA. PLS component 1 (PLS1) for the MSN model explained 13.5% of the covariance between regional CHR vs. unaffected comparison subject MSN effect sizes and gene expression. Key markers of synaptic neurotransmission, including parvalbumin, GABAA receptor subunit delta, and sodium and potassium channel subunits were in the top 2% of PLS1 loadings. Functional annotation of all genes in the top 5% of loadings on PLS1 were associated with synaptic signaling, including glutamate, GABA and endocannabinoid signaling.

Conclusions: These findings suggest that differences in MSN topology between individuals at CHR for psychosis and unaffected comparison subjects cluster in specific cortical areas, are identifiable at the baseline MRI scan, and associate with markers of GABA neurotransmission and synaptic transmission-related biological pathways.

Keywords: Clinical High Risk for Psychosis, Human Neuroimaging, Transcriptomics

Disclosure: Nothing to disclose.

P550. Cerebellar-Cortical Connectivity and Prediction of Antipsychotic Response in First-Episode Psychosis

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Background: The prediction of antipsychotic response among patients with early psychosis remains a clinical challenge. Our recent work has provided converging evidence that functional connectivity of the cerebellar-cortical circuitry may serve as a promising prognostic biomarker for psychosis (Cao et al., Schiz Bull, 2021; Cao et al., AJP, 2023). However, which cerebellar system would show the best prediction value remains unclear.

Methods: This study included two independent clinical samples with a longitudinal design (discovery sample: n = 31, mean age 23.4 years, 19 males; replication sample: n = 69, mean age 21.8 years, 52 males). In both samples, patients with first-episode psychosis received baseline resting-state fMRI scans before entering a 12-week randomized clinical trial (treated with either

risperidone or aripiprazole). In line with our prior publication (Sarpal et al., AJP, 2015), treatment response was evaluated as two consecutive visits with a Clinical Global Impression (CGI) Scale improvement score of 1 or 2 (much or very much improved) and a rating of 3 (mild) or less on the Brief Psychiatric Rating Scale (BPRS) Psychosis items (i.e., conceptual disorganization, grandiosity, hallucinatory behavior, and unusual thought content). We used a state-of-the-art parcellation scheme to define nine cerebellar functional systems (auditory, visual, sensorimotor, frontoparietal, cingulo-opercular, default-mode, language, attention, multimodal), and seed-based analysis was performed on each cerebellar system to examine its functional connectivity with the entire cerebral cortex. Group-level analysis was subsequently conducted to compare the resulting connectivity patterns between responders and non-responders.

Results: A total of 9 patients in the discovery sample and 23 patients in the replication sample were classified as non-responders. Comparing with responders, non-responders showed significantly decreased connectivity between the cerebellar language system (chiefly includes the right cerebellar crus 2 and lobule 8) and the inferior frontal gyrus in the discovery sample (cluster-based $P < 0.05$ after 5000 permutations). A similar effect focusing on the cerebellar language seed was also observed in the replication sample.

Conclusions: These findings support our prior work that cerebellar-cortical connectivity is a prognostic biomarker for psychosis and suggest that the function of the cerebellar language system may be particularly relevant to antipsychotic treatment response.

Keywords: First-Episode Psychosis, Treatment Response, Neuroimaging Biomarkers, Cerebellum, Resting-State Functional Connectivity

Disclosure: Nothing to disclose.

P551. Associations With Premature Termination of Treatment by Epinet Participants at Coordinated Specialty Care for First Episode Psychosis Clinics

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Background: The NIMH-funded EPINET project includes 101 clinics in the United States that provide a Coordinated Specialty Care (CSC) treatment model for first episode psychosis (FEP). Clinics administer a harmonized assessment battery (named CAB) to their EPINET participants every 6 months. The final data set will be an important source of information for the field. Prior studies of CSC treatment have demonstrated that patients in CSC are more likely to remain in treatment than those in standard care. However, even with CSC, many patients terminate treatment before completing a CSC program. Knowing the characteristics of patients who prematurely terminate treatment is important both from a clinical perspective and for the field when analyzing data on CSC outcomes.

Methods: The CAB includes data on reason for CSC termination. EPINET clinics are grouped into consortiums named hubs. Data for our analysis were available from the ESPRITO hub with 13 clinics in 6 US states. We were interested in treatment terminations that were premature and contrary to the advice of the CSC team (e.g. stopping treatment entirely). Treatment terminations that were consistent with CSC team suggestions (e.g. program completion, recommended treatment at another program) were not counted as premature terminations. The follow-up period for each participant for assessing whether a premature termination had occurred was 6 months. Univariate logistic regression analyses adjusted for site were conducted to examine the association of the following variables with premature treatment termination: participant age, length of time in CSC (=6 months and =12 months and =18 months) gender, racial background, ethnicity, highest education level completed by either parent, highest education level completed by the participant, current housing situation, type of health insurance (commercial, public, none), enrolled in school, having a job, legal issues in the past 6 months, alcohol use, marijuana use, other drug use, prescription of an antipsychotic, prescription of a long-acting injectable antipsychotic (LAI), total number of medications prescribed, side effect burden, medication adherence, family involvement in treatment, scores on the Global Functioning: Social Scale, severity of positive symptoms, BAC composite cognition score and scores on the Intent to Attend scale in which participants rate the likelihood that they will 1) attend their next visit and 2) complete the CSC program.

Results: Data were available for 565 CSC participants. Over a 6-month observation period, 87 (15.4%) prematurely terminated treatment. Controlling for site, the following variables were associated with increased likelihood of premature termination: being homeless or having unstable housing versus living alone (OR = 7.3, 95% CI = (1.5, 34.1), $p = .01$); having no (OR = 1.97, 95% CI = (1.00, 3.87), $p = .05$) or public insurance (OR = 1.88, 95% CI = (1.04, 3.39), $p = .04$) versus commercial insurance; not being prescribed versus being prescribed a LAI (OR = 1.87, 95% CI = (1.03, 3.39), $p = 0.04$). The following variables were associated with decreased likelihood of premature termination: longer duration of CSC treatment with those in CSC >= 12 months and < 18 months (OR = 0.29, 95% CI = (0.11, 0.78), $p = .01$) and those in CSC 18 months or longer (OR = 0.45, 95% CI = (0.25, 0.79), $p < .01$) having lower rates compared to those in CSC < 6 months; better scores on the Global Functioning: Social Scale (OR = 0.81, 95% CI = (0.70, 0.94), $p = < .01$) and reporting higher likelihood to attend on the Intent to Attend scale (OR = 0.88, 95% CI = (0.79, 0.98), $p = 0.02$).

Conclusions: Factors that increase likelihood of premature discontinuation such as insurance status, inadequate housing and not receiving LAI medication and those that decrease it such as longer duration of CSC participation, better social functioning and the patient's expectation of attending the CSC program can guide development of strategies to improve retention. Further, these associations will be important to consider when examining dropout effects in analyses of longitudinal data with first episode psychosis study participants.

Keywords: First Episode Psychosis, Coordinated Specialty Care, Premature Treatment Discontinuation

Disclosures: Teva, Amalyx, Otsuka, Acadia: Consultant (Self).

P552. NAYAB: A Randomised Double-Blind Placebo-Controlled Trial of Minocycline and/or Omega-3 Fatty Acids Added to Treatment as Usual for At Risk Mental States

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Background: Inflammatory mechanisms are thought to contribute to the onset of psychosis in persons with an at-risk mental state (ARMS). We investigated whether the anti-inflammatory properties of minocycline and omega-3 polyunsaturated fatty acids (omega-3), alone or synergistically, would prevent transition to psychosis in ARMS.

Methods: NAYAB was a multicentre, double-blind, randomised placebo-controlled study of minocycline and omega-3 using a 2×2 factorial design. The four treatment groups took either minocycline, omega-3, their combination, or their matching placebos for 6 months followed by a 6-month follow-up period. The primary outcome was transition to psychosis at 12 months. 10,173 help-seeking individuals aged 16-35 years were screened using the Prodromal Questionaire-16. Individuals scoring 6 and over were interviewed using the Comprehensive Assessment of At-Risk Mental States (CAARMS) to confirm ARMS. Participants were randomly assigned in a 1:1:1:1 ratio to either placebo, minocycline, omega- 3 or minocycline and omega-3 in combination. We chose to trial 200 mg of minocycline as this was the dose used in a previous trial in early-phase schizophrenia and 1.2g of omega-3 based on trials in ARMS populations. A sample size of 59 per treatment arm was required to detect placebo-active differences with a power of 80% and at the 5% significance level. To allow for a 25% drop-out rate and to ensure there were 59 completing participants per arm, a minimum sample size of 80 subjects per treatment group was required requiring a total sample size of 320 participants. The 2x2 factorial analysis for omega-3 compared the proportion or 'risk' of psychosis onsets in 'all omega-3' exposed participants whether taken alone or in combination with minocycline, versus onset risk in the 'no-omega-3' participant group taking double placebo or minocycline alone. The same approach was used for 'all minocycline' versus 'no-minocycline'. Chi-square analysis was used to test for significant deviation from equality of risk. Risk ratios (RR) with 95% confidence intervals (CIs) are presented below. We used analysis of variance to analyse the post-treatment ratings at 6 and 12 months (repeated 'time' measure) in the non-transitioning group, co-varying for baseline with fixed factors for omega-3 ('all omega-3' vs. 'no omega-3') and minocycline ('all minocycline' vs. 'no minocycline'). We looked for evidence of synergy in positive interactions between omega-3 and minocycline factors. Last observations for drop-outs were carried forward to the post treatment ratings.

Results: 326 participants were recruited, and 80-82 participants were randomised to each of the four treatment groups. 29 participants dropped out of the study resulting in a 91% retention rate. Drop-outs were evenly distributed between the treatment groups with the exception that 4 participants in the omega-3 + placebo group self-harmed by overdose or self- cutting, requiring brief medical attention but not continued contact with health services. The randomised sample included 60% male participants with a mean age of 24 years at trial entry. There were no statistically significant or numerically important differences in age, sex or other social and occupational demographics between the 4 treatment groups. All participants rated positive for attenuated psychotic symptoms (APS) and none had brief limited psychotic symptoms (BLIPS). 15% met criteria for the vulnerability risk group, defined by family history and declining performance.

Forty-five (13.8%) participants transitioned to psychosis. The risk of transition was non-significantly greater in those randomised to omega-3 alone or in combination with minocycline at 17.3.%, compared to 10.4% in those not exposed to omega-3 a risk-ratio (RR = 1.67, 95% CI [0.95, 2.92]; $p = 0.07$). The risk of transition was non-significantly lower at 12.8% in those randomised to minocycline alone or in combination with omega-3 compared to those not exposed to minocycline at 14.8% (RR = 0.86, 95% CI [0.50, 1.49]; $p > 0.10$). In participants who did not become psychotic, CAARMS and depression symptom scores were reduced at six and twelve months (SMD = 1.43; 95% CI [0.33, 1.76]; $p < 0.01$) in those exposed to omega-3. There were no effects of minocycline on CAARMS or depression scores.

Conclusions: We found no evidence that treatment with minocycline or omega-3, either alone or in combination, reduced transition to psychosis. However, the power of the study was substantially reduced as the overall transition rate of 14% was half the 30% anticipated. Nevertheless, we infer that the lack of a protective effect of omega- 3 is unlikely to be a false negative since it was associated with a near significant increase RR of transition. The estimated effect of minocycline was marginally less than unity (RR = 0.85) but the CI extends to RR = 0.50 so we cannot conclude that minocycline is definitively without a clinically relevant effect. Omega-3 supplementation appeared to benefit non-psychotic general and transdiagnostic symptoms. In a recent ARMS study, only the non -transitioning majority had raised blood IL-6 levels at baseline (PMID 36940754). This finding raises the possibility that non-transitioning ARMS symptoms may have a distinct inflammatory pathogenesis that may have been helped by the anti-inflammatory effects of omega-3 supplementation in our study.

Keywords: Early Intervention, Psychosis-Risk, Minocycline, Omega-3, Psychosis

Disclosure: Nothing to disclose.

P553. Pharmacokinetics and Tolerability of a Once-Daily Controlled-Release Formulation of MK-8189, an Investigational PDE10A Inhibitor for Treating Schizophrenia

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Background: MK-8189 is an investigational phosphodiesterase 10A (PDE10A) inhibitor for treating schizophrenia. Data from a first-in-human study and a positron emission tomography study in healthy participants using an immediate-release (IR) formulation of MK-8189 demonstrated that doses of 3mg and 6mg achieved peak enzyme occupancies of ~62% and ~78% respectively. However, dystonia was reported in 3 out of 12 (3mg) and 1 out of 6 (6mg) participants and appeared to be related to time of maximum concentration (Tmax; 0.5-2h). The ~6h half-life led to a high peak-to-24h-trough concentration ratio (~17), suggesting that rapid changes in enzyme inhibition may be poorly tolerated. Here we report findings with an improved controlled-release (CR) formulation of MK-8189.

Methods: The pharmacokinetics of three MK-8189 2mg tablet CR formulations were evaluated in healthy participants (n = 14). One CR formulation was then selected and evaluated for tolerability and pharmacokinetics in three Phase-1 multiple-dose studies in participants with and without schizophrenia using once-daily dosing. Study 1 was placebo-controlled and participants were titrated from 2mg to 12mg as monotherapy (n = 26) or from 2 or 4mg to 16mg (n = 19) as adjunctive therapy to prescribed antipsychotic therapy. In Study 2, participants were administered MK-8189/placebo as monotherapy (n = 32), or adjunctive therapy (n = 17), and titrated from 4mg to 24mg. An additional panel were administered MK-8189/placebo as monotherapy and titrated from 8mg to 48mg (n = 26). In Study 3, one panel (n = 8) were titrated from 16mg to 24mg or placebo, while another panel (n = 18) received 24mg/placebo without titration.

Results: In non-elderly healthy participants, the selected CR formulation had a Tmax of 10-24h and peak-to-24h-trough ratio of ~1.3; doses ≤24mg did not result in dystonia (0 out of 36 participants), although maximum concentration (Cmax) values were similar or greater than those associated with dystonia in the first-in-human study using the IR formulation. Results in schizophrenia participants were as follows: In Study 1, dystonia was reported in 5 out of 33 participants receiving MK-8189 (3 following monotherapy [2mg, 4mg, 12mg] and 2 following adjunctive

therapy [8mg, 16mg]). All events responded immediately to benztropine. Three of these 5 participants continued in the trial and 2 discontinued. In Study 2, with titration from 4mg to 24mg and 8mg to 48mg, no MK-8189-related dystonia (0 out of 38 participants) was observed. Cmax values of ~1.8μM were achieved, substantially higher than the 0.376μM Cmax associated with dystonia in healthy participants in the first-in-human study using the IR formulation. In Study 3, 2 out of 6 participants had transient dystonia following the first dose of 16mg, but both responded to treatment and participants continued dosing without recurrence. No dystonia (0 out of 14 participants) was reported when dosing was initiated at 24mg. Other adverse events across all three studies were mostly mild or moderate in intensity and there was no clear dose-related increase in specific events.

Conclusions: MK-8189 CR was generally well-tolerated in schizophrenia participants. Dystonia was observed at lower doses but not at doses ≥20mg. Based on the enzyme occupancy-concentration relationship, 24mg MK-8189 CR is expected to produce sustained enzyme occupancy ≥80%, while being well-tolerated. Preclinical studies suggest that with higher PDE10A inhibition, the D1-mediated direct striatonigral pathway is activated, which could account for the counter-intuitive findings of lower dystonia at higher doses. Alternatively, fluctuations in PDE10 inhibition may promote dystonia compared to sustained inhibition. Doses up to 24mg are being evaluated in a Phase 2b trial [NCT04624243].

Keywords: Schizophrenia Novel Treatment, PDE10A, Clinical Psychopharmacology

Disclosure: Merck and Co., Inc.: Employee, Stock/Equity (Self).

P554. Subcortical Functional and Molecular Contributions to Antipsychotic Efficacy: A Preliminary 7-Tesla Examination

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Background: Evidence implicates abnormalities in subcortical structures of the cortico–basal ganglia–thalamo–cortical system in schizophrenia. However, the relationship between these abnormalities and response to antipsychotic treatment remain largely unknown. The advent of 7-Tesla (7T) multivoxel spectroscopy (MRSI) facilitates higher resolution assays of neurometabolites and multimodal assays from regions such as the caudate and thalamus in relation to the clinical course of antipsychotic treatment. In this preliminary, longitudinal study, we use 7T imaging to examine relationships between antipsychotic efficacy and 1) changes in concentrations of glutamate and GABA; 2) changes in functional connectivity of subcortical structures; and 3) exploratory multimodal MRSI-fMRI relationships.

Methods: A cohort of 20 treated at UPMC Western Psychiatric Hospital with early phase schizophrenia were included in this study. Patients were scanned in a 7-Tesla Magnetom scanner upon initiating and after 8 weeks of second-generation antipsychotic treatment. Symptoms were quantified using the Brief Psychiatric Rating Scale (BPRS). fMRI data was preprocessed using fMRIPrep and group-level analyses examined changes in thalamic functional connectivity with cluster correction at $p = 0.05$. Magnetic resonance spectroscopy imaging (MRSI) data was used to quantify levels of neurometabolites, including glutamate and GABA, with LCModel.

Results: Preliminary results do not show significant changes in glutamate for GABA in the caudate or thalamus in relation to treatment efficacy ($p > 0.05$). With greater efficacy, decreased functional connectivity was observed between the thalamus and left superior parietal lobule and left precentral gyrus ($p < 0.05$, corrected). Exploratory analyses indicate a negative association between change in GABA in the right thalamus and connectivity to the right lingual gyrus ($p < 0.05$, corrected).

Conclusions: These preliminary analyses leverage high field 7T neuroimaging to examine molecular and functional correlates of antipsychotic efficacy in early phase schizophrenia. Participant enrollment and analyses for this multimodal and longitudinal analysis are ongoing. Further MRSI and functional connectivity will be conducted to expand upon these preliminary findings.

Keywords: Schizophrenia Spectrum Illness, MRSI, Basal Ganglia, Antipsychotic Response, Functional Connectivity

Disclosure: Nothing to disclose.

P555. Academic-Community Early Psychosis Intervention Network (AC-EPINET) and Health Outcomes Network and Education (HONE): A Learning Health System for Research and Real-World Dashboarding of Outcomes in First Episode Psychosis Care

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Background: Specialized team-based care for First Episode Psychosis (FEP) has been established as the standard of care in the US, where it is called Coordinated Specialty Care (CSC). Similar models have demonstrated the potential to improve clinical outcomes across the globe. Despite this, there exists no universal standard to which clinics may design and benchmark their CSC services. The HONE (Health Outcomes Network and Education) Learning Health System (LHS) model was developed at the Yale STEP clinic, specifically for FEP. It focuses on a core set of multi-stakeholder derived outcomes and facilitates rapid cycles of continuous quality improvement. This poster presentation reports on the dissemination of HONE to 6 clinical-academic FEP sites, with the development of a clinical data dashboard, co-designed to enhance treatment effectiveness and track the effectiveness of quality improvements.

Methods: The HONE LHS method was implemented as part of the AC-EPINET project across a consortium of 6 FEP services embedded in clinical-academic centers across the Midwest and coordinated by a central hub located at Indiana University (Principal Investigator: Breier). The sites are: Indiana University, University of Rochester, Ohio State University, Tulane University, Vanderbilt University and University of Michigan. This work was supported by, and is nested within the larger, national NIMH Early Psychosis Intervention Network (EPINET) program comprised of 8 such hubs, linked to over 100 clinics. All EPINET clinics use the same standardized, Core Assessment Battery (CAB) which combines clinician-administered and self-report instruments. The CAB is administered at the enrollment baseline and every 6 months throughout the study (details can be found at https://nationalepinet.org).

Clinician-centered implementation balanced science, informatics, incentives and culture to integrate LHS activities as deeply as possible into everyday clinic workflows. A series of site engagement meetings explored similarities and differences between the clinics, identifying core common values and objectives for the network. A pragmatic data capture pipeline was designed and launched, capitalizing on legacy systems to minimize measurement burden and regulatory barriers. A core set of visualizations, piloted at STEP, were adapted to the AC-EPINET project and launched to both academic and clinical users at the sites. The initial objectives were to minimize: Duration of

Untreated Psychosis (measured as time to first antipsychotic, and time to CSC enrollment); pre-referral hospitalizations, post-referral hospitalizations, symptoms, cardiovascular risk and suicide attempts; and to maximize: equity in access (by birth sex and race), vocational engagement, and successful transitions of care at discharge. Inclusion of Wilson Confidence Intervals and proportion of missing data aids pragmatic, real-time interpretation. Patients were provided information and given the opportunity to opt-out of LHS activities and the de-identified data-sharing - without impacting upon their eligibility for other elements of care.

Results: At the 2-year mark AC-EPINET has 30 clinical users, having collected 1,397 CABs from 596 enrollees. 70.6% of enrollees reported Male birth sex, 29.4% Female and 0.3% Other. 45.3% reported race as White, 40.1% Black, 3.2% Asian, 3.2% Other and 2.2% unsure. 72.6% of the sample were prescribed an oral antipsychotic medication at baseline, 19.2% an injectable. At 12 months, 63.9% are prescribed an oral, and 25.9% an injectable antipsychotic. Within the network as a whole, at least 59% of enrollees received their first antipsychotic within 3 months of psychosis onset, and 72.1% were admitted to specialty care within a year. Although 57.9% are currently overweight, less than 18.5% became newly overweight within the first 6 months of care.

Users can interactively compare their clinic to network outcomes, and across a curated set of patient sub-grouping variables (e.g., race, gender, medication status). The feasibility of this approach will be demonstrated via live demonstration of the production system. A recorded version will also be accessible online.

Conclusions: By balancing science, informatics, incentives, and culture it is possible to collaboratively design dashboards intended to help organize and continuously improve FEP care via clinical-academic collaborations. Whilst remaining mindful of inferential limitations, this quality improvement infrastructure may be disseminated and extended to support practice-based research, including providing pragmatic and rapid estimations of real-world treatment effectiveness.

Keywords: First Episode Psychosis, Prescribing, Real-world Clinical Outcomes, Learning Health System

Disclosure: STEP Forward LLC: Consultant (Self)

P556. Effect of Ulotaront on Brain Dopamine Synthesis Capacity in Subjects With Schizophrenia on Stable Doses of a D2 Antipsychotic: Results of an 18F-DOPA Pet Study

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Background: 18F-DOPA positron emission tomography (PET) imaging studies have found higher striatal dopamine synthesis capacity is associated with symptoms of psychosis and is correlated with severity [1].

In a mouse model, ketamine-induced elevations in dopamine synthesis capacity were reduced by single doses of ulotaront (3 mg/kg i.p.) while no effect on baseline dopamine synthesis capacity was observed [2]. Ulotaront is a trace amine-associated receptor 1 (TAAR1) agonist with 5-HT1A activity that has demonstrated efficacy in a Phase 2 trial in patients with schizophrenia [3]. The aim of the current study was to evaluate the effect of ulotaront adjunctive to a D2 antipsychotic on brain dopamine synthesis capacity, as measured by 18F-DOPA PET imaging, in adults with schizophrenia.

Methods: Male or female subjects, ages 18-45, were enrolled who met DSM-5 criteria for schizophrenia and were on a stable dose of antipsychotic medication (excluding clozapine) for a ≥3 weeks prior to screening. At screen, an 18F-DOPA PET scan was performed. Subjects were continued on their current antipsychotic medication, and treatment with ulotaront was added for a period of 14 days, consisting of an initial dose of 50 mg/d for 3 days followed by titration to 75 mg/d for the remaining time period. A one-time dose reduction (from 75 mg to 50 mg) for tolerability purposes was allowed; subjects remained on the reduced dosage for the remaining time. On day 14, the 18F-DOPA PET scan was performed approximately 2-4 h after the final dose. The primary endpoint was change from baseline in dopamine synthesis capacity in the striatum at day 14 by 18F-DOPA PET scan. Imaging data were analyzed with Patlak modelling with cerebellum as reference region, resulting in the influx constant Kicer reflecting dopamine synthesis capacity. For the whole striatum and its subregions (associative, motor, limbic), averaged regional Kicer values were calculated, and correlated with PANSS total and subscale scores (Marder Positive). Change from baseline in dopamine synthesis capacity was calculated, with 95% confidence interval, and a repeated measures ANOVA used to test the effect of treatment and if there was a treatment by region interaction. Efficacy measures included the PANSS total and subscale scores. Sample size calculation: Measurement of dopamine synthesis capacity has shown good test-retest reliability [4], with a reported within-subject standard deviation of 0.00056/min in dopamine synthesis capacity. The absolute elevation in dopamine synthesis capacity for schizophrenia cases versus normal healthy controls is estimated to be approximately 0.001/min and to have a correlation of approximately 0.6 with symptoms [4]. Given this relationship between dopamine synthesis capacity and symptoms, it is estimated that a decrease in dopamine synthesis capacity of 0.0005/min in patients is the smallest reduction likely to be clinically meaningful (translating to an estimated 20% reduction in PANSS score). A sample size of 16 treated subjects will provide >90% power to detect a reduction from baseline of 0.0005/min in dopamine synthesis capacity.

Results: 22 subjects were enrolled; 19 had useable baseline and day 14 scans: male ($n = 14$), female ($n = 5$); mean (SD) age, 32.9 (6.7); mean (SD) age at initial diagnosis, 28.6 (6.5); baseline mean (SD) PANSS total 79.3 (7.3), PANSS positive subscale 19.2 (4.6), PANSS negative subscale 22.8 (6.1). There was a significant effect of treatment ($p < 0.05$) and striatal subregion ($p < 0.05$) on Kicer but no treatment by subregion interaction ($p > 0.8$). Mean percent changes from baseline, and respective treatment effect size were: whole striatum $= -3.98\%$ (95% CI: -8.68%, 0.72%) & Cohen's $d = -0.46$ (95% CI: -0.97, 0.05), associative striatum $= -3.38\%$ (95% CI: -8.09, 1.34) & Cohen's $d = -0.39$ (95% CI: -0.89, 0.11) ; motor striatum $= -3.89\%$ (95% CI: -9.61, 1.81%) & Cohen's $d = -0.39$ (95% CI: -0.89, 0.11); and limbic striatum $= -5.79\%$ (95% CI: -9.66, -1.92%) & Cohen's $d = -0.74$ (95% CI: -1.28, -0.19). The mean reduction in dopamine synthesis capacity for all 3 striatal subregions was greater than the estimated minimal threshold (0.0005/min) likely to be clinically meaningful. There was a significant moderate correlation between change in striatal 18F-DOPA Kicer and change in Marder Positive Factor score on the PANSS ($r = 0.5$, $p < 0.05$) but no significant relationship with change in PANSS total symptom score ($r = 0.2$, $p > 0.05$).

Conclusions: The results of this 18F-DOPA PET study found that 14 days of treatment with ulotaront adjunctively administered to subjects with schizophrenia on stable doses of a D2 antipsychotic medication resulted in potentially clinically meaningful reductions in dopamine synthesis capacity in the striatum, and specifically the associative, motor, and limbic subregions. These clinical results translate the preclinical findings of ulotaront reducing striatal dopamine synthesis capacity in mice. Notably, there was a significant correlation between change in dopamine synthesis

capacity and change in positive symptoms of psychosis. The magnitude of the reduction from baseline in dopamine synthesis capacity (and additional, correlated reduction in positive symptoms of psychosis) despite stable baseline treatment with a D2 antipsychotic, provides confirmation of the potential efficacy of the novel TAAR1 agonist mechanism of action of ulotaront.

Keywords: Schizophrenia (SCZ), PET, Dopamine, Treatment, Imaging

Disclosures: Angellini, Autifony, Biogen, Boehringer-Ingelheim, Eli Lilly, Heptares, Karuna, Global Medical Education, Invicro, Jansenn, Lundbeck, Neurocrine, Otsuka, Sunovion, Recordati, Roche and Viatris/ Mylan: Honoraria (Self).

P557. Impact of MK-8189 in Preclinical Models of Cognition and Electroencephalography (EEG) Spectral Power

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Background: MK-8189 is potent and selective phosphodiesterase 10A (PDE10A) inhibitor in clinical studies for the treatment of schizophrenia. In preclinical models, we have previously demonstrated that MK-8189 produces robust activation of striatal signaling pathways and antipsychotic efficacy. Here we evaluated MK-8189 in assays measuring two different types of cognitive processing, episodic-like memory and executive function, both of which are severely disrupted in schizophrenia. We employed qEEG to further characterize the impact of MK-8189 on cortical activity.

Methods: Model 1 (episodic-like memory): Male Wistar Hannover rats were used to examine the influence of MK-8189 on novel object recognition. On the day of testing each rat explored two identical objects for 3 min (T1). Exploration was recorded then animals were removed and 48 hours later each rat was again placed in the testing arena for 3 min and exposed to one identical object and one novel object (T2). The amount of time animals spent exploring the novel object relative to the familiar object was the primary measure.

Model 2 (executive function): Rhesus monkeys were used in the objective retrieval task. Ketamine was used to induce executive function deficits in this task. Sessions consisted of a fixed arrangement of "easy" ($n = 8$) and "difficult" ($n = 11$) trials. Easy trials were used to detect potential adverse drug effects, such as drug-related motor or visuospatial impairment. Difficult trial performance was used as the dependent measure for assessing cognitive function since it requires attention and impulse control and is mediated by the prefrontal cortex. Trials were scored "correct" if subjects successfully reached into the open plane of the box and retrieved the reward on their first attempt. Once vehicle and ketamine baseline performance stabilized MK-8189 (0.15mg/kg) characterization was initiated. MK-8189 or vehicle was given p.o. for 4 days prior to testing and on day 5 MK-8189 was administered orally 2 h prior to testing and ketamine was administered intramuscularly 30 min prior to testing.

EEG studies were conducted in rhesus monkeys implanted with subcutaneous telemetric devices to permit the simultaneous recording of electrocorticogram (ECoG), electrooculogram (EOG), and electromyogram (EMG) activity.

Results: MK-8189 produced a significant improvement in the rat novel object recognition task at 0.16 and 0.25 mg/kg. Effects were similar in magnitude to the acetylcholinesterase inhibitor donepezil. The potential of MK-8189 to influence executive function was examined using the rhesus monkey object retrieval task. MK-8189 reversed a ketamine-induced deficit in this task. Importantly, easy trials were not affected in this study demonstrating that MK-8189 was well tolerated. qEEG analysis revealed a significant decrease in power in the high-frequency gamma and beta bands from 2–7 h following dosing with MK-8189. Reduction in gamma has been observed following administration of atypical antipsychotics in preclinical species and gamma synchronicity is known to be markedly altered in schizophrenia, supporting a link between gamma and this disorder. In contrast, modest increases in the spectral power of mid-frequency bands (theta and alpha) were observed following administration of MK-8189.

Conclusions: These preclinical findings suggest that PDE10A inhibition via MK-8189 could potentially impact cognitive impairment in humans. MK-8189 has a significant impact on executive function and episodic memory at similar PDE10A enzyme occupancies (~25 – 50% PDE10A enzyme occupancy). Accordingly, MK-8189 produced significant effects on qEEG spectral power further demonstrating the ability of MK-8189 to modulate signaling in cortical areas associated with cognition.

Keywords: Cognitive Impairment Associated with Schizophrenia, Preclinical Pharmacology, EEG Biomarkers

Disclosure: Merck: Employee (Self).

P558. Exploring the Potential Role of Synergistic Psychedelic – NMDA Receptor Modulator Treatment for Refractory Neuropsychiatric Disorders

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Background: A growing body of data supports a potential therapeutic role for serotonergic psychedelics in depression and other neuropsychiatric disorders. However, hallucinogenic effects may limit their utilization in disorders where induction of psychosis is a particular concern. Serotonergic psychedelics enhance neuroplasticity via serotonin 2A receptor (5HT2AR) activation and complex serotonergic-glutamatergic interactions involving ionotropic glutamate receptors, tropomyosin receptor kinase B (TrkB) and the mammalian target of rapamycin (mTOR). N-methyl-d-aspartate receptors (NMDAR) channel antagonists, i.e. ketamine, and glycine modulatory site full and partial agonists, i.e. D-serine (DSR) and D-cycloserine (DCS), share some of these mechanisms of action and have neuroplastic and antidepressant effects. Moreover, pro-cognitive effects have been reported for DSR and DCS and 5HT2AR-NMDAR interactions modulate neuronal excitability in prefrontal cortex and represent a target for new antipsychotics. We have hypothesized that synchronous administration of a psychedelic and a NMDAR modulator may increase the therapeutic impact of each of the treatment components and allow for dose adjustments and improved safety. We evaluated the effects of DSR and DCS on psilocybin-induced head twitch response (HTR), a rodent proxy for human psychedelic effects, and on acute MK-801-induced hyperlocomotion, a test modeling positive symptom of schizophrenia. Our findings provide initial support for the potential therapeutic applicability of synergistic psychedelic – NMDA receptor modulator treatment in treatment refractory psychiatric disorders, including schizophrenia characterized by prominent negative symptoms.

Methods: To measure drug induced HTR, male ICR mice (8-10 weeks, 26-30 g) were injected intraperitoneally with psilocybin (4.4 mg/kg), DSR (3000 mg/kg) or DCS (320 mg/kg); a combination of DSR and psilocybin or DCS and psilocybin; or vehicle (saline) and were immediately placed inside a magnetometer to determine HTR over 20 minutes. To measure MK-801-induced Open Field Test (OFT) hyperactivity, mice were injected intraperitoneally with psilocybin (4.4 mg/kg), DSR (3000 mg/jg) or DCS

(320 mg/kg); a combination of DSR and psilocybin or DCS and psilocybin; or vehicle (saline). After 30 minutes they were injected IP with MK-801 (0.5 mg/kg), and after another 30 minutes were placed in the OFT arena for 60 minutes. Activity was monitored by the Noldus Ethovision system.

Results: HTR analysis showed that psilocybin induced a significant increase in head twitches compared to saline control ($p < 0.0001$). DSR alone did not affect HTR. However, in mice co-administered DSR and psilocybin, HTR was completely blocked ($p = 0.0022$ - DSER + psilocybin vs. psilocybin). Similarly, DCS did not affect HTR while the combination of psilocybin and DCS attenuated HTR and induced a significantly lower number of head twitches compared to psilocybin alone ($p = 0.0001$). In the acute MK-801-induced hyperlocomotion test, a significant difference ($p = 0.02$) was observed in the distance travelled between mice administered saline prior to MK-801 injection and mice administered DSR + psilocybin. The DSR + psilocybin group moved a significantly shorter distance, indicative or lower potential of the DSR + psilocybin treatment combination to induce or exacerbate positive psychotic symptoms. Similar results were obtained in the DCS experiment in which co-administration of DCS and psilocybin significantly reduced the hyperactivity induced by MK-801 ($p = 0.008$). Psilocybin alone did not increase MK-801 induced hyperactivity suggesting that it may not exacerbate psychotic features.

Conclusions: The findings of our HTR experiments suggest that both DSR and DCS have the potential to limit the acute hallucinogenic effects of psilocybin as reflected by the head twitch response in mice. This supports the potential feasibility of co-administration of these compounds to patients at risk for induction or exacerbation of psychosis. Moreover, the combination of DSR and psilocybin and of DCS and psilocybin significantly reduced MK-801-induced hyperlocomotion, indicating a potential therapeutic effect on positive symptoms of schizophrenia. Furthermore, psilocybin alone did not exacerbate MK-801-induced hyperactivity. This novel combined approach has the potential to significantly improve the treatment of refractory psychiatric disorders by addressing NMDA receptor-mediated effects and leveraging the reported neuroplastic properties of psilocybin. Further studies are warranted to elucidate the mechanisms underlying these effects.

Keywords: Psilocybin, NMDA Modulators, Psychedelic Therapy

Disclosure: Hadasit Medical Research Corporation: Patent (Self).

P559. The Bidirectional Effect of 2-AG on Hyperdopaminergic States: Implications for Therapeutic 2-AG Modulation in Psychosis

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Background: Several serious, debilitating, and lifelong conditions, including psychosis in schizophrenia (SCZ), mania in bipolar disorder (BD), and attention deficit/hyperactivity disorder (ADHD) are believed to be related to a dysregulation in dopamine (DA) signalling (DA pathologies). Furthermore, the endocannabinoid system (ECS) is suggested to be dysregulated in DA pathologies: enzymes in the biosynthesis pathway of 2-arachidnoylglycerol (2-AG), a major endocannabinoid neurotransmitter, were shown to be altered in SCZ; DAGL (diacylglycerol lipase; 2-AG synthesis) levels are decreased in patients with first episode psychosis; MAGL (monoacylglycerol lipase; primary 2-AG metabolism) expression levels are significantly lower in patients with SCZ; and elevated 2-AG was observed in individuals at high risk of psychosis. Despite the mixed findings, the elevation of 2-AG is coveted in certain clinical contexts, with clinical trials of MAGL inhibitors (MAGLi) currently underway for post-traumatic stress disorder (PTSD) and Tourette syndrome. However, some evidence suggests that increasing 2-AG might be detrimental in hyperDA pathologies. Before wide therapeutic use, it's imperative to understand the effect of MAGLi in vulnerable populations, and whether decreasing 2-AG is therapeutic. A subpopulation with dysregulated 2-AG and/or DA may be vulnerable to psychiatric effects of MAGLi. Therefore, we assessed pre-clinical effects of MAGLi (increase 2-AG) and DAGLi (decrease 2-AG) in two models of hyperDA; based on well-established associations between psychopathologies and increased subcortical DA.

Methods: Genetic (adult dopamine transporter-knockout (DATKO)) and pharmacological (C57Bl/6J with amphetamine) models of hyperDA were treated acutely with a MAGLi (MJN110, 5mg/kg) or DAGLi (DO34, 30mg/kg), and tested on several behavioural assays. Lipidomic and molecular analyses were completed (striatal brain samples), and partial correlation networks were generated. Using the novel positron emission tomography (PET) radiotracer for MAGL, [18F]MAGL-2102, and an established radiotracer for imaging the cannabinoid receptor type-1 (CB1), [18F]FMPEP-d2, we interrogated the status of MAGL and CB1 in vivo in hyperDA states for the first time, quantifying and comparing the distribution between DATKO and WT littermate controls. Binding of radiotracers was used to infer expression levels of targets probed. Data were analyzed using three-way ANOVA (behaviour), Student's t-test for lipidomics, and repeated measures ANOVA for PET quantification (with appropriate post hoc analyses for all tests).

Results: DATKO have 5 times more extracellular DA in the striatum; exploratory hyperactivity, impaired sensorimotor gating, blunted response to psychostimulants, and disrupted lipid profiles. Brain uptake of [18F]MAGL-2102 (male > female) was similarly and significantly decreased in both sexes in DATKO (whole brain AUC: −21% and −17% in female and male DATKO, respectively). [18F]FMPEP-d2 (CB1), on the other hand, showed opposite sex-dependent binding in WT (female > male), with a sex-dependent significant decrease in tracer uptake in female DATKO (-27% in whole brain AUC), but not males (-11%, non-significant). When treated with a MAGLi, DATKO mice showed exacerbation of hyperlocomotion, sensorimotor deficits, and further disruption of lipid networks. MAGLi increased reward association in DATKO, but not WT, suggesting an addiction liability in certain populations. MAGLi effects weren't limited to DATKO; it exacerbated psychostimulant responses in C57BL/6J. Data suggests that increasing 2-AG via MAGLi exacerbates states of hyperDA, mediated by CB1. Interestingly, decreasing 2-AG synthesis (via DAGLi) presented opposite effects on all measured hyperDA behavioural outputs in both DATKO and C57BL/6J. As such, DAGLi may offer a novel therapeutic approach in hyperDA states.

Conclusions: The present study applied behavioural pharmacology combined with molecular, lipidomic, and PET imaging studies to demonstrate profound brain-region specific remodelling of 2-AG in hyperDA states. The work highlights hitherto unrecognized potential for detrimental effects of MAGLi in certain disease states or hyperDA pathologies. It also revealed a potential therapeutic approach to ameliorate hyperDA pathologies with indirect modulation of DA by reducing 2-AG synthesis via DAGLi.

Keywords: Endocannabinoids, Dopamine, Psychosis, 2-AG

Disclosure: Nothing to disclose.

P560. Potentiation of CB2 Receptor Signaling Mediates Antipsychotic-Like Efficacy in Mice

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Background: Novel therapeutic strategies that are mechanistically distinct from currently utilized monoamine-based drugs are desperately needed for treating schizophrenia. Muscarinic acetylcholine receptors represent one of the more promising novel targets currently being tested both clinically and preclinically for alleviating schizophrenia symptoms. Preclinical studies suggest that the antipsychotic-like efficacy of muscarinic acetylcholine receptors may be mediated, at least in part, by endocannabinoid signaling through the cannabinoid type-2 receptor (CB2). Although CB2 is highly expressed in the immune system, emerging evidence points to CB2 playing a key role in regulating neuronal function in the brain, including being expressed on midbrain dopamine neurons. Collectively, these findings have led to the hypothesis that the CB2 receptor could represent a novel therapeutic target for the treatment of schizophrenia.

Methods: A CB2-selective PAM (Ec21a) was tested for antipsychotic-like efficacy in several previously validated assays, including amphetamine-induced disruption of prepulse inhibition (PPI; 20 mice/group). C57BL/6 mice (Taconic) were dosed with either Ec21a (10 mg/kg, i.p.), the M4 PAM VU0467154 (10 mg/kg, i.p.), or vehicle (20% β-CD, i.p.) 30 minutes prior to being dosed with amphetamine (4 mg/kg. s.c.). For some studies, mice were dosed with the CB2 antagonist AM630 (10 mg/kg, i.p.) or vehicle (10% Tween-80) 30 minutes prior to Ec21a to test if the effects were CB2 receptor-dependent. PPI was assessed 30 minutes after the amphetamine dose using a startle pulse (55 dB above background, 40-msec broadband noise pulse) either alone or preceded by 50 msec by a prepulse at 5, 11, and 17 dB above background. We also assessed the ability of Ec21a and VU0467154 to alter psychostimulant-induced hyperlocomotion (20 mice per group). For these studies, mice were habituated to an open filed environment for 90 minutes prior to dosing with Ec21a, VU0467154, or vehicle as outlined above and then 30 minutes later were dosed with either vehicle, amphetamine (2 mg/kg, s.c.), or MK801 (0.2 mg/kg, s.c.). Locomotion was tracked for an hour after amphetamine or MK801 hyperlocomotion.

Results: Administration of Ec21a significantly reversed amphetamine-disrupted PPI at all three of the prepulse intensities examined ($p < 0.05$ between vehicle/amphetamine group compared to the Ec21a/ amphetamine group via a One-way ANOVA followed by a Dunnett's multiple comparisons test). This effect was blocked by pretreatment with the CB2 antagonist AM630 indicating that the Ec21a-mediated effects were CB2-dependent. Dosing with Ec21a also significantly reversed both amphetamine- and MK801-induced hyperlocomotion ($p < 0.05$ between vehicle/psychostimulant group compared to the Ec21a / psychostimulant group via a One-way ANOVA followed by a Dunnett's multiple comparisons test).

Conclusions: The ability of Ec21a to mediate antipsychotic-like efficacy in numerous behavioral assays suggests that potentiating CB2 receptor signaling could represent a novel strategy for treating schizophrenia. Ongoing and future studies will be performed with CB2f/f mice to generate tissue selective knockouts to determine what populations of CB2 receptors mediate these antipsychotic effects. These studies will test the hypothesis that CB2 receptors expressed on dopamine neurons may mediate antipsychotic efficacy by regulating dopamine signaling.

Keywords: Neuropsychiatric Disorders, CB2 Receptor, Preclinical

Disclosure: Nothing to disclose.

P561. Agonist Activation of Orphan Receptor GPR52 Opposes Dopamine D2 Receptor Signaling and Neuromodulation in the Nucleus Accumbens

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Background: GPR52 is an orphan G protein-coupled receptor and recently identified GWAS schizophrenia risk gene. GPR52 is a Gs/olf class receptor that activates cAMP signaling and it is expressed primarily in the ventral striatum of the human brain in dopamine D2 pathway medium spiny neurons (D2 MSNs). This unique brain expression profile of GPR52 suggests the receptor may functionally regulate cAMP signaling to oppose the signaling of dopamine D2 receptors (D2R). This distinguishes GPR52 as an attractive, druggable target for psychiatric disorders including schizophrenia and substance use disorders. Here we utilize a novel GPR52 selective agonist to elucidate GPR52 neuronal signaling in the nucleus accumbens (NAc) and GPR52 functional crosstalk with D2 receptors to modulate D2 MSN excitability.

Methods: To examine potential signaling crosstalk between GPR52 and D2R, human GPR52 and D2 receptors were co-expressed in HEK293 cells, or in cells lacking Gs/olf G proteins, and agonists or antagonists for both receptors were examined for cAMP signaling in living cells (Glosensor assay). Primary rat striatal neurons were cultured and agonist activation of GPR52 to modulate neuronal cAMP levels was determined using a cAMP HTRF assay (Cisbio). To determine if GPR52 activation modulates NAc MSN excitability and D2R signaling responses, a whole cell patch clamp study was performed measuring evoked action potentials (APs) in D1 or D2 MSNs using mouse NAc brain slices ($n = 6$-12 cells analyzed per treatment group).

Results: In molecular signaling studies, expression of human GPR52 in wildtype HEK293 cells elevated basal cAMP levels by over 100 fold, with further elevation of cAMP in response to the selective GPR52 agonist PW0787, which was eliminated by stable knockout of Gs/olf G proteins using CRISPR/Cas9 genome editing. Due to this robust GPR52/Gs/cAMP signaling, we hypothesized GPR52 activation would crosstalk and modulate Gi/o-coupled cAMP inhibition by the D2R. Expression of D2R alone in HEK293 cells produced low basal cAMP levels (~4000 light counts/sec, Glosensor assay), and treatments with agonist quinpirole and antagonist haloperidol both yielded minimal changes to cAMP levels (~1000-2000 light counts/sec). However, co-expression of GPR52 with D2R substantially increased basal cAMP levels which allowed a larger window of agonism by the D2R agonist quinpirole (decrease of ~500,000 light counts/sec) and revealed robust inverse agonism by D2R antagonist haloperidol (increase of ~150,000 light counts/sec). Agonist activation of GPR52 by PW0787 in cultured rat striatal neurons also significantly elevated cAMP levels indicating Gs/cAMP pathway signaling. In whole cell patch clamp studies using mouse NAc brain slices, the GPR52 agonist PW0787 significantly increased the frequency and number of evoked action potentials in D2, but not D1 MSNs. In addition, PW0787 reversed the inhibitory effect of the D2 agonist quinpirole on evoked APs, indicating GPR52 activation functionally opposes D2R signaling and D2R neuromodulation in NAc MSNs.

Conclusions: Taken together, these findings indicate the schizophrenia risk gene GPR52, via activating Gs/Golf cAMP signaling, is an excitatory receptor selectively expressed in D2 MSNs. GPR52 functionally opposes D2R signaling via the cAMP pathway and reverses inhibitory neuromodulation by the D2R in the NAc. These findings characterize the neuropharmacology of the novel, and selective, GPR52 agonist PW0787 as a valuable tool to study GPR52 activation in both health and disease. Functional alterations in GPR52 cAMP signaling and crosstalk with D2R

signaling in the NAc may also help explain why GPR52 is a schizophrenia risk gene. These findings further support that GPR52 is a druggable target with therapeutic potential for treating neuropsychiatric disorders involving dysregulated NAc signaling, with therapeutic possibilities for treating psychosis and substance use disorders.

Keywords: Orphan Receptors, Schizophrenia, Dopamine, Antipsychotics, cAMP Signalling, Nucleus Accumbens Core, Dopamine D2 Receptors

Disclosures: MapLight Therapeutics: Contracted Research (Self). Sensorium Therapeutics: Consultant (Self).

P562. Pharmacological Characterization of Potent Human Trace Amine-Associated Receptor 1 (TAAR1) Agonists

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Background: TAAR1 is a promising novel drug target for psychosis and schizophrenia treatment, with several developed agonists entering clinical trials. Pharmacological characterization of these compounds may aid in understanding the difference in their clinical efficacy and side effects and in the development of additional TAAR1 agonists.

Methods: The pharmacological profile of two such candidates, Ulotaront and Ralmitaront, are expanded on using a combination of in vitro second messenger, G-protein/β -arrestin recruitment and GIRK channel activation assays using heterologous overexpression systems. We further use computational models of TAAR1 for developing highly potent TAAR1 agonists and evaluate their selectivity profile using PRESTO-TANGO screening.

Results: Compared to Ulotaront, Ralmitaront showed lower efficacy at TAAR1 in G protein recruitment (81% ± 1%, n = 3), cAMP accumulation (60% ± 13%, n = 3), and GIRK activation (69% ± 12%, n = 3-6) assays. Furthermore, Ralmitaront displayed slow receptor activation kinetics in each of these assays, reaching a steady state 10 – 50 minutes after Ulotaront, depending on the assay used. Likewise, the most potent TAAR1 ligands designed by structure-based modeling were more potent than either Ulotaront or Ralmitaront, as assessed by our unique TAAR1 G-protein recruitment assay. These compounds displayed a similar receptor interaction pattern as Ulotaront, which was evaluated using a PRESTO-TANGO screen comprising all human aminergic receptors. Specifically, our new potent TAAR1 agonists were observed to partially or fully activate the HTR1A, HTR1D, ADRA2A and the dopamine D2 receptors.

Conclusions: We specifically identify ligand kinetics and receptor selectivity of TAAR1 agonists as key features to consider for clinical development in the treatment of psychiatric disorders, particularly schizophrenia.

Keywords: GPCR, TAAR 1, Schizophrenia- Novel Treatment

Disclosure: Nothing to disclose.

P563. Reduced Slow Wave Density is Associated With Worse Psychotic Experiences in Individuals at Clinical High Risk for Psychosis

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Background: Subjective sleep disturbances have been consistently reported in individuals at clinical high risk for psychosis (CHR). Furthermore, we recently demonstrated objective sleep abnormalities, including increased gamma activity and reduced sleep spindle duration in CHR individuals compared to healthy control (HC) subjects. Besides spindles, slow waves are the main oscillations occurring during NREM sleep. Deficits in sleep slow wave density have been reported in early-stage psychotic patients and were associated with the severity of their positive symptoms. However, the presence of slow wave abnormalities and their relationship with positive symptoms has not been examined in CHR individuals.

Methods: Overnight high-density (hd)-EEG recordings were collected in 37 CHR individuals and 32 HC subjects. To analyze and characterize slow wave activity (SWA) features, a customized algorithm was applied for the automatic detection of various slow wave parameters. To detect slow waves, the EEG signal was re-referenced to the average of the two mastoid electrodes and band-pass filtered at 0.5–4.0 Hz. Slow waves were identified as negative deflections occurring between two zero crossings and were selected based on their period. Specifically, only waves with consecutive zero crossings spanning 0.25 to 1.0 seconds, detected in artifact-free NREM epochs were considered as slow waves. SWA and several other slow wave parameters, including density, amplitude, up- and down-slopes were computed and compared across CHR and HC groups. Furthermore, in CHR individuals the Scale of Prodromal Symptoms (SOPS) positive symptoms assessment and the scale for the assessment of the positive symptoms (SAPS), which provides a more comprehensive evaluation of positive symptoms were collected.

Results: Compared to HC subjects, CHR individuals had a significantly higher sleep onset latency; $p = 0.031$, t-stat = 2.200 as well as a lower sleep efficiency, $p = 0.017$, t-stat = −2.448. CHR also had a higher percentage of wake after sleep onset (WASO), with a trend towards significance ($p = 0.085$, t-stat = 1.743). No differences in any other sleep architecture parameters were found between the two study groups. Topographic analyses show no differences in SWA in any EEG electrodes between CHR and HC individuals. Also, no significant differences were found in any of the slow-wave measures when comparing the two study groups. However, when examining two CHR subgroups based on positive symptom severity, we found that CHR individuals with higher positive symptoms showed lower slow wave density compared to their matching HC counterparts, whereas no significant differences in slow wave density were observed between CHR individuals with low positive symptoms and matched HC individuals. Furthermore, we found a significant negative correlation between the average slow wave density and the sum z-score of SAPS and SOPS-PS ($R = 0.47$, $p = 0.003$).

Conclusions: Altogether, these findings indicate that reduced sleep slow wave density may represent a neurophysiological biomarker reflecting the severity of symptoms in individuals at risk for schizophrenia and related psychotic disorders.

Keywords: Clinical High Risk for Psychosis, Slow-Wave Sleep, High Density Sleep EEG, Positive Symptoms

Disclosure: Nothing to disclose.

P564. Cross-Sectional and Longitudinal Analysis of Cognitive Deficits Among People Aging With Schizophrenia: Associations With Mental health, Physical Health, and Aging Biomarkers

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Background: Cognitive functioning is a key predictor of disability and functioning among people with schizophrenia (PwS). Cognitive deficits occur during the premorbid phase with additional declines at the onset of psychosis. As they age, PwS have non-linear progression of cognitive deficits with a 10-20 fold higher rate of dementia compared to the general population.

This study evaluated cognitive functioning among a cohort of 155 community dwelling individuals with and without schizophrenia, with follow-up in a subset (n = 54) over 2.7 years (range 1.5-7 years). We hypothesized that 1) adults with cognitive impairments will have worse mental and physical health compared to non-impaired participants and 2) having cognitive impairments will be associated with worse aging biomarkers. We explored longitudinal stability of cognitive deficits.

Methods: We included 92 PwS (mean age 49.7 ± 10.3) and 63 NCs (mean age 52.3 ± 12.2), age range 27-70 years. PwS and NC groups were age- and sex-comparable (51% women), with similar racial background. PwS had fewer years of education. All participants completed assessments for clinical symptoms (SAPS and SANS, Calgary Depression Scale), antipsychotic dosage, physical health (comorbidities, BMI), and assays for oxidative stress (F2-isoprostanes) and inflammatory cytokines. Trained staff administered the MATRICS Cognitive Consensus Battery (speed of processing, attention/vigilance, verbal learning, working memory, reasoning/problem solving, social cognition) and the Delis-Kaplan Executive Functioning System (DKEFS, executive functioning).

We calculated deficit scores based on MATRICS and DKEFS t-scores to quantify level of impairment (0 = "no impairment" to 5 = "severe impairment") across domains. This approach more accurately assesses deficits, i.e., above average scores in one domain cannot cancel out below average performance in another. We compared sociodemographic, mental and physical health, and biomarker levels between the impaired and non-impaired groups. In a subset, we used Spearman's correlations to examine the relationships between baseline and follow-up cognitive functioning. We present Cohen's d effect sizes.

Results: Among NCs, 22% had global cognitive impairment (5% processing speed, 26% attention/vigilance, 22% verbal learning, 18% working memory, 11% reasoning/problem-solving, 18% social cognition, 0% executive functioning). Global impairment among NCs was associated with lower functioning (d = −.87) and more physical comorbidities (d = .49). Compared to the non-impaired NC group, the impaired NCs had a trend for being older ($p = 0.06$, d = 0.58). The two groups had similar sex, education level, depressive symptoms, and BMI.

Among PwS, 71% (n = 65) had global cognitive impairment (44% had impaired processing speed, 55% attention/vigilance, 64% verbal learning, 50% working memory, 32% reasoning/problem solving, 65% social cognition, 29% executive functioning). Compared to the non-impaired PwS group, the impaired PwS had similar age, sex, and education level. The two subgroups had similar positive, negative, and depressive symptoms; daily antipsychotic dosages; physical comorbidities; and BMI. The impaired subgroup had F2-isoprostane levels ($t(40.8) = 2.03$, $p = .049$, d = .53), with a trend toward higher interleukin-6 levels ($t(43) = 1.75$, $p = .094$, d = .53).

For the subset of 39 PwS with follow-up cognitive assessments, baseline and follow-up cognitive functioning were highly correlated (Spearman's r = .55-.85, p's < .001).

Conclusions: We found high rates of cognitive impairment among community-dwelling PwS. Impairments were associated with cardiovascular disease, oxidative stress, and inflammation –all potential mechanisms of neurodegeneration among PwS.

Keywords: Psychosis, Cognitive Impairment, Oxidative Stress, Inflammation

Disclosure: Nothing to disclose.

P565. AMPA Receptor Density Dependent Local and Global Functional Network Are Associated With the Significant Difference Between Healthy Subjects and Patients With Schizophrenia

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Background: Neurobiological factors that determine the degree or strength of functional connectivity measured with resting-state functional MRI (rsfMRI) remain unknown. To address these challenges, we utilized [11C]K-2 PET that was developed to visualize AMPA receptors in the living humans since these receptors have been identified as integral to the pathology of psychiatric disorders in animal studies. Concurrently, we applied local functional connectivity density (lFCD) and global functional connectivity density (gFCD) metrics, representing brain functional hubness in proximal and distal regions of each voxel. The aim of this study was to test the hypothesis that an increase in AMPA receptor density corresponds to higher lFCD and gFCD. Additionally, we explored the difference in the relationship between AMPA receptor density and these indices in both healthy subjects and those with schizophrenia.

Methods: [11C]K-2 PET and rsfMRI scans were performed in 31 healthy subjects (the healthy controls) and 14 patients with schizophrenia (the schizophrenia patients). Standard Uptake Value Ratio (SUVR) was employed as a metric for AMPA receptor density, with white matter serving as the reference region, and both SUVR and rsfMRI images were normalized to MNI coordinates in the same manner. RsfMRI data were processed to extract lFCD and gFCD values across all brain regions, Yeo's 7 network Atlas regions, and 83 regions from the Hammers-Smith Atlas. Subsequently, in each region of these ROIs or networks, the voxel-wise SUVR-lFCD and SUVR-gFCD correlations were ascertained using Spearman's rank correlation coefficient test. The correlations between SUVR, and lFCD, and gFCD within the anterior cingulate gyrus were contrasted between the healthy controls and the schizophrenia patients.

Results: In the healthy controls, the average lFCD correlation coefficients were 0.39 in the whole brain, 0.44 in the Default Mode Network (DMN), and 0.56 in the Visual Network (VN), 0.76 in the anterior cingulate gyrus (ACC), 0.67 in the posterior cingulate gyrus, 0.56 in the pre-subgenual ACC, and 0.52 in the lateral occipital lobe, while in the schizophrenia patients those values were 0.33 in the whole brain, 0.40 in the DMN, 0.47 in the VN, 0.66 in the anterior cingulate gyrus, 0.69 in the posterior cingulate gyrus, 0.61 in the pre-subgenual ACC and 0.47 in the lateral occipital lobe. The mean gFCD correlation coefficients of the healthy controls were 0.47 in the whole brain, 0.55 in the DMN, 0.55 in the VN, 0.79 in the anterior cingulate gyrus, 0.73 in the posterior cingulate gyrus, 0.76 in the anterior knee, 0.66 in the caudate nucleus, 0.65 in the inferior knee, 0.64 in the globus pallidum, 0.52 in the lateral occipital lobes. On the other hand, those of the schizophrenia patients were 0.33 for whole brain, 0.40 for DMN, 0.47 for VN, 0.66 for anterior cingulate gyrus, 0.77 for posterior cingulate gyrus, 0.77 for pre-subgenual ACC, 0.70 for caudate nucleus, 0.65 for subgenual ACC, 0.63 for globus pallidum, and 0.52 for lateral occipital lobe. Nonlinear least squares modeling revealed distinct patterns in lFCD and gFCD relative to SUVR in both the left and right ACC within healthy subjects. Conversely, the schizophrenia patients demonstrated varied trends, including a decreasing trend of increase for lFCD and a monotonically decreasing trend for gFCD in certain SUVR ranges.

The relationships between SUVR and lFCD/gFCD in the right and left ACCs were significantly different between the healthy controls and schizophrenia patients (all p-values < 0.0001).

Conclusions: The present study found a positive correlation between AMPA receptor density and functional connectivity hubness in a wide range of brain regions, and in light of proximal hubness, especially highly correlated in regions whose hubness degree has been reported to be high. Furthermore, the functional connectivity hubness specification according to AMPA receptor density in the ACC was significantly different in the schizophrenia patients compared to the healthy controls.

Keywords: AMPA Receptors, PET Imaging Study, rsfMRI Functional Connectivity, Schizophrenia (SCZ), Resting State Networks

Disclosure: Nothing to disclose.

P566. Evaluation of M4 Muscarinic Receptor Occupancy by Emraclidine Using [11C]MK-6884 PET in Healthy Volunteers

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Background: Emraclidine is a novel, highly-selective M4 muscarinic acetylcholine receptor-positive allosteric modulator currently in development for the treatment of schizophrenia and Alzheimer's disease psychosis (Krystal et al. Lancet. 2022;400:2210-2220). Preclinical characterization of emraclidine in rodents showed favorable brain penetration, direct target engagement, and robust in vivo activity in animal models of psychosis, including reversal of amphetamine-stimulated locomotion and prepulse inhibition. Furthermore, in vivo target engagement and an overall exposure-occupancy relationship of emraclidine was recently confirmed using the [11C]MK-6884 radioligand in nonhuman primate positron emission tomography (PET) imaging studies evaluating M4 receptor occupancy (RO) as a function of emraclidine dose and plasma concentration (Duvvuri et al. Presented at ACNP 2021). Here, we report RO data for emraclidine obtained in a human healthy volunteer PET study.

Methods: This phase 1 open-label trial (NCT04787302) enrolled healthy adult volunteers in an adaptive study design across 3 cohorts (n = 3 per cohort). Participants in the first cohort received a single 30-mg dose of emraclidine (maximal dose, anticipated to achieve ~80-90% M4 RO based on in vitro binding affinity data). M4 RO from each cohort informed dose selection in the subsequent cohort. All participants received a baseline PET/magnetic resonance imaging (MRI) scan up to one week prior to emraclidine dosing and a second PET/MRI scan immediately after dosing using 370 MBq [11C]MK-6884 intravenous administration and 90-minute scanning with arterial blood sampling and metabolite analysis for full tracer pharmacokinetic (PK) modeling. Blood samples for emraclidine PK analysis were obtained at 30, 60, and 90 minutes after emraclidine dosing during the PET scan. The primary endpoint was estimation of emraclidine RO in the striatum (averaging values from caudate and putamen), with the cerebellum as a reference region. The overall M4 RO (%) was calculated as the difference from baseline in M4 nondisplaceable binding potential (BPnd) after emraclidine dosing (BPnd [baseline] and BPnd [dose]) divided by BPnd [baseline]. The relationship between M4 RO and emraclidine plasma exposure was assessed using an exposure-response model informed by the maximum estimated RO (Emax), emraclidine plasma concentration (C), and the concentration at which 50% of maximum RO is achieved (EC50), assuming a Hill coefficient (γ) of 1: $RO = (Emax \times C\hat{}\gamma)/(EC50\hat{}\gamma + C\hat{}\gamma)$

Results: Preliminary results suggest target engagement of emraclidine at the M4 receptors in caudate and putamen as evidenced by displacement of [11C]MK-6884 by emraclidine at the maximum dose. Additional results at lower doses (emraclidine 15 mg and 5 mg) and exposure/dose responsiveness of M4 occupancy will be informed by the forthcoming study results.

Conclusions: These data confirm the target binding of emraclidine to M4 receptors in the striatum of healthy volunteers and corroborate recent findings in primates using the same radiotracer.

Keywords: Emraclidine, Muscarinic M4, Schizophrenia, Positron Emission Tomography

Disclosure: Cerevel Therapeutics: Employee, Stock/Equity (Self).

P567. Cortical White Matter Microstructural Alterations Underlying Impaired Gamma-Band Auditory Steady-State Response in Schizophrenia

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Background: Gamma-band auditory-steady state response (ASSR), primarily generated from the auditory cortex, has received significant attention as a potential brain marker to investigate pathophysiology of schizophrenia. Previous studies have shown reduced gamma-band ASSR in patients with schizophrenia and its correlations with impaired neurocognition and psychosocial functioning. Recent studies from clinical and healthy populations suggested that the neural substrates of reduced gamma-band ASSR may be more widespread in the cortices surrounding the auditory cortex especially in the right hemisphere. This study aimed to investigate associations between gamma-band ASSR and white matter alterations in the bundles broadly connecting the right frontal, parietal and occipital cortices to clarify the networks underlying reduced gamma-band ASSR in schizophrenia.

Methods: 40 Hz ASSR measured by electroencephalography and diffusion tensor imaging of 42 patients with schizophrenia and 22 healthy comparison subjects were obtained in this study. Intertrial coherence was used as an index of gamma-band ASSR, and fractional anisotropy was used as an index of DTI.

Results: Gamma-band ASSR was reduced in patients ($t = 1.7$, $p = 0.049$, $d = 0.44$). The fractional anisotropy as white matter integrity index in the regions connecting the right frontal, parietal and occipital cortices was reduced in patients ($\beta = -0.36$, $p < 0.001$). Gamma-band ASSR was positively correlated with the fractional anisotropy only in healthy subjects ($\beta = 0.41$, $p = 0.038$), but not in patients ($\beta = 0.17$, $p = 0.23$).

Conclusions: These findings coincide with our hypothesis that the generation of gamma-band ASSR is supported by white matter bundles that broadly connect the cortices and that the relationships may be disrupted in schizophrenia. Our study may further help characterize and interpret reduced gamma-band ASSR as a useful brain marker in the pathophysiology of schizophrenia.

Keywords: Diffusion Tensor Imaging (DTI), Electroencephalography (EEG), Gamma-Band Auditory Steady-State Response (ASSR), Schizophrenia, White Matter Microstructural Alteration

Disclosure: Nothing to disclose.

P568. Acoustic Startle and Mismatch Negativity Impairments in 22q11.2 Deletion Syndrome

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Background: 22q11.2 deletion syndrome (22q11DS) is one of the most robust genetic predictors of the development of psychosis and other psychiatric illnesses. In this study, we examined the performance of 22q11DS subjects on two putative psychophysiological biomarkers of psychosis risk. Latency of the acoustic startle response (ASR) is a putative marker of neural processing speed and is prolonged (slower) in schizophrenia. Mismatch negativity (MMN) is an auditory evoked potential in response to unusual or "oddball" acoustic stimuli embedded within a train of repetitive acoustic stimuli and indexes brain processing of novel stimuli. ASR latency and MMN are impaired in schizophrenia, associated with an increased risk of psychosis, and dependent on NMDA receptor signaling. These electrophysiological assessments could serve as translational biomarkers in interventions for groups at high risk for psychosis.

Methods: We recruited 30 22q12DS subjects (mean age = 29, M/F = 17/13) and 32 healthy comparison subjects (HCs; mean age = 32, M/F = 14/18) from the community. Subjects completed a comprehensive cognitive battery and clinical symptom assessments. Startle magnitude, latency, and prepulse inhibition were assessed with a standard acoustic startle paradigm. Subjects with significant hearing loss were excluded. The eyeblink component of the startle response was recorded using surface electrodes placed on the orbicularis oculi to capture the electromyographic signal. Startling stimuli were 115dB 40 msec white noise bursts, and non-startling stimuli were 85dB 20 msec white noise bursts delivered through headphones. The startle session began and ended with habituation blocks consisting of six pulse-alone trials. Block 2 consisted of 4 trial types presented in pseudorandom order: pulse alone trials, and pre-pulse trials that consisted of a non-startling stimulus presented 30, 60, or 120 msec prior to the startling stimuli (9 of each trial type). After data processing and cleaning, acoustic startle latency for each trial type and amplitude to pulse-alone trials were log-transformed. Individuals were classified as being a startler if they had measurable startle blinks in at least four out of the first six trials in the first habitation block; otherwise they were classified as a non-startler. For the auditory MMN task, each participant completed the Double-Deviant target detection paradigm, which presents a pseudorandom sequence of frequent standard tones (85% of trials; 633 Hz and 50 ms) and three types of infrequent deviant tones (15% of trials). Deviants differed from standards based on Frequency (1000 Hz), Duration (100 ms) or both (1000 Hz and 100 ms). MMN signals were recorded by EEG from the Fz electrode for the standards, frequency, duration, and double deviant trials. MMN responses were generated by subtracting the average of the standard trials from the average of each deviant trial types.

Results: Across the three blocks of the startle session, the magnitude of the acoustic startle response to pulse-alone trials was robustly lower in 22q11DS subjects than HC (repeated measures ANOVA with age, sex, hearing acuity as covariates: $F(1,54) = 4.02$, $p = 0.05$). A post hoc pairwise comparison for Block 2 for pulse-alone trials were significant for lower magnitude in 22q11DS subjects ($p = 0.041$). 22q11DS subjects also had a significantly higher proportion of non-startlers than HC subjects ($\chi 2 = 6.46$; $p = 0.011$).

Onset latency of the startle responses to pulse-alone stimuli in the initial habituation block was significantly slower in 22q11DS than HC subjects (ANOVA; $F(1,45) = 5.27$, $p = 0.026$; age, sex, hearing acuity and pulse-alone magnitude included as covariates). Latency to other trial types and prepulse inhibition could not be analyzed because the 22q11DS subjects had inadequate blink responses to these other trial types consistent with their overall lower startle magnitudes.

Individuals with 22q11DS showed significantly smaller absolute magnitudes of evoked responses to the Duration Deviant trials from 270-290 ms (Group x Sex ANOVA; $F(1,61) = 4.33$, $p = .042$; age entered as a covariate) and the Double Deviant trials from 250-330 ms (Group x Sex ANOVA; $F(1,61) = 6.525$, $p = .013$; age entered as a covariate).

Conclusions: Our results of reduced magnitude and slow latency of startle responses and reduced EEG-evoked responses to oddball stimuli in 22q11DS subjects suggest reduced central nervous system and neuronal responsiveness. These findings are consistent with robust cognitive impairments observed in 22q11DS subjects. Further research is needed to untangle the complex relationship between basic NMDA dysfunction, psychophysiological responsiveness, and cognitive impairment.

Keywords: 22q11 Deletion Syndrome, Mismatch Negativity, Acoustic Startle

Disclosure: Nothing to disclose.

P569. Characterization of Four Major Psychiatric Disorders Based on AMPA Receptor Distributions Measured With [11C]K-2: A Novel PET Tracer Study

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Background: Synaptic phenotypes in living patients with psychiatric disorders are poorly characterized. Excitatory glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) is a fundamental component for neurotransmission. We recently developed a positron emission tomography (PET) tracer for AMPAR, [11C]K-2, the first technology to visualize AMPARs in living human brain.

Methods: One hundred forty-nine patients with psychiatric disorders (schizophrenia, n = 42; bipolar disorder, n = 37; depression, n = 35; and autism spectrum disorder, n = 35) and 70 healthy participants underwent a PET scan with [11C]K-2 for measurement of AMPAR density. Brain regions with correlation between AMPAR density and symptomatology scores of each disease and those that showed differences in AMPAR density between patients and healthy participants were identified by using a voxel-wise analysis.

Results: Specific brain regions that showed correlation between AMPAR density and symptomatology scores were detected in each of four disorders. We also found brain areas with significant differences in AMPAR density between patients with each psychiatric disorder and healthy participants. Some of these areas were observed across diseases, indicating that these are commonly affected areas throughout psychiatric disorders.

Conclusions: Schizophrenia, bipolar disorder, depression, and autism spectrum disorder are uniquely characterized by AMPAR distribution patterns. Moreover, the presence of the commonly affected regions in comparison to healthy controls suggests that these disorders may share the biological characteristic in terms of

AMPAR. Our approach to psychiatric disorders using [11C]K-2 can elucidate the biological mechanisms across diseases and pave the way to develop novel diagnostics and therapeutics based on the synapse physiology.

Keywords: AMPA Receptors, PET Imaging, Schizophrenia (SCZ), ASD, Major Depressive Disorder

Disclosure: Nothing to disclose.

P570. Poster Withdrawn

P571. Biomarkers of Cognitive Dysfunction in Schizophrenia: Using TMS-EEG to Assess Prefrontal Oscillatory Deficits in Early-Course Patients

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Background: Accumulating evidence suggests that patients with schizophrenia have a reduced ability to generate fast oscillatory activity in the dorsolateral prefrontal cortex (DLPFC). It is believed that this deficit underlies some of the cognitive dysfunctions commonly observed in these subjects. Chronic patients with schizophrenia have a reduction in several oscillatory parameters evoked by transcranial magnetic stimulation (TMS) and simultaneously recorded with electroencephalography (TMS-EEG) in the DLPFC, with the main oscillatory frequency (or "natural frequency") showing the largest reduction compared to healthy controls (HCs). However, it remains to be established whether a slowing of the DLPFC natural frequency is present in the early phases of schizophrenia and is associated with the cognitive dysfunctions of these patients.

Methods: We used TMS-EEG to investigate local oscillatory dynamics elicited by stimulation of the left DLPFC in twenty-five subjects with early-course schizophrenia (ECSCZ, i.e. within three years from the onset of psychosis) and twenty-five age and gender-matched HCs. Single-pulse TMS was delivered at 120% of the resting motor threshold and a neuronavigation system was used to precisely target the DLPFC. State-of-the-art real-time monitoring of the EEG response and noise masking procedures were employed to ensure data quality. In each subject, an automated algorithm was employed to identify the oscillatory frequency with the highest cumulate spectral power, i.e. the natural frequency, at the electrode closest to the stimulation site. Local cortical synchronization following TMS was quantified by calculating the EEG spectral power in each frequency band relative to the broadband power (i.e. the relative spectral power, RSP). Goal-directed working memory performance was assessed using the "AX" Continuous Performance Task (AX-CPT). Group differences were investigated with Wilcoxon ranksum tests. Correlations between neurophysiological and cognitive parameters were performed using Spearman's rhos.

Results: The natural frequency of the left DLPFC was significantly reduced in ECSCZ patients compared to HCs ($p = .58$*10-7), which corresponded to a large effect size (Cohen's $d > 2.0$). Patients also showed a higher frontal left RSP in the beta band relative to HCs ($p = .0003$). The AX-CPT performance was significantly worse in ECSCZ patients ($p = .006$). In ECSCZ patients, the DLPFC natural frequency was inversely correlated to negative symptoms ($\rho = -0.54$; $p = .006$). Furthermore, across all participants, the beta-band RSP in frontal left channels correlated inversely with the AX-CPT performance ($\rho = -0.33$; $p = .043$).

Conclusions: Our results suggest that a reduction in the natural frequency of the prefrontal cortex is an early neural signature associated with schizophrenia. Furthermore, abnormalities in the intrinsic oscillatory properties of the DLPFC appear to reflect worse negative symptoms and goal-directed working memory performance. This evidence may provide background for the development of novel therapeutic strategies targeting these core clinical dimensions of patients with schizophrenia.

Keywords: Schizophrenia (SCZ), TMS, EEG, Cognitive Dysfunction, Negative Symptoms

Disclosure: Nothing to disclose.

P572. Associations Between Nonlinear Dynamics During Rest and Cognitive Functioning in Schizophrenia

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Background: Brain dynamics are inherently nonlinear, however the contribution of this activity to cognitive functioning and relevance to SZ is not well known. During rest, power in the alpha frequency (7-13 Hz), is the strongest activity recorded with EEG and has shown mixed findings in schizophrenia (SZ). Here, we report the extent nonlinear dynamics of resting EEG are associated with cognitive functioning and whether this information is distinct from canonical alpha.

Methods: Patients with SZ ($n = 38$, mean age = 47.8 yrs, M/F 19/18) underwent EEG testing that included two 5 minute recordings of resting state activity with eyes open and eyes closed, respectively. Power spectral densities were calculated to derive estimates of alpha power. Delay Differential Analysis was used to model nonlinear structure directly from the time series. To reject the null hypothesis that nonlinear features estimated from the observed time series were generated by a linear, stochastic process, we compared the observed features with those calculated from surrogate data that preserved the original spectral content. Cognitive domains were assessed via MATRICS Consensus Cognitive Battery and reported as age and sex adjusted t-scores. Exploratory analyses using bootstrapped spearman correlations ([95% CI] reported) and post-hoc linear regression were performed.

Results: Greater evidence for nonlinear dynamics of eyes open rest, but not eyes closed, robustly correlated with multiple cognitive domains (Visual Learning: $r = -.62$ [-.79 -.4]; Verbal Learning: $r = -.47$ [-.66 -.2]; Reasoning and Problem Solving: $r = -0.4$ [-.6 -.17]; Speed of Processing: $r = -.36$ [-.57 -.09]; Working Memory: $r = -.36$ [-.62 -.07]). The only associations for alpha power meeting 95% CI criteria during eyes open rest included Visual Learning: $r = -.46$ [-.64 -.23]. Notably, patients with more apparent nonlinear dynamics during eyes open rest showed lower Visual Learning ($B = -2.9$ $SE = 0.97$, $p = 0.006$) after controlling for alpha power.

Conclusions: Nonlinear dynamics detected during resting state EEG recordings, particularly with eyes open condition, are strongly associated with multiple cognitive domains in patients with SZ. Traditional spectral measures of alpha activity did not show strong associations with cognitive performance during this condition. Nonlinear measures thus provide a unique window for understanding cognitively-relevant functioning in SZ.

Keywords: Nonlinear Analysis, Schizophrenia (SCZ), Electroencephalography (EEG)

Disclosure: Nothing to disclose.

P573. A Novel Circuit Underlying Amotivation in the 22q11DS Model of Schizophrenia

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Background: Schizophrenia is a multifaceted neurodevelopmental disorder characterized by notoriously intractable positive, cognitive, and negative symptom categories. The severity of the negative symptom amotivation is negatively correlated with social and occupational functioning and is not currently met with effective treatment options. Advances in treatment are hampered by a lack of mechanistic understanding of the neural circuits underlying this symptom. Despite being implicated in motivational states, the dorsal striatum is largely overlooked when studying motivated behavior. Further, disruptions in thalamic nuclei are involved in all facets of schizophrenia symptomology, but the role of the thalamus in amotivation remains unknown.

Methods: Here, we use a mouse model of 22q11 deletion syndrome (22q11DS), a genetic disorder in which 30% of individuals develop symptoms that are indistinguishable from idiopathic schizophrenia, to test the role of the thalamostriatal pathway in motivated behavior. We use the Progressive Ratio behavior task to test motivation in female and male 22q11DS mice and wildtype littermates (on average $N = 12$/genotype). To investigate the circuits underlying amotivation we use ex vivo optogenetics with whole-cell patch-clamp electrophysiology (n = 16 cells/5 mice of both sexes per group) and manipulate these circuits in vivo using chemogenetics ($N = 7$/sex).

Results: We find that 22q11DS mice of both sexes display amotivation (Unpaired t test, $^{**}p = 0.0053$) and exhibit weakened excitatory inputs to the dorsomedial striatum (DMS) from the parafascicular nucleus of the thalamus (Pf) (Unpaired t test, $^{*}p = 0.013$). Mimicking the weakened Pf-DMS with chemogenetics in wildtype mice produces the amotivation phenotype (Paired t test, $^{**}p = 0.0075$), causally implicating this circuit in amotivational states. Striatal cholinergic interneurons (CHIs) modulate Pf signaling onto the principal medium spiny neurons of the DMS and provide a basal cholinergic tone in the DMS by firing tonically. We show that, compared to wildtype mice, there are substantially more spontaneously active CHIs in 22q11DS mice (Chi-square test, $^{*}p = 0.03$). Moreover, blocking signaling through acetylcholine receptors rescues the weakened Pf-DMS drive (Unpaired t test, $^{**}p = 0.0012$).

Conclusions: These findings are the first to implicate the Pf-DMS pathway in amotivation symptoms arising in 22q11DS and point to intra-striatal acetylcholine signaling as a possible mechanistic linchpin in the etiology of amotivation.

Keywords: Motivation, Schizophrenia (SCZ), Dorsomedial Striatum, Acetylcholine, 22q11 Deletion Syndrome

Disclosure: Nothing to disclose.

P574. Striatal and Dopaminergic Potentiation Regulates Auditory Fear Learning

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Background: Dopamine and its striatal counterparts has been shown to play a central role in learning. Yet, it is unclear how these circuits interface with classic sensory neural pathways for sensory associative learning. The tail portion of the striatum, the auditory striatum, receives a variety of sensory and prefrontal inputs and has been shown to be involved in tone-reward associations. However, whether and how the auditory striatum is integrated into aversive learning circuits has yet to be investigated. In order to explore this, we used a combination of viral tracing, live fluorescent cellular and dopamine microendoscopic imaging, and optogenetic interventions to dissect the role of the auditory striatum in an auditory fear conditioning paradigm.

Methods: All animal procedures were approved by the Stony Brook University Animal Care and Use Committee. All animal procedures were further conducted in accordance with U.S. National Institutes of Health standards. C57BL/6J (The Jackson Laboratory) mice were used for this study. Both male and female 2-4-month-old mice were used.

The auditory fear conditioning paradigm consisted of a single habituation-conditioning session followed by two probe sessions 24 and 48 h afterward, respectively. Fear conditioning and probe sessions occurred in Context A and Context B within a sound-attenuating box (Ashburn, VA). Context A and B were differentiated by distinct patterns on chamber walls and different floor textures. All data and videos were collected via an automated video processing system (Freezeframe, Actimetrics). Experimenters were blinded to all ablation and intervention conditions. For optogenetic interventions, lasers with calibrated power settings (approximately 10 mW at fiber tip) were used to deliver pulses of light during tone presentation.

For live imaging studies of the auditory striatum, mice underwent simultaneous GCaMP6f (AAV2/9-CAG-GCaMP6f virus, n = 6 mice) or DA2m (AAV2/9-CAG-DA2m virus, n = 6 mice) viral infusion and GRIN lens implantation in the auditory striatum. Continuous imaging was performed for animals undergoing fear conditioning. For optogenetic studies, mice underwent simultaneous optic fiber implantation and ArchT (AAV2/9-CAG-DIO-ArchT-GFP) infusion to either the auditory striatum (n = 4 mice) or the substantia nigra pars compacta (n = 4 mice). For control, a similar surgery was performed, except with the expression of GFP instead (AAV2/9-CAG-EGFP, n = 4 mice for auditory striatum intervention; n = 4 mice for substantia nigra pars compacta intervention). For genetic ablation studies, a caspase approach was used to target the auditory striatum (AAV2/5-EF1a-DIO-taCasp3-TEVp). Both normal and non-normal distributions were found for this study and when appropriate, unpaired Student t-tests, Mann-Whitney test, and Wilcoxon rank sum tests were used.

Results: We found that optogenetically inhibiting auditory striatal neurons during tone presentation impaired subsequent freezing behavior and thus fear memory formation (n = 4 mice per control and intervention group, unpaired Mann-Whitney test, $p < 0.0001$). Genetic ablation of amygdala-projecting auditory striatal neurons also decreased freezing behavior of animals subjected to conditioning (n = 4 mice per control and ablation group, unpaired Mann-Whitney test, $p < 0.0001$). Using calcium imaging in behaving mice, we found that auditory striatal neuronal responses to conditioned tones were potentiated across memory acquisition and expression (n = 262 neuronal ROI from six mice, Wilcoxon Rank-sum test, $p < 0.001$). Furthermore, nigrostriatal dopaminergic projections also displayed heighted responses across auditory fear conditioning (n = 6 separate field-of-view recordings across six mice, Wilcoxon Rank-sum test, $p < 0.001$). Optogenetic inhibition of this nigrostriatal pathway during tone presentation decreased freezing behavior (Unpaired Mann-Whitney test, $p < 0.01$).

Conclusions: We found that the auditory striatum is necessary for auditory fear memory acquisition. Additionally, we demonstrate that the auditory striatum appears to conduct this behavior through a novel striatal-amygdala pathway. Furthermore, the neurons in the auditory striatum and its dopaminergic inputs

appear to display neuronal potentiation across learning, akin to other brain regions known to be involved in memory formation. Together, these findings demonstrate the neural circuit and physiological properties of the auditory striatum in mediating auditory fear learning and memory formation.

Keywords: Auditory Striatum, Dopamine, Auditory Fear Conditioning, Auditory Perception, Basolateral Amygdala

Disclosure: Nothing to disclose.

P575. Adolescent Stress Induces Behavioral Changes and Hippocampal Excitatory/Inhibitory Imbalance: Involvement of Redox Dysregulation and Mitochondrial Dysfunction

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Background: The developing adolescent brain is highly susceptible to social experiences and environmental insults, influencing how personality traits emerge. We previously found that adolescent stress leads to long-lasting behavioral changes and excitatory/inhibitory (E/I) balance dysregulation in the ventral hippocampus (vHip) associated with neurodevelopmental disorders, such as schizophrenia and bipolar disorder. The neurobiological mechanisms of psychiatric disorders have been linked with oxidative damage and reduced antioxidant capacity in the brain. However, the impact of severe stressors during adolescence, a critical neurodevelopmental period, on mitochondrial function, redox balance, and their functional consequences are not completely understood. We hypothesized that mitochondrial respiratory function and redox homeostasis in the vHip are affected by adolescent stress, leading to behavioral and electrophysiological changes associated with psychiatric disorders.

Methods: We performed a behavioral characterization during late adolescence (postnatal day, PND 47 – 50), including naïve animals and animals exposed to stress from PND 31 until 40 (10 days of footshock and 3 restraint sessions) by assessing sociability (social interaction test) and cognition function (novel-object recognition test). Then, we uncovered changes in E/I balance by analyzing the activity of glutamate pyramidal neurons, and the number of parvalbumin (PV)-containing GABAergic interneurons and their possible association with oxidative stress. To address the dynamic impact of stress on mitochondrial redox homeostasis, we performed high-resolution respirometry, DHE staining, MitoSox™ and AmplexRed® assays one (PND 41) and ten days (PND 51) after stress protocol. Also, we evaluated glutathione (GSH) and glutathione disulfide (GSSG) levels at PND 51. Finally, we assess the genome-wide transcriptomic signature of vHip of stressed animals by performing a bulk RNA-sequencing following the behavioral tests.

Results: One week after stress, adolescent-stressed animals exhibited: (1) loss of sociability and cognitive impairment; (2) enhanced vHip pyramidal neuron activity; and (3) reduction in the number of PV-positive cells and their associated perineuronal nets. These changes were associated with an increased marker of oxidative stress in the vHip, in which was co-localized with PV interneurons. By performing high-resolution respirometry analysis, we found that stress impacted mitochondrial uncoupled efficiency (PND 41) and the phosphorylation capacity (PND 51). In addition, stressed animals displayed long-lasting redox dysregulation in the vHip, as revealed by molecular analysis. GSSG levels were increased in the vHip and serum of stressed animals and negatively correlated with social and cognitive performance, indicating that GSH was previously oxidized by ROS in stress conditions, and may affect behavioral phenotype. In another cohort of animals, we identified three cluster subgroups by performing principal component analysis of behavioral assessment: naïve higher-behavioral z-score (HBZ), naïve lower-behavioral z-score (LBZ), and stressed animals. Genes encoding subunits of oxidative phosphorylation complexes were significantly down-regulated in both naïve LBZ (Cox7c) and stressed animals (Coa5), while the Txnip gene that encoded thioredoxin-interacting protein were up-regulated in stressed animals and negatively correlated with behavioral performance.

Conclusions: Our results identify mitochondrial genes associated with distinct adolescent behavioral phenotypes and highlight the negative impact of adolescent stress on vHip mitochondrial respiratory function and redox regulation, in which are partially associated with E/I imbalance and behavioral abnormalities.

Keywords: Adolescent Stress, Mitochondria, Parvalbumin Interneurons, Oxidative Stress

Disclosures: FAPESP, CAPES, CNPq: Grant (Self).

P576. Social Engagement Moderates Relations Between Hippocampal-TPJ Connectivity and Neighborhood Social Fragmentation in the Clinical High Risk for Psychosis Syndrome

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Background: Hippocampal hyperactivity, as measured during resting-state functional magnetic resonance imaging (rsfMRI), is a risk factor for psychotic disorder and an indicator of acute psychotic illness. Hippocampal activity has implications for hippocampal functional connectivity (h-FC), and the degree to which hippocampal activation covaries with activation in other areas of the brain, which has further implications for global patterns of brain connectivity. Consistent with evidence from prospective studies that exposure to stress is associated with increased risk of subsequently developing a psychotic disorder, patterns of brain connectivity observed in those with psychosis spectrum disorders converge with those observed in stressor-exposed individuals to suggest that h-FC with well-connected regions of large-scale brain networks is altered in these samples. Hippocampal-FC with the temporoparietal junction (TPJ) is of special interest not only because the hippocampus and TPJ maintain especially robust connections, but also because their connectivity has been associated with hippocampal volume, exposure to simulations of losses of agency, and experiences of auditory hallucinations, all of which are relevant features of psychotic disorders. To our knowledge, no prior work has interrogated relations between hippocampal-TPJ connectivity and characteristics of the social environment in the psychosis prodrome. The present study aimed to address this gap in literature. The social environment was characterized by the neighborhood-level social fragmentation index (SFI), which has been positively associated with rates of psychotic disorders and symptom severity.

Methods: The study sample included participants at clinical high-risk for psychosis ($N = 92$) between the ages of 12 and 30 years (55% male) from the second phase of the North American Prodrome Longitudinal Study who completed rsfMRI at baseline and had available addresses at baseline suitable for geocoding. Fisher's z-statistics corresponding to bilateral h-FC with regions of

the TPJ—bilateral angular gyri (AG), inferior parietal lobule (IPL), and superior temporal poles (STP)—were entered as dependent variables in separate linear regression models with SFI as the predictor. Consistent with prior work, SFI was operationalized as the average z-score of four characteristics germane to the census tract of baseline residence: residential instability (i.e., percentage of people who moved), percentage of renter-occupied housing, percentage of single-person households, and percentage of people divorced. For each h-FC relation shown to be significantly associated with SFI, the covariates of age, biological sex, presence of antipsychotic medication, and z-statistic corresponding to area-level neighborhood poverty were entered stepwise in hierarchical regression models. Those models were directly compared using analysis of variance to determine if adding the covariates improved model fit according to residual sums of squares. The optimized model for each h-FC relation was maintained in the final step of analysis, which tested for the moderating role of social engagement (SE) on the relation between h-FC and SFI. Consistent with prior work, SE was operationalized as the total count of five items endorsed on the Life Events Stress scale that concerned desirable social activities. This procedure was repeated using bilateral middle occipital lobes as control region seeds because this area is seldom implicated in the etiologies of psychotic disorders or the large-scale brain networks including the hippocampus and TPJ.

Results: Among the 12 hippocampal-TPJ relations tested for association with SFI ($n = 6$ for left h-FC and $n = 6$ for right h-FC), three relations were nominally significant and one survived correction for multiple comparisons using the Benjamin-Hochberg method at $p < .05$. In all three cases, hippocampal-TPJ connectivity was significantly and positively associated with SFI; specifically, right hippocampal-left STP ($b = .08$, $p = .003$, 95% CI = .03–13), left hippocampal-left STP ($b = .07$, $p = .018$, 95% CI = .01–.13), and right hippocampal-right AG connectivity ($b = .05$, $p = .042$, 95% CI = .01–.10). None of the occipital-TPJ relations were associated with SFI. In addition, none of the covariates accounted for a significant improvement in model fit and were not maintained in the final model for each h-FC relation that included an interaction term between SE and SFI. Our findings suggest that SE significantly moderates the relation between SFI and left hippocampal-left STP connectivity, as well as the relation between SFI and right hippocampal-right AG connectivity. As determined by calculating simple slopes for the relations between SFI and the implicated h-FC association, we observed a positive and significant relation between SFI and h-FC for those with low and mean levels of social engagement, and no relation between SFI and h-FC for those with high levels of social engagement.

Conclusions: Our findings build upon the growing body of literature indicating that individual-level social engagement buffers against deleterious effects of adverse socioenvironmental characteristics on neurobiological features germane to the etiologies of psychotic disorders. Further work characterizing the links between hippocampal hyperactivity and functional outcomes in psychosis spectrum disorders is warranted.

Keywords: Systems Neuroscience, Default Mode Network, Hippocampal Connectivity, Social Fragmentation Index, Clinical High Risk for Psychosis

Disclosure: Nothing to disclose.

P577. Code Morphing Preserves Prefrontal Representations in a Thalamocortical Model of Schizophrenia

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Background: Schizophrenia (SCZ) causes a diverse and debilitating symptomatology, including positive, negative, and cognitive symptoms. Because of few therapeutic options, cognitive symptoms (including deficits in working memory and executive functions) are particularly defining of SCZ prognosis. Improved understanding of the neural basis of schizophrenia could help inform development of treatments for cognitive symptoms.

In humans, decreased connectivity between the thalamus and prefrontal cortex is observed in individuals classified as high risk for eventually developing schizophrenia. Notably, however, not all of these individuals go on to develop full-blown illness, suggesting that on a neural level, there may be differences in how prefrontal cortex may compensate for weakened thalamic input.

Methods: Adult mice ($n = 9$) were trained in a novel spatial working memory task in an automated, 8-arm radial arm. During task execution, prefrontal cortex activity was recorded at cellular-resolution using miniscope-based calcium imaging. On a fraction of trials, projections to the prefrontal cortex from the thalamus were optogenetically inhibited, to transiently decrease thalamocortical connectivity.

Results: Optogenetic manipulations of thalamocortical projections had weak global effects on task behavioral performance, but degraded performance differentially across different phases of the task, supporting the temporal-specificity of thalamocortical input for spatial working memory. Under manipulation, prefrontal representations of spatial information remained present, but morphed, suggesting that flexibility of neural representation underlies resilience to changes in circuit connectivity.

Conclusions: A temporally-specific inhibition of thalamocortical circuitry influenced prefrontal encoding patterns without profoundly disrupting behavior, suggesting that the prefrontal cortex may possess adaptive mechanisms to compensate for short-term changes in inputs. Irregularities in the capacity to reliably morph representations maybe underlie the progression of particular individuals from clinical high risk for schizophrenia to full-blown illness.

Keywords: Working Memory, Schizophrenia (SCZ), Prefrontal Cortex, Thalamo-Cortical Interactions, Neural Populations

Disclosure: Nothing to disclose.

P578. Neurobiological Basis of Male Sexual Orientation

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Background: The causal factors related to human sexual orientation have remained largely unknown. In several countries, homosexuality has been considered as a degenerative cultural phenomenon imported from liberal Western democracies and is prohibited under threat of death penalty. Homosexual individuals face discrimination and have substantially higher risk of suicide and mental disorders than heterosexual individuals. Political attitudes about sexual orientation correlate with the views on the causes of homosexuality, and those who believe that sexual orientation is determined by neurobiological causes tend to have a more permissive attitude towards homosexuality.

Methods: We studied the gene expression phenotype in induced pluripotent stem cell (iPSC)- derived cortical neurons, corresponding to maturity at the late first/early second trimester,

among Finnish homosexual men ($N = 6$), and heterosexual men ($N = 6$) and women ($N = 5$).

Results: Homosexual orientation was strongly linked to down-regulation of HHIP, GLI1, and several histone genes, and up-regulation of ZP3, FIRRE and several microRNAs (the lowest p-value corrected for multiple testing 4.6 x 10-4). One of the six statistically significantly upregulated microRNAs was miR-206 which regulates cellular response to estrogen stimulus, and another was miR-199a-5p which targets DDX3Y in Y chromosome. The most robust findings in canonical pathways were observed in DNA methylation and transcriptional repression, sonic hedgehog, and IL-33 signaling pathways (p-values down to 9.6 x 10-5).

Conclusions: Since no differentially altered gonadal hormonal or endocrine factors affect iPSC-derived neurons, our findings suggest that sexual orientation is primarily attributed to neuronal development per se and associated with the differential expression of genes coding for histone modification, left-right asymmetry, sperm binding, and X chromosome inactivation. The results show that male homosexuality is a neurobiological phenotype determined already during the late first/ early second trimester of pregnancy. Elucidating the causal mechanism of homosexuality may help to reduce stigma and discrimination against sexual minorities.

Keywords: Homosexuality, Induced Pluripotent Stem Cells (iPSCs), Sexual Orientation

Disclosure: Nothing to disclose.

P579. Maternal Kynurenine 3-Monooxygenase Genotype in Mice Directs Sex-Specific Behavioral Outcomes in Offspring

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Background: Both genetic and environmental influences impact outcomes of neurodevelopmental disorders (NDD), including autism spectrum disorder (ASD), schizophrenia (SZ), and bipolar disorder (BD). Cognitive impairments, sleep disturbances, and anxiety are highly prevalent comorbidities in these patients. Kynurenine 3-monooxygenase (KMO) is a pivotal branch-point in the kynurenine pathway (KP) of tryptophan metabolism. Reduced Kmo mRNA expression and KMO enzyme activity are found in postmortem brains of individuals with SZ.

Methods: To further characterize the relationship between NDD endophenotypes and alterations in Kmo, we presently used mice with heterozygous (HET-Kmo+/-) parental origin to generate wild-type offspring (WT-Kmo+/+), while offspring from C57BL/6J wild-type parental origin were the control (WT-Control) group. Adult offspring were used for behavioral testing, either in Barnes maze (BM) or in elevated zero maze (EZM), or sleep experiments, by implanting telemetry transmitters to continuously monitor electroencephalogram (EEG) and electromyogram (EMG) in freely moving mice. Sleep-wake behavior was also evaluated in HET-Kmo +/- mice. KP metabolites and corticosterone in breast milk were analyzed in HET-Kmo+/- and WT-Control females, the parental genotypes.

Results: Female WT-Kmo+/+ offspring were impaired in BM spatial learning traveling a significantly longer distance ($P < 0.01$) and committing a higher number of errors ($P < 0.05$) concurrently with increased immobility in the maze ($P < 0.05$). WT-Kmo+/+ females exhibited increased anxiety-like behavior in EZM with fewer entries to the open area ($P < 0.05$). Sleep duration was decreased in female WT-Kmo+/+ mice along with significantly prolonged wakefulness ($P < 0.05$). Male WT-Kmo+/+ offspring were impaired in BM reversal learning when compared to their counterpart controls ($P < 0.05$). Both female and male HET-Kmo+/- mice displayed reduced sleep duration ($P < 0.01$) and altered EEG power spectra (genotype x frequency: $P < 0.0001$) relative to WT-Control mice. Breast milk kynurenine was significantly higher in HET-Kmo+/- than in WT-Control mothers ($P < 0.0001$).

Conclusions: Taken together, our results suggest that maternal Kmo genotype imposes adverse behavioral outcomes in offspring in parallel with enhanced kynurenine exposure during the early postnatal period.

Keywords: Kynurenine Metabolism, 3-Hydroxykynurenine, REM Sleep, NREM Sleep, Maternal Genotype

Disclosure: Nothing to disclose.

P580. Effects of Persistent Inflammatory Pain on Sleep and Peripheral Biomarkers

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Background: Classical behavioral outcomes such as exploratory behavior and stress coping have proven insufficient in aiding the identification of biomarkers, treatment targets, and novel therapeutics, particularly in preclinical models of persistent and chronic pain. Objective and continuous measures, such as sleep telemetry recordings, provide a more complete understanding of how pain may act as a stressor, over periods of time that more accurately reflect chronic conditions as they appear in humans. Stress and pain both engage limbic and mesolimbic circuits involved in regulation of sleep and circadian rhythms. Stress activates the HPA axis to a degree that can cause changes in peripheral systems that reflect damage, including adrenal hypertrophy, elevated cortisol (CORT), and involution (shrinkage) of the thymus, an organ involved in T-cell production and inflammatory processes. It is not currently known if chronic pain alone is sufficient to recapitulate the effects of traumatic stress on sleep or peripheral systems, and the degree to which the changes are linked (coupled) to one another. To examine this question, we used a laboratory model of persistent pain (Complete Freund's adjuvant injection [CFA]) in mice.

Methods: For sleep studies, male and female C57BL/6J mice were implanted with wireless transmitters to measure EEG, EMG, body temperature, and locomotor activity, followed by a week of recovery. Continuous telemetry was collected for 7 days of baseline, 7 days after isoflurane exposure (to control for the anesthesia-related aspect of the CFA procedure), 7 days after saline injection into the left hind-paw (to control for the injection-related aspect of the CFA procedure), and 21 days after CFA injection into the right hind-paw. Active wake, paradoxical sleep (REM), and slow wave sleep (SWS) vigilance states, as well as activity and body temperature, were quantified as percent of baseline. Data were analyzed using one-way and two-way repeated measures ANOVAs. In a separate experiment, male and female C57BL/6J mice were injected with either saline or CFA into the right hind-paw, and three weeks later the adrenal glands and thymus were collected, together with trunk blood (for future analyses). Gland weights were normalized to body weight and analyzed with unpaired t-tests.

Results: In the 24 hours after CFA injection, mice spent less time awake than in the 24 hours after isoflurane and saline exposures ($F2,28 = 2.694$, $p = 0.0001$) and, consequently, more time in both REM ($F2,30 = 1.681$, $p = 0.0014$) and SWS ($F2,31 = 0.7456$, $p = 0.0115$). Mice also exhibited more bouts of REM ($F2,28 = 4.181$,

$p = 0.0001$) and SWS ($F2,31 = 4.304$, $p < 0.0001$). CFA injection also increased time and bouts spent asleep in the dark phase, when mice are typically more active. These changes corresponded to transient decreases in activity ($t6 = 2.528$, $p = 0.0448$) and body temperature ($t6 = 4.238$, $p = 0.0055$). While there were no effects on adrenal gland weights, thymus weights were decreased in CFA-treated mice relative to saline-treated controls ($t6 = 2.636$, $p = 0.0387$).

Conclusions: Persistent inflammatory pain, as modeled by CFA treatment in mice, produced changes in sleep patterns. Effects on REM and SWS and bouts broadly resemble the effects of chronic social defeat stress. This model of pain also produced thymic involution without causing adrenal hypertrophy, indicating that these changes can occur independently and are thus uncoupled. These data suggest that persistent inflammatory pain recapitulates some, but not all, features of chronic stress.

Keywords: Sleep, Pain Models, Telemetry, Inflammation

Disclosure: Nothing to disclose.

P581. Effect of Lemborexant on Sleep Architecture in Adult and Elderly Subjects With Moderate to Severe Chronic Obstructive Pulmonary Disease

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Background: Patients with chronic obstructive pulmonary disease (COPD) commonly experience disruption of sleep and, accordingly, negative impacts on sleep architecture, initiation, and maintenance of sleep. These effects can include reduction and temporal redistribution of rapid eye movement (REM) and non-REM sleep. Lemborexant (LEM) is a competitive dual-orexin receptor antagonist (DORA) approved in the United States, Japan, Canada, Australia, and several Asian and Middle Eastern countries to treat adults with insomnia. In Study E2006-G000-303 (NCT02952820), a pivotal phase 3 study of older adults with insomnia, subjects who received LEM treatment had increased total sleep time (TST), with increases in REM sleep. Study E2006-A001-113 (Study 113; NCT04647383) was a phase 1 clinical trial investigating the respiratory safety of LEM versus placebo (PBO) in adult and elderly subjects with moderate to severe COPD. There was no adverse impact on peripheral oxygen saturation and the apnea-hypopnea index (AHI) during TST on Day 8 of daily treatment for subjects who received LEM 10 mg (LEM10) versus PBO, demonstrating respiratory safety with single and multiple dosing. This post-hoc analysis of Study 113 evaluated sleep architecture in subjects with moderate to severe COPD and without insomnia who received LEM10.

Methods: Study 113 was a multicenter, multiple-dose, randomized, double-blind, PBO-controlled, 2-period crossover study in adult (age ≥ 45 to < 65 y) and elderly (age ≥ 65 to ≤ 90 y) subjects with moderate to severe COPD and an AHI < 15 events/hour. Subjects were screened for COPD severity by spirometry performed based on Global Initiative for Obstructive Lung Disease recommendations. Subjects were randomized to two 8-night treatment periods with LEM10 or PBO; treatment periods were separated by ≥ 14-day washout prior to subject treatment group crossover. In-laboratory polysomnography (PSG) was performed at screening and on the first and last nights of both treatment periods. Comparisons were made between the PBO and LEM10 conditions. PSG was performed on Day 1 (after a single bedtime dose) and Day 8 (after multiple bedtime doses) during both treatment periods to assess non-REM (N1, N2, N3, and total) and REM sleep durations along with TST (duration of sleep from sleep onset until awakening, in minutes) and REM latency (time from sleep onset to REM onset, in minutes).

Results: The analysis set comprised 30 subjects, mean age (SD) 69.2 (6.3) y, 21/30 (70.0%) were female, with moderate to severe COPD. Five (16.7%) subjects had severe COPD and 25 (83.3%) had moderate COPD. On both days, TST was significantly higher with LEM10 vs PBO: mean (SD), in minutes, on Day 1: 388.60 (46.87) vs 318.97 (81.95); $P < 0.0001$; Day 8: 369.93 (63.92) vs 331.95 (83.39); $P = 0.003$. Total non-REM sleep was significantly higher only on Day 1 for LEM10 vs PBO: mean (SD), in minutes, on Day 1: 298.10 (38.43) vs 260.65 (57.13); $P < 0.0001$; Day 8: 283.98 (53.75) vs 269.15 (59.68); $P = 0.145$. Total REM sleep was significantly higher for LEM10 vs PBO on both Day 1 and Day 8: mean (SD), in minutes, on Day 1: 90.50 (40.04) vs 58.32 (37.63); $P < 0.0001$; Day 8: 85.95 (36.517) vs 62.80 (38.29); $P = 0.001$. REM latency was numerically decreased on Day 1 and significantly decreased on Day 8 for LEM10 vs PBO: mean (SD), in minutes, on Day 1: 100.38 (104.60) vs 130.10 (93.97); $P = 0.057$; Day 8: 94.38 (89.05) vs 126.61 (106.91); $P = 0.022$. LEM was well tolerated; most treatment-emergent adverse events were mild.

Conclusions: In subjects with moderate to severe COPD but without confirmed insomnia, LEM increased total sleep, non-REM, and REM sleep compared with PBO as assessed after single or multiple doses of LEM. In conjunction with evidence of respiratory safety, this suggests that lemborexant may be a useful option for the treatment of insomnia in patients with moderate to severe COPD.

Sponsor: Eisai, Inc

Keywords: Dual Orexin Receptor Antagonists, COPD, Sleep Architecture, Insomnia

Disclosure: Eisai, Inc.: Employee (Self)

P582. Chronic Morphine Treatment Induces Opposing, Sex-Specific Adaptations on Morphine Signaling Within the Presynaptic and Somatic Compartments in a Thalamo-Striatal Circuit

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Background: Opioid drugs induce both desired analgesia, or pain relieving effects, and adverse effects including respiratory depression, constipation and reward. Activation of the mu-opioid receptor (MOR) mediates both the desired and undesired effects. Repeated exposure to opioids results in tolerance to their analgesic effects. However, tolerance to many adverse effects develops to a much lesser extent. Additional adaptations can counteract opioid tolerance and lead to facilitation of some opioid-mediated behaviors. This differential development of tolerance complicates the use of opioids for long term pain management. Opioid tolerance is thought to result from down-regulation and functional uncoupling of MORs, but the specific cellular drivers of these processes are not understood. MORs regulate neuronal function in soma and presynaptic compartments of neurons and MOR signaling in these compartments may be differentially affected by long-term opioid exposure. In this study, we investigated whether chronic morphine treatment induced adaptations in morphine signaling in presynaptic versus somatic MORs within the same neuronal population in both male and female mice. Glutamate releasing projection neurons in the medial thalamus (MThal) project to the dorsomedial striatum (DMS) and express high levels of MOR. These brain regions are important mediators of opioid-regulated processes including pain aversiveness. Here, we investigated the effects of chronic

morphine treatment on subsequent morphine signaling within MThal-DMS presynaptic terminals and MThal somata. We also investigated the role of MOR phosphorylation in driving the observed adaptations.

Methods: We used ex vivo patch clamp electrophysiology to measure the effects of chronic morphine treatment on subsequent morphine signaling at MThal-DMS presynaptic terminals and MThal cell bodies. Both male and female mice were used for all experiments (n = 10-12 cells/sex/treatment condition). The first set of experiments were performed using wild-type C57Bl/6J mice. Morphine treated mice were implanted with an osmotic minipump to continuously deliver morphine (80 mg/kg/day) for 7 days prior to experimentation. For presynaptic responses, mice were stereotaxically injected in the MThal with a virus encoding channelrhodopsin2 to allow for selective activation of MThal terminals in the DMS. Whole cell patch clamp recordings were performed in DMS medium spiny neurons to record optically evoked excitatory postsynaptic currents (oEPSCs), and inhibition of oEPSCs by bath perfusion of morphine (3 μM) was quantified. For somatic responses, mice were stereotaxically injected in the DMS with the retrograde tracer choleratoxin subunit B conjugated to Alexa 488 for targeting of DMS-projecting MThal neurons. Whole cell patch clamp recordings were performed in MThal neurons, and morphine-mediated conductance of G protein-gated inwardly rectifying potassium (GIRK) channels was quantified and normalized to baclofen-mediated GIRK conductance. To investigate the role of MOR phosphorylation in driving effects of chronic morphine treatment on MThal-DMS morphine signaling, presynaptic recordings were performed in drug-naïve and chronically treated MOR 10 S/T-A mice, a knockin mouse line in which mice express serine or threonine to alanine mutations at 10 phosphorylation sites on the MOR C-terminus.

Results: Within MThal-DMS presynaptic terminals, chronic morphine treatment increased subsequent inhibition of oEPSCs by morphine (morphine facilitation) in wild-type mice. This effect was sex-specific, occurring in males but not females ($p = 0.0052$, treatment x sex interaction, 2-way ANOVA; male naïve vs chronic morphine: $p = 0.0004$, female naïve vs chronic morphine: $p = 0.9957$, Šidák's multiple comparisons test). Conversely, at MThal cell bodies, chronic morphine treatment decreased subsequent morphine mediated GIRK conductance in wild-type mice (morphine tolerance). This effect was small but significant in both males and females ($p = 0.0266$, main effect of treatment, 2-way ANOVA). Surprisingly, in MOR phosphorylation-deficient 10 S/T-A mice, chronic morphine treatment resulted in tolerance to, rather than facilitation of, subsequent morphine signaling at MThal-DMS presynaptic terminals in both male and female mice ($p = 0.0108$, main effect of treatment, 2-way ANOVA).

Conclusions: This study demonstrated opposing effects of chronic morphine treatment within different compartments of the same medial thalamic neuronal population. Surprisingly, chronic morphine treatment induced facilitation of morphine signaling within MThal-DMS presynaptic terminals, but tolerance to morphine signaling at MThal cell bodies. The presynaptic effects occurred only in males, while somatic effects were present in both sexes. These findings suggest that the effects of chronic opioid exposure are not ubiquitous, but instead depend on several factors such as subcellular receptor distribution and the sex of the animal. The finding that in MOR phosphorylation-deficient mice, chronic morphine treatment resulted in presynaptic morphine tolerance, rather than facilitation, indicates that receptor phosphorylation may be a driver of the facilitation observed in wild-type mice.

Keywords: Slice Electrophysiology, Dorsomedial Striatum, Opioid Tolerance, Mu Opioid Receptor

Disclosure: Nothing to disclose.

P583. Sex-Specific Mechanisms in Fentanyl Use and Relapse in the Ventral Tegmental Area

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Background: Like other substances with abuse potential, opioid exposure disrupts connections between the ventral tegmental area (VTA) and the nucleus accumbens (NAc). Both NAc and VTA undergo molecular and physiological changes in response to opioid use that are heavily implicated in drug relapse. Numerous studies have established the importance of the dopaminergic VTA to NAc projection, however the GABAergic projection from NAc back to VTA is understudied in opioid use. Our previous work found fentanyl abstinence increases excitability and reduces dendritic complexity of NAc D1 neurons, the primary neuron subtype projecting from NAc to VTA. We thus hypothesized altered activity in D1 neurons would modulate opioid related behaviors through its projections to the VTA, and either directly or indirectly influence VTA dopamine neuron activity, dopamine release, and gene expression. In parallel, we sought to ascertain the VTA cell types receiving NAc input, and to characterize molecular adaptations in VTA neuron subtypes after fentanyl self-administration.

Methods: All experiments were conducted in 8–10-week-old mice using sex as a biological variable. NAc->VTA projections in fentanyl relapse: Mice were trained to self-administer fentanyl (1.5μg/kg/infusion IV) in operant chambers equipped with nosepokes. Mice received retrograde AAV-Cre in the VTA and Cre-dependent DREADDs or mCherry in the NAc after fentanyl self-administration (n = 5-7/sex/virus). Two-weeks later, mice were tested for fentanyl-seeking under extinction conditions with 1 mg/kg CNO. NAc->VTA projections in fentanyl reward: D1-Cre mice received Cre-dependent DREADDs or mCherry in the NAc, and were implanted with an intracranial cannula in the VTA prior to conditioning (n = 5-9/sex/virus). Two-weeks later, mice underwent saline and fentanyl conditioning sessions over three days in a conditioned place preference arena. On the post-test day, mice were allowed free exploration after intracranial 0.1 mM DCZ or PBS infusion, using a counterbalanced within-subjects design, with IC drug infusions separated by 4h. Systemic 0.1 mg/kg DCZ used an identical design with n = 5-12/sex/virus. Molecular adaptations after fentanyl self-administration: mice received transynaptic AAV-Cre in the NAc prior to fentanyl or saline self-administration training (n = 7-8/sex/drug). VTA nuclei were isolated from half the mice (pooled 4 mice/condition) for single nuclei RNAseq with the 10X Genomics platform. Brains from remaining mice were flash frozen and 16μm sections containing VTA were processed with RNAscope and imaged on a confocal microscope. Kcnab2 in fentanyl reward and relapse: For conditioned place preference, mice received intracranial AAV-Kcnab2 or mCherry in the VTA prior to conditioning (n = 5-7/sex/virus). For self-administration, half of the mice received intracranial AAV-Kcnab2 or mCherry prior to training to determine the effects on acquisition and subsequent seeking under extinction conditions at 24hr and 14d abstinence. The other half received intracranial AAVs after self-administration training, and were tested for seeking under extinction conditions 14d later.

Results: We found female mice self-administered more fentanyl compared with males (Day x Sex $F1,31 = 3.5$, $p = 0.007$), and female mCherry exhibited greater fentanyl-seeking behavior compared with male mCherry (Sex F1,31, $p < 0.001$). Increased Gq signaling in NAc->VTA neurons abolished fentanyl seeking in

both sexes (Virus $F2,31 = 29.6$, $p < 0.001$; female: $p < 0.0001$; male, $p = 0.002$; Dunnet's). Inhibiting NAc->VTA neurons showed a trend towards decreased fentanyl seeking in female mice ($p = 0.059$). Inhibiting NAc D1 neurons, or their terminals in the VTA, reduced fentanyl conditioned place preference in females. (Sex: $F1,36 = 4.96$, $p = 0.03$, DCZ: $F1,36 = 4.3$, $p = 0.04$, saline vs DCZ in Gi females, $p = 0.003$). We detected transsynaptic Cre from NAc in 2% of all VTA nuclei, with the most enrichment in cluster 3, a population of dopamine-GABA-glutamate co-releasing neurons. We next examined gene expression in VTA after fentanyl self-administration using single nuclei RNAseq. We found altered expression of numerous, transcriptionally co-regulated genes important for synaptic function in VTA, including potassium channel subunit Kcnab2. Kcnab2 overexpression in VTA decreased fentanyl conditioned place preference in male, but not female mice (Virus x Sex $F1,23 = 4.4$, $p = 0.04$; male mCherry vs Kcnab2, $p = 0.0002$, Sidak's). In preliminary studies, we expressed Kcnab2 in VTA prior to self-administration training, and found no influence on intake nor fentanyl seeking behavior at 24hr and 14d abstinence. Kcnab2 also had no significant effect on fentanyl seeking after 14d abstinence when expressed after self-administration training.

Conclusions: First, our data in mice replicate rat findings where females self-administer more opioids compared with males. Second, the DREADDs experiments indicate that activity in the NAc->VTA projection is important for fentanyl seeking behavior after abstinence, and has sex-specific effects on fentanyl place preference distinct from fentanyl seeking. Fentanyl self-administration alters expression of genes important for neuronal structure and physiology in the VTA. Ongoing experiments are determining how these and other molecular manipulations in specific VTA cell types alters acquisition of fentanyl self-administration, fentanyl-seeking, and dopamine release in NAc from both sexes.

Keywords: Fentanyl, Intravenous Drug Self-Administration, DREADDs, RNAseq, Opioid

Disclosure: Nothing to disclose.

P584. Investigating the Interaction Between Drug Exposure and Estrous Cycle Fluctuations on NAC Opioid Receptor Expression in Oxycodone- vs. Cocaine-Exposed Rats

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Background: While sex and ovarian hormones are known to influence substance use and relapse vulnerability across multiple classes of drugs of abuse, increasing evidence indicates that estrous cycle fluctuations impact opioid intake and seeking behavior in a manner distinct from that observed for psychostimulants. While cue-induced cocaine seeking increases during the estrus stage of the estrous cycle (Estrus Females) compared to both males and females in other cycle stages (Non-Estrus Females), we have recently found that cue-induced oxycodone seeking decreases selectively in Estrus Females following prolonged withdrawal (>40 days) from extended-access oxycodone self-administration. One potential mechanism mediating these opposite behavioral findings across different drug classes could be distinct interactions between drug exposure, ovarian hormones and opioid receptor signaling within the mesolimbic reward circuitry. Indeed, it is known that ovarian hormones can influence opioid receptor expression in several brain regions including the nucleus accumbens (NAc) and that drug-induced changes of opioid receptor expression or function can influence drug seeking and relapse vulnerability.

Methods: Here we assessed protein expression levels of opioid receptors (mu, delta and kappa) in the nucleus accumbens (NAc) in males and females and across the estrous cycle following withdrawal from extended-access (6 h/day for 10 days) saline, cocaine or oxycodone self-administration.

Results: Preliminary findings indicate distinct oxycodone-induced changes in opioid receptor expression in NAc core versus shell along with an interaction between oxycodone and sex/estrous cycle phase on opioid receptor expression.

Conclusions: Together these studies may contribute to our understanding of the cellular mechanisms underlying distinct estrous cycle-dependent changes in drug seeking and relapse vulnerability between stimulants and opioids.

Keywords: Cocaine and Opioid Use Disorders, Opioid Receptor Expression, Sex and Estrous Cycle Effects

Disclosure: Nothing to disclose.

P585. Sex Differences in Metabotropic Glutamate mGlu1 and mGlu5 Regulation of Prefrontal Cortex Parvalbumin Interneurons and Behavioral Responses to Alcohol

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Background: Proper function of the prefrontal cortex (PFC) is essential for the top-down regulation of motivated and affective behaviors, and population-level differences in PFC function have been related to alcohol use disorder (AUD) diagnosis. Women who develop AUD proceed through disease milestones more rapidly than male counterparts, thus there is great motivation to identify cellular and synaptic mechanisms that guide sex differences in PFC function. Parvalbumin-expressing inhibitory neurons (PV-INs) are a key subpopulation of GABAergic cells within PFC. Previous mouse studies from our lab found that PFC PV-INs display basal sex differences in membrane physiology parameters and undergo sex-dependent adaptations following chronic drinking; however, the cellular elements and synaptic mechanisms that mediate these sex-dependent effects remain unclear.

Methods: In these studies, we investigated Group 1 metabotropic glutamate receptors (mGlu1 and mGlu5), two G protein-coupled receptors highly expressed in PFC PV-INs. We made whole-cell patch clamp recordings of layer 5 prelimbic PV-INs to examine basal sex differences in mGlu1 and mGlu5 modulation of PV-IN membrane and synaptic physiology.

Results: PV-INs from male mice displayed greater depolarizing currents than females following stimulation with the mGlu1/5 agonist DHPG. Using selective antagonists, we found that the depolarizing currents are mediated by both mGlu1 and mGlu5 receptors in male mice, whereas only mGlu5 receptor activation modulated PV-INs in females ($p < 0.05$ Bonferonni post-tests following 2-way ANOVA). Similarly, we found that mGlu1 receptor activation enhances excitatory drive onto PV-INs from male mice, but this effect is mediated by mGlu5 receptors in female mice ($p < 0.05$ Bonferonni post-tests following 2-way ANOVA). We then examined depolarization-induced suppression of excitation (DSE), a form of endocannabinoid-mediated short-term plasticity. While PV-INs from both sexes displayed comparable DSE under basal conditions, mGlu5 receptor activation enhanced DSE in male but not female PV-INs ($p < 0.05$ Bonferonni post-test following 2-way ANOVA).

Conclusions: Ongoing studies are examining effects on the top-down control of voluntary drinking, changes in working

memory, and anxiety-like behaviors. Preliminary analyses suggest that PV-mGlu5-KO mice display decreased binge drinking, and that female KO mice resist the development in asocialiaty following chronic drinking in female mice. Taken together, these studies identify mGlu1 and mGlu5 receptors as candidate signaling molecules involved in sex differences in PV-IN activity, PFC function, and behaviors relevant for AUD.

Keywords: Prefrontal Cortex, Synaptic Plasticity, Inhibitory Interneuron, Parvalbumin, mGlu5

Disclosure: Nothing to disclose.

P586. E-Cigarette Vapor Induces Changes in Addiction- and Inflammation- Associated Genes in Rat Brains and Cultured Astrocytes

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Background: People with HIV (PWH) and people with psychiatric disorders use e-cigarettes and other addictive substances at higher rates than the general population. PWH are at risk for developing HIV-associated dementia (HAND) despite anti-retroviral therapy. Neuroinflammation contributes to the development of HAND and other psychiatric disorders. E-cigarette liquid is composed of a psychoactive substance (ex: nicotine, THC) and a solvent (ex: propylene glycol). Understanding how e-cigarette vapor affects brain cells, particularly neuroimmune cells like astrocytes, is important to the general population of e-cigarette users and to individuals with HIV or other psychiatric disorders whose brains have higher baseline levels of neuroinflammation and may be more vulnerable to insult. The goal of this study was to investigate the effects of propylene glycol (PG), nicotine (NIC), and THC on mRNA and protein expression in rat brain and cultured astrocytes. The anti-retroviral dolutegeravir (DTG) in combination with PG, NIC, and THC was also assessed due its relevance to PWH.

Methods: 6-month old, male, Sprague-Dawley rats (n = 6/group) were placed into vaporization chambers for 30-min sessions twice per day for 10-days and exposed to room air (AIR), PG (100%), PG + NIC (30mg/ml), or PG + THC (200mg/ml). Rats were euthanized and frontal cortex was homogenized. Half was processed for RNA extraction and half for protein isolation. RNA was sent for RNA sequencing. Differential gene expression was analyzed using Illumina's BaseSpace Cloud software. Differentially expressed genes were confirmed by RT-qPCR. Rat astrocyte (C6; ATCC, cat# CCL-107) cultures were exposed to PG (5mM, 50mM, 150mM) for 6h or 18h. Rat astrocyte cultures were exposed to PG (150mM), NIC (10μM), THC (10μM), dolutegeravir (200ng/ml) for 18h in the following combinations: PG, PG+NIC, PG+THC, DTG, PG+DTG, PG+NIC+DTG, PG+THC+NIC. RNA was extracted from astrocytes. RT-qPCR was used to assess fold change of EGR2 and ARC mRNA using the comparative CT method and ACTB as the control probe. Protein concentrations of EGR2 and ARC in rat brain were determined by Western blot and normalized to actin. Statistical significance was determined by one-way ANOVA.

Results: RNA sequencing of whole brain lysate obtained from rats exposed to PG, AIR, or NIC identified 14 genes significantly differentially expressed between PG and AIR, 13 genes between PG and NIC and 3 genes between NIC and THC. Early growth receptor 2 (EGR2) and activity-regulated cytoskeletal gene (ARC) were chosen for validation. RT-PCR of rat frontal cortex showed a significant decrease of EGR2 mRNA in the PG group compared to AIR, THC and NIC. For ARC, mRNA from the PG-exposed rats was non-significantly decreased compared to AIR, THC, NIC.

PG also non-significantly decreased EGR2 and ARC protein concentrations. In vitro experiments of rat astrocytes revealed that at 6h PG decreases EGR2 but increases ARC mRNA levels. By 18h PG increases both EGR2 and ARC mRNA levels in a dose-dependent manner. NIC but not THC decreased this affect. The addition of DTG reversed the effect NIC had on decreasing PG-induced upregulation of EGR2 mRNA in astrocytes.

Conclusions: 10-days of PG vapor exposure alters gene expression in rat brain. PG decreases expression of EGR2 and ARC mRNA in frontal cortex. Non-significant decreases in EGR2 and ARC protein levels showed the same trend. However, 10-days of RNA change may not be long enough to alter protein levels. More studies are needed. EGR2 and ARC are involved in addiction-related gene expression networks. It is concerning that PG alone is sufficient to modulate these genes absent the addition of addictive substances. EGR2 is important in control of inflammation. ARC modulates synaptic plasticity and is altered in neurodegenerative diseases. It is possible that chronic exposure to PG vapor enhances neuroinflammation and impairs synaptic plasticity which is concerning given the high rate of e-cigarette use in PWH and other psychiatric disorders. NIC and THC reversed PG-induced decreases in EGR2 and ARC. Given the popularity of recreational and medicinal cannabis future studies investigating the role THC has on these pathways is needed. Similar to the in-vivo findings, PG down regulated EGR2 in cultured rat astrocytes at 6h. However, by 18h PG upregulated EGR2 and ARC, an effect that was again attenuated by NIC. One possibility for this difference is cell type and the in vivo environment which contains many cell-types interacting together to maintain homeostasis. Future in-vivo studies evaluating the effects of e-cigarettes on astrocytes are needed. The addition of DTG reversed the effect NIC had on decreasing PG-induced upregulation of EGR2. This suggests that for PWH DTG further modulates e-cigarette-induced alterations in gene expression. However, further in-vivo studies are needed.

Keywords: Neuroinflammation, Electronic Cigarette (e-cigarette), HIV and Inflammation, Addiction, Cannabis

Disclosure: Nothing to disclose.

P587. Early-Life Stress and Ovarian Hormones Alter Transcriptional Regulation in the Nucleus Accumbens Resulting in Sex-Specific Responses to Cocaine

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Background: Substance use disorders affect people across genders, although a significant body of evidence shows that women and men develop these disorders differently. Specifically, cocaine use disorder is a major global health issue which affects ~1 million individuals over age 12 in the United States alone and there is overwhelming evidence that females are more sensitive to the effects of this drug. Women are reported to transition to addiction faster, have more difficulty remaining abstinent, and experience more adverse consequences of cocaine use. Clinical studies revealed early-life stress and ovarian hormones as two factors contributing to female-specific vulnerability to cocaine. However, the underlying molecular mechanisms remain poorly understood.

Methods: To examine the effects of early-life stress on later behavior and gene expression, we used an early-life stress

(maternal separation, MS) paradigm in C57Bl/6J mice, which included a 3-hour daily mother-pup separation combined with maternal unpredictable stress from postnatal day (PD) 1-14. MS-induced risk for cocaine addiction in both male and female offspring was examined in the late adolescent period (from PD 54-60) using cocaine conditioned place preference (CPP) with low (2.5 mg/kg) and high (10 mg/kg) cocaine doses ($N = 15$-25 mice per sex/group/dose). To address the effect of ovarian hormones on the CPP phenotype, the estrous cycle stage of female mice was determined daily, using vaginal cytology. We analyzed the CPP data using a three-way ANOVA with experimental group (control or MS), sex (female or male), and cocaine dose (high or low) as factors. For females, we analyzed the data accounting for the estrous cycle stage. To determine the transcriptional signatures of early-life stress that preceded the cocaine-induced behavioral phenotypes, we performed gene expression (RNA-seq) analysis in the key reward area, the nucleus accumbens (NAc), of control and MS females and males at PD50 ($N = 6$/group/sex). To address epigenetic mechanisms through which the estrous cycle and sex affect acute cocaine response, at PD54, we performed chromatin accessibility analysis (ATAC-seq) on neuronal nuclei from the NAc of proestrus (high-estrogenic, $N = 9$) females, diestrus (low-estrogenic, $N = 9$) females, and males ($N = 9$), following 1-hr 10 mg/kg cocaine treatment. Bioinformatics analysis of RNA-seq and ATAC-seq data included: differential gene expression and chromatin accessibility analysis; gene enrichment (gene ontology, KEGG pathway, gene set enrichment analysis); gene coexpression clustering (Clust) analysis; and motif analysis. Finally, to address the importance of the inactive X chromosome in female-specific response to cocaine, we performed the CPP test using female mice that have only one X chromosome (39, XO; $N = 12$), wildtype female controls (40,XX; $N = 12$) and males (40,XY; $N = 5$) of the same genetic background (C57Bl/6J x CBA/CaGnLeJ), using 10 mg/kg cocaine dose.

Results: We show that cocaine can induce CPP in both males and females, but the effect is dose-dependent and more consistent in males, while it varies with the estrous cycle stage and is more strongly affected by early-life stress in females (group by sex by dose interaction; $(F(1, 132) = 4.32, p = 0.04$; three-way ANOVA). Analyzing gene expression (Padj < 0.1) and chromatin (Padj < 0.05) in the NAc, we find shared involvement of X chromosome inactivation- and estrogen signaling-related gene regulation in enhanced conditioning responses seen after early-life stress and during the low-estrogenic (and more anxious) state in females. During the low-estrogenic state, females respond to acute cocaine exposure by increasing the accessibility of neuronal chromatin enriched for the binding sites of ΔFosB, a transcription factor implicated in chronic cocaine response and addiction. Conversely, high-estrogenic females respond to cocaine by preferential closing of neuronal chromatin, providing a mechanism for limiting cocaine-driven chromatin and synaptic plasticity. We find that physiological estrogen withdrawal, exposure to early-life stress, and absence of the second X chromosome all nullify the protective effect of high-estrogenic state on cocaine conditioning in females.

Conclusions: Our study highlights that cocaine use in women and other menstruating individuals involves complex interactions of ovarian hormones with internal factors (such as negative affective state) and external risk factors such as stress. We offer a molecular framework to understand sex-specific neuronal mechanisms underlying cocaine use disorder, opening new avenues for future sex- and gender-informed treatments for these disorders.

Keywords: Cocaine Sex Differences, Ovarian Hormones, Early Life Stress, Gene Expression, Epigenetic

Disclosure: Nothing to disclose.

P588. Adaptor Protein Complex 2 in the Orbitofrontal Cortex Predicts Alcohol Use Disorder

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Background: Alcohol use disorder (AUD) is a life-threatening disease characterized by compulsive drinking, cognitive deficits, and social impairment that continue despite negative consequences. The inability of individuals with AUD to regulate drinking may involve functional deficits in cortical areas that normally balance actions that have aspects of both reward and risk. Among these, the orbitofrontal cortex (OFC) is critically involved in goal-directed behavior and is thought to maintain a representation of reward value that guides decision making.

Methods: In the present study, we analyzed post-mortem OFC brain samples collected from age- and sex-matched control subjects and those with AUD using proteomics, bioinformatics, machine learning, and reverse genetics approaches.

Results: Of the 4,500+ total unique proteins identified in the proteomics screen, there were 47 proteins that differed significantly by sex that were enriched in processes regulating extracellular matrix and axonal structure. Gene ontology enrichment analysis revealed that proteins differentially expressed in AUD cases were involved in synaptic and mitochondrial function, as well as transmembrane transporter activity. Alcohol-sensitive OFC proteins also mapped to abnormal social behaviors and social interactions. Machine learning analysis of the post-mortem OFC proteome revealed dysregulation of presynaptic (e.g., AP2A1) and mitochondrial proteins that predicted the occurrence and severity of AUD. Using a reverse genetics approach to validate a target protein, we found that prefrontal Ap2a1 expression significantly correlated with voluntary alcohol drinking in male and female genetically diverse mouse strains. Moreover, recombinant inbred strains that inherited the C57BL/6J allele at the Ap2a1 interval consumed higher amounts of alcohol than those that inherited the DBA/2J allele.

Conclusions: Together, these findings highlight the impact of excessive alcohol consumption on the human OFC proteome and identify important cross-species cortical mechanisms and proteins that control drinking in individuals with AUD.

Keywords: Alcohol Use Disorder, Orbitofrontal Cortex (OFC), Proteomics

Disclosure: Nothing to disclose.

P589. Dissecting Ventral Midbrain Dopamine Neuron Heterogeneity to Opioid Reward

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Background: The development of opioid-use disorders (OUD) is hypothesized to be based on brain circuit-specific pathological adaptations following repeated cycles of opioid intake, withdrawal, and abstinence. It is important to understand how the neural circuits that control reward processing are regulated across the opioid-exposure cycle. Dopamine neurons of the ventral tegmental area (VTA) and their projections to subdivisions of the nucleus accumbens (NAc), such as the NAc core or NAc shell, have been demonstrated to be critical for discrete components of reward processing and reward learning. Classically, VTA dopamine

neuron activity and dopamine release has also been shown to be regulated indirectly by opioids through disinhibitory mechanisms of local and distal GABA signaling. However, these is an increasing recognition of VTA dopamine neuron diversity. Whether opioids uniformly or distinctly modulate these dopamine subpopulations remains largely unknown. It was previously established that VTA dopamine neurons that express the corticotrophin releasing hormone receptor-1 (Crhr1VTA) project to the NAc Core and impact acquisition of natural reward learning while cholecystokinin-expressing VTA (CckVTA) dopamine neurons project to the NAc shell to impact motivation and performance of learned reward behaviors.

Methods: To determine whether opioids distinctly regulate the heterogeneous midbrain dopamine system, we used combinatorial genetic strategies to isolate functionally-distinct dopamine subpopulations that project to the NAc core or NAc shell. We expressed cre-inducible fluorescent proteins (AAV-FLEX-eYFP) in the VTA of Crhr1-Cre or Cck-Cre mice to isolate Crhr1-VTA and Cck-VTA neurons. We then exposed opioid-naïve mice to a single injection of the opioid morphine (10 mg/kg). Brain tissue was fixed 90 min later to capture the induction of Fos, an immediate early gene indicative of elevated neural activity. In a separate cohort of mice, we. also injected AAV-FLEX-eYFP in the VTA of Crhr1-Cre or Cck-Cre mice visualize these dopamine subpopulations for electrophysiological profiling of baseline intrinsic activity, synaptic activity, and modulation by the mu-opioid receptor (MOR), DAMGO.

Results: Using immunohistochemistry for Fos, we found that a single injection of morphine selectively activates cFos in Cck-VTA dopamine neurons when compared to Crhr1-VTA dopamine neurons (Crhr1-Cre mice $n = 3$, Cck-Cre mice $n = 3$). Further, Cck-VTA dopamine neurons have higher baseline excitability (Crhr1-VTA $n = 13$ cells, Cck-VTA $n = 15$ cells; 2-way ANOVA, ****$p < 0.0001$) and inhibitory synaptic transmission (Crhr1-VTA $n = 10$ cells, Cck-VTA $n = 12$ cells; t-test, *$p < 0.05$) when compared to Crhr1-VTA dopamine neurons. Finally, inhibitory post synaptic currents onto Cck-VTA dopamine neurons are more susceptible to DAMGO inhibition than Crhr1-VTA dopamine neurons (Crhr1-VTA and Cck-VTA $n = 4$ cells, paired t-test, *$p < 0.05$).

Conclusions: Our findings suggest distinct intrinsic or extrinsic neurophysiological regulation of dopamine subpopulations by opioids. The increased sensitivity to DAMGO inhibition of inhibitory synaptic activity onto the Cck-VTA dopamine neuron population may reveal a mechanism of regulation for the increased neural activity observed in this population following an acute morphine exposure.

Keywords: Dopamine, Mu Opioid Receptor, Cell- and Circuit-Selectivity

Disclosure: Nothing to disclose.

P590. Role of Projections From Orbitofrontal Cortex to Dorsal Striatum in Incubation of Oxycodone Craving

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Background: Prescription opioids are the main driver of opioid epidemic that involves drug misuse, addiction, and even overdose death. The high relapse rate is a major challenge in treating drug addiction, including oxycodone. In rats, oxycodone seeking progressively increases during abstinence and maintains for an extended period, a phenomenon termed incubation of oxycodone craving. We previously found that the orbitofrontal cortex (OFC) plays a causal role in this incubation. Here, we aimed to identify critical downstream regions of OFC in incubation of oxycodone craving by focusing on the central to medial portion of the dorsal striatum (DS), based on previous anatomical evidence.

Methods: In Exp.1, we first injected fluorescence-conjugated cholera toxin subunit B (CTb-555) into DS. Next, we trained male Sprague-Dawley rats to self-administer oxycodone (0.1 mg/kg/infusion, 6 h/d) for 10 days. We then either tested (Seeking-test) or did not test (No-test) rats for oxycodone seeking on abstinence day 15. Immediately after the test, we perfused the rats for immunohistochemistry to label Fos (a neural activity marker) in OFC. In Exp.2, we assessed the effect of pharmacological inactivation of DS on incubated oxycodone seeking. In Exp.3, we used an anatomical disconnection procedure (muscimol + baclofen, 50 + 50 ng/ 0.5 µl/side in OFC; SCH23390, 0.75 µg in 0.5 µl/side in DS) to examine the causal role of OFC to DS projections in incubated oxycodone seeking.

Results: We found that the number of Fos + CTb double-labeled cells in OFC was significantly higher in Seeking-test group than No-test group on abstinence day 15. In addition, DS inactivation and contralateral disconnections of OFC to DS projections decreased oxycodone seeking on abstinence day 15.

Conclusions: Taken together, our data showed that the activation of OFC to DS projections was associated with oxycodone seeking on abstinence day 15, and both DS and OFC to DS projections play critical roles in incubated oxycodone seeking. Ongoing studies are assessing the effect of ipsilateral disconnections on incubated oxycodone seeking and whether the role of OFC to DS projections in oxycodone seeking is time-dependent during abstinence.

Keywords: Incubation of Oxycodone Craving, Orbitofrontal Cortex, Dorsal Striatum

Disclosure: Nothing to disclose.

P591. Sex-Specific Effects of Nicotine on Neural Circuit Mechanisms in the Nucleus Accumbens Underlying Sensory Reinforcement

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Background: Substance Use Disorder (SUD) is characterized by dysfunction in the neural circuits underlying motivation leading to maladaptive behaviors associated with reward processing. Biological sex has been shown to be a critical variable in SUD and epidemiological evidence shows that women and men are differentially vulnerable, with women being most at risk. Tobacco use disorder is one of the most common SUDs due to the highly addictive properties of nicotine and it remains one of the leading causes of preventable death worldwide. The current resurgence in prevalence of nicotine use, because of the recent increase in e-cigarette products consumed, has caused a tobacco and nicotine use epidemic that was previously on the decline. Importantly, nicotine has been shown to be a weak primary reinforcer in the absence of cues, but in the presence of cues produces high rates of responding in reinforcement tasks. Therefore, the goal of this project was to understand how biological sex contributed to the behavioral effects of nicotine and to define the mechanism underlying the interaction between sensory stimuli in the environment and nicotine's pharmacological effects in mice.

Methods: We took a multifaceted approach to determine sex-dependent neurochemical mechanisms that underlie the role of

repeated nicotine exposure on operant sensory reinforcement in male (n = 44) and female (n = 44) mice. First, mice were trained for sensory reinforcement, where they emitted a nose poke response for the presentation of a visual and auditory stimulus. Next, mice were treated with saline or nicotine via subcutaneous injection for five days. Additionally, we repeated the sensory reinforcement experiment in mice that were pre-treated with nicotine or saline to determine how nicotine treatment preceding sensory reinforcement affected sex-specific behavioral responses. Finally, we assessed dopamine release dynamics and the effects of nicotine on these dynamics using fast scan cyclic voltammetry in the nucleus accumbens. We then correlated the effects of nicotine on dopamine release with behavior in the same animals during the sensory reinforcement task.

Results: Mice showed high rates of responding that were significantly higher than the unreinforced condition. (Two-way ANOVA, main effect of active or inactive nose poke: $F(1, 70) = 21.31$, $p < 0.0001$; Sidak's multiple comparisons test: males – active vs inactive, $t(18) = 2.304$, $p < 0.0001$; females – active vs inactive, $t(19) = 4.251$, $p = 0.0009$). Interestingly, sensory stimuli were more reinforcing in female mice as compared to males. (Two-way ANOVA, main effect of session: $F(4, 144) = 45.44$, $p < 0.0001$; session vs sex interaction: $F(4, 144) = 3.758$, $p = 0.0061$). After mice were treated with saline or nicotine for five days, we found a significant increase in sensory reinforcement in response to nicotine in males, but not female mice. (Two-way ANOVA, main effect of session: $F(1.366, 68.29) = 94.61$, $p < 0.0001$; nicotine: $F(3, 50) = 8.401$, $p = 0.0001$; session vs nicotine interaction: $F(9, 150) = 4.846$, $p < 0.0001$. *$p < 0.05$. Data are presented as mean ± SEM). Notably, mice that underwent sensory reinforcement following nicotine pretreatment did not display sex differences in behavioral responses. Correlating the effects of nicotine on dopamine release with behavior revealed a significant positive correlation between the rate of responding in the sensory reinforcement task and the effects of low dose nicotine on dopamine release in females, but not males. Additionally, we found that reinforced responses to sensory cues after repeated nicotine injections positively predicted dopamine release in response to dopamine release mechanisms following bath application of a higher desensitizing dose of nicotine. Furthermore, sex differences in the effects of nicotine on dopamine release were not observed in mice that underwent sensory reinforcement following nicotine pre-treatment.

Conclusions: Overall, these data suggest that sex differences in nicotine regulation of dopamine release underlies sex-specific behavior in sensory reinforcement. Importantly and in accordance with behavior, the effects of nicotine on dopamine release after repeated nicotine injections was not sex specific. This suggests that nicotine's ability to activate vs desensitize receptors may play differential roles in sex-specific behavioral effects.

Keywords: Nicotine, Sensory Processing, Sex Differences, Fast Scan Cyclic Voltammetry, Reinforcement Learning

Disclosure: Nothing to disclose.

P592. Epigenetic Mechanisms Underlying Drug-Seeking Behavior: Role of HDAC3 in Regulating Gene Expression Within the MHb Cholinergic Population for Cocaine- Induced Relapse-Like Behavior

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Background: Previous data from the Wood lab has shown that chemogenetic activation of a population of cholinergic neurons located in the Medial habenula (MHb) was sufficient to drive cocaine-primed reinstatement of conditioned place preference. Moreover, it was found that the immediate early gene and transcription factor, NR4A2 was necessary in the cholinergic population within the MHb to drive such behaviors. Nr4a2 is an HDAC3 target gene. Both Nr4a2 and Hdac3 are expressed in the MHb, suggesting that HDAC3 may have a key role in reinstatement as well, and perhaps even more important as a higher level epigenetic regulator of gene expression underlying reinstatement.

Methods: To examine the role of HDAC3's deacetylase activity within the cholinergic population of MHb neurons, and whether HDAC3 is critical for cocaine-primed reinstatement of CPP I will deliver a Cre-dependent (DIO) AAV into the MHb ChAT-Cre mice to express the deacetylase dead mutant of HDAC3 (DIO-HDAC3-Y298H-v5). We will inject, HDAC3-Y298H-v5 (n = 16), or DIO-GFP control Vector (n = 19) into the MHb of ChAT-Cre animals to limit HDAC3 manipulations to the cholinergic neurons of the ventral MHb. Mice will be trained and tested in cocaine-induced Conditioned Place Preference reinstatement. Briefly, mice will be conditioned using 10mg/kg (cocaine-HCl), followed by a 5 day extinction phase. Following extinction training, animals will be reinstated with a 5mg/kg cocaine-HCl injection to test the conditioning memory.

Results: Our recent work shows that HDAC3 physically comes off the Nr4a2 promoter (using HDAC3-ChIPqPCR) during reinstatement, suggesting that HDAC3 negatively regulates Nr4a2. We predict that this change in HDAC3 binding of specific genes in the MHb is critical for regulating cocaine-primed reinstatement and therefore expression of the deacetylase dead HDAC3-Y298H mutant is known to block deacetylase activity, increase histone acetylation, and enhance memory. Thus, expression of HDAC3-Y298H is predicted to reduce HDAC3-mediated repression of gene expression in the MHb leading to an increase in cocaine primed reinstatement (measured by an increase in CPP score), which is what we observed in our preliminary data (HDAC3-Y298H n = 16; GFP, n = 19, multiple comparison Two way Anova *$p = 0.0288$). A trend for impaired extinction was observed in the animals with the point mutation.

Conclusions: This preliminary study indicates that animals expressing an HDAC3-Y298H point mutation have increased cocaine-primed reinstatement of CPP, as compared to controls. Further experiments are required to study the observed extinction effect, to parse out whether the enhancement in cocaine-primed reinstatement is due an enhancement of the conditioning memory, or an impairment in the formation of extinction memory.

Keywords: Epigenetics, Cocaine Addiction, Conditioned Place Preference

Disclosure: Nothing to disclose.

P593. Dopamine Signaling in Nucleus Accumbens Core Contributes to Incubation of Cocaine Craving

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Background: During abstinence from cocaine self-administration (SA), cue-induced cocaine seeking in rats progressively intensifies and then remains high for months. This 'incubation of craving' provides a translationally relevant model for the persistence of vulnerability to craving and relapse in persons recovering from substance use disorder. We have shown that expression of incubation depends on strengthening of AMPAR transmission in the nucleus accumbens core (NAcc). However, despite the importance of NAcc dopamine (DA) for motivated behavior, little

is known about its role in incubation. We investigated this using DA biosensors and pharmacological approaches.

Methods: Male (M) and female (F) rats nose poked to receive IV cocaine (0.5 mg/kg/infusion; 6 h/d x 10 d; infusion paired with light cue). Cue-induced seeking tests, in which responding in the previously active port delivered the cue but no cocaine, were performed on forced abstinence day (FAD) 1 (before incubation) and FAD40-50 (after incubation). Prior to drug SA, GRAB-DA2m was expressed in NAcc and a fiber optic cannula was implanted (Study 1), or a guide cannula was implanted for intra-NAcc infusion of DA antagonists (Study 2).

Results: Study 1: Photometry recordings during cue-induced seeking tests demonstrated DA transients time-locked to nose pokes in the previously active hole on both FAD1 and FAD40-50 ($p < 0.0001$; $n = 23$, 9 M/14 F, within subject design). The magnitude of the response (AUC) did not differ between FAD1 and FAD40-50 ($p = 0.5090$). Study 2: The D1 DA receptor antagonist SCH39166 (1µg/0.5µL/hemisphere) or vehicle was infused into NAcc 15 min prior to the FAD40-50 seeking test (between subject design). Expression of incubation was significantly reduced by SCH39166 ($n = 17$, 7 M/10 F) vs. vehicle ($n = 13$, 7 M/6 F), with a more pronounced effect in males (post hoc tests: M + F, $p = 0.0005$; M, $p = 0.0004$; F, $p = 0.0549$). D2 DA receptor antagonist studies are underway, but preliminary data suggest that D2-R antagonism also reduces incubated cocaine seeking.

Conclusions: Cue-induced cocaine seeking is accompanied by a similar magnitude of NAcc DA release in early and late abstinence, suggesting that 'incubated' seeking does not reflect enhancement of DA release (i.e., a presynaptic effect). Rather, 'incubated' seeking appears to depend on postsynaptic strengthening of AMPAR transmission (demonstrated previously) and on D1 receptor signaling (demonstrated here) in the NAcc. It is possible that incubation is associated with alterations in postsynaptic DA receptor signaling in the NAcc. Future studies will examine how glutamate and DA interact to set the gain on cue reactivity during cocaine abstinence.

Keywords: Dopamine, Incubation of Cocaine Craving, In Vivo Fiber Photometry

Disclosure: Nothing to disclose.

P594. A Novel Investigation of Tobacco Co-use on Endocannabinoid Activity in People With Cannabis Use

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Background: Cigarette smoking is the most common form of tobacco use and remains the leading cause of preventable disease and death in the United States. Nicotine is the addictive ingredient in tobacco and its rewarding effects on the brain make cigarette smoking difficult to quit. While 70% of people who use tobacco want to quit, current treatment options for tobacco cessation (e.g., nicotine patch) show limited efficacy, with most attempts ending in relapse (~77%). Thus, there is a clear need for more efficacious treatments to help people quit tobacco and remain abstinent for the long-term. A better understanding of how tobacco use affects the brain may help develop new medications to treat people with tobacco use disorder.

Accumulating preclinical evidence suggests that nicotine interacts with the endocannabinoid system. Preclinical evidence shows that deficient signaling of the major endocannabinoid, anandamide, through upregulated activity of its catabolic enzyme, fatty acid amide hydrolase (FAAH) may be implicated in tobacco use disorder. Studies in animals models of tobacco use disorder have demonstrated that blocking the actions of FAAH mitigated addictive-like behaviors (e.g., decreases nicotine consumption), while administering nicotine to rats reduced anandamide levels in the brain, which may reflect higher brain FAAH concentrations. Thus, higher FAAH levels may be linked to tobacco use disorder. However, no study has investigated FAAH levels in individuals with tobacco use disorder.

Study Aim: In a previously collected sample of individuals with current cannabis use, we explored if tobacco co-use increased FAAH activity compared to cannabis-only use in brain regions implicated in tobacco use disorder [striatum (associative, sensorimotor), amygdala, hippocampus, and cerebellum]. We predicted that brain FAAH levels would be higher in these regions in individuals with co-use compared to cannabis only use.

Methods: Our sample consisted of 12 participants (8 men and 4 women) with current cannabis use between the ages of 18 and 35. Four participants had daily tobacco co-use (2 men and 2 women) and 8 individuals used cannabis only (6 men and 2 women). [11C] CURB is a positron emission tomography (PET) radioligand that can be used to quantify the levels of FAAH in brain in vivo. Participants underwent a one-hour PET imaging session using a High Resolution Research Tomograph (HRRT). A structural magnetic resonance imaging (MRI) scan was completed on a different day for anatomical parcellation of the brain. Arterial blood samples were collected during the PET scan to measure radioactivity in plasma and to provide a metabolite-corrected input function for kinetic modeling. [11C]CURB binding was quantified using the composite rate constant λk3 ($\lambda = K1/k2$), as derived from an irreversible two-tissue compartment model. We used a repeated-measures ANCOVA, controlling for genetic variability known to affect [11C]CURB binding (FAAH rs324420 C > A) and sex.

Results: Groups did not differ on age or cannabis use parameters (recency of use, cumulative use, presence of cannabis use disorder diagnosis). Interestingly, a significant group x ROI interaction was observed $F(4,32) = 2.74$; $p = 0.046$. Bonferroni-corrected pairwise comparisons revealed that [11C]CURB λk3 was significantly higher in the sensorimotor striatum [co-users, $M = 0.167$ (0.17); cannabis-only users, $M = 0.156$ (0.26), $p = 0.03$] and cerebellum [co-users, $M = 0.173$ (0.21); cannabis-only users, $M = 0.145$ (0.29), $p = 0.003$] in co-users compared to cannabis-only users. No other regions showed between-group differences.

Conclusions: In line with preclinical evidence and our hypothesis, preliminary findings suggest that tobacco use may be associated with elevated FAAH activity in people with cannabis use. Our future studies (funded by a Brain and Behavior Research Foundation Young Investigator grant) will examine tobacco use in a sample of individuals with tobacco use disorder, without the confound of concurrent cannabis use, to determine if the same pattern is observed.

Keywords: Tobacco Smoking, Endocannabinoid System, Fatty Acid Amide Hydrolase, Cannabis Use, Positron Emission Tomography (PET)

Disclosure: Nothing to disclose.

P595. Role of the Epigenetic Enzyme EZH2 in Compulsive Alcohol Intake

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Background: Alcohol use disorder (AUD) is a major public health problem accounting for about 5% of the global disease burden. However, treatment for AUD remains challenging, which warrants

the need to better understand AUD and its underlying mechanisms. Alcohol use despite adverse consequences (i.e., compulsive use) is a core feature of AUD and can be modelled in rats using a contingent footshock-punished alcohol self-administration procedure. In a recent study, we have identified the activation of neurons expressing protein kinase C delta (PKCδ) in the central amygdala (CeA) as a key mechanism in regulating this behavior. Here, we aim to identify the molecular mechanisms that promote the activation of the CeA PKCδ neuronal ensemble during compulsive alcohol intake. Epigenetic mechanisms are known to regulate long term transcriptomic changes. Thus, their dysregulation may lead to a transcriptomic reprogramming within the CeA PKCδ neuronal ensemble, and drive "compulsive" alcohol intake.

Methods: To address this hypothesis, we used the NanoString® technology to measure the differential expression of about 22 epigenetic enzymes. We then used a viral vector approach to knockdown the epigenetic enzyme EZH2, which was found upregulated in "compulsive" rats.

Results: We found an increased expression of the epigenetic enzyme Enhancer of Zeste 2 (EZH2) in the CeA of punishment-resistant rats when compared to rats that decreased alcohol intake in presence of footshock ($p < 0.05$). EZH2 is a histone 3 lysine 27 (H3K27) methyltransferase and is a catalytic component of the polycomb repressive complex 2 (PRC2). Using a viral vector approach to knockdown Ezh2, we demonstrated the functional role of EZH2 in compulsive alcohol intake. Two way ANOVA indicated a main effect of group (scrambled vs. EZH2 KD; $F(1,20) = 6.65$; $p = 0.02$) and a significant interaction (group X treatment; $F(1,20) = 5.46$; $p = 0.03$). Post hoc analysis showed a significant decrease in the resistance score in compulsive KD rats compared to compulsive scrambled rats ($p = 0.001$). In accordance with these findings, we also found that pharmacological inhibition of EZH2 using tazemetostat, a potent and selective Ezh2 inhibitor, decreased resistance to punishment in "compulsive" rats and showed no effect in "non-compulsive" rats. Two-Way ANOVA showed a significant interaction: group (non-compulsive vs. compulsive) X treatment (vehicle vs. tazemetostat); $f(1,33) = 5.41$; $P = 0.026$. Post hoc analysis (Newmans keuls) showed significant increase in the number of reinforcements in compulsive vehicle compared to non-compulsive vehicle and EZH2 rats (respectively, $p = 0.002$; $P = 0.004$); as well as compared to compulsive tazemetostat rats ($P = 0.04$).

Conclusions: Together our findings highlight the contribution of epigenetic mechanisms and more particularly EZH2, in mediating compulsive alcohol intake.

Keywords: Alcohol Use Disorder and Drug Addiction, Central Amygdala, Epigenetic Modification

Disclosure: Nothing to disclose.

P596. Cholinergic Signaling in the Habenula-Interpeduncular Nucleus Explains Sex Differences in Alcohol Reinforcement

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Background: Alcohol use disorder (AUD) is the third leading cause of preventable death in the United States and accounts for >$220 billion in healthcare costs and lost productivity each year. Between 2000 and 2016, the prevalence of AUD has increased more prominently in females than males (6% vs. −0.2%), with heavy drinking increased by 14% in females and by 0.5% in males. Females are also at elevated risk of experiencing the negative health effects of alcohol. Indeed, females who suffer from AUD develop alcohol-related liver injury, cardiovascular conditions, and cancers at higher rates than males. The reasons for sex-dependent differences in alcohol consumption are multifaceted and not fully understood. Females report greater stress-provoked negative affect, including anxiety, than males and are more likely to use alcohol for its subjective anxiolytic properties. Female-related gonadal hormones such as progesterone and estrogen also influence the reinforcing effects of alcohol. Nevertheless, the neurobiological mechanisms that explain sex-dependent differences in AUD vulnerability remain unclear. Here, we used cutting-edge cellular and molecular approaches to better understand the underlying mechanisms.

Methods: All experiments involved C57BL/6 and Chrna5-KO mice aged 12-20 weeks. Drinking-in-the-dark and operant responding for ethanol rewards were used to assess alcohol consumption. Anxiety-like behavior was assessed using the light-dark box and open-field procedures. Chromium platform from 10x Genomics with ScanPy-based analysis was used to profile single-cell RNA (scRNA-seq) transcriptomics. Visium with Seurat-based analysis was used to obtain spatial transcriptomics. Multiplexed Analysis of Projections by Sequencing (MAP-seq) was used to profile the connectomes and connectivity strength of targeted neurons with single-cell resolution. Whole-cell recording with optogenetics was used to assess neural activity and synaptic transmission.

Results: Female mice exhibited higher anxiety-like behavior ($p < 0.05$) and ethanol consumption ($p < 0.01$) than males. Spatial transcriptomics identified the interpeduncular nucleus (IPN) as a brain region demonstrating striking differences in transcriptional profiles between female and male mice. scRNA-seq confirmed these sex-differences in transcriptional profiles, with genes involved in cholinergic transmission particularly impacted. Ex vivo recordings revealed that habenula-derived cholinergic transmission in the IPN was mediated predominately by α5 subunit-containing nicotinic acetylcholine receptors (α5 nAChRs) in female mice, and by muscarinic acetylcholine receptors (mAChRs) in males. Ethanol enhanced GABA transmission in the IPN to a greater extent in female than male mice ($p < 0.05$). This sex-dependent difference was abolished in α5 nAChR knockout (KO) mice. Elevated alcohol drinking and anxiety-related behaviors in female relative to male mice were abolished in α5 nAChR KO mice and in female wild-type mice in which α5 nAChR expression in IPN was disrupted using the CRISPR/Cas9 system. Conversely, these sex-dependent differences in behavior were restored in female α5 nAChR KO mice by virus-mediated re-expression of α5 nAChRs in the IPN ($p < 0.05$). α5 nAChR-expressing IPN neurons are known to project prominently to the median raphe nucleus (MRN) and other stress-related brain midbrain and hindbrain regions. Using MAP-Seq, we found that the overall connectomes of IPN neurons did not differ between male and female mice. However, MAP-seq predicted that the strength of synaptic connectivity between the IPN and MRN was weaker in female than male mice. This sex-difference in IPN-MRN connectivity strength was confirmed using whole-cell recordings with optogenetics ($p < 0.05$). Furthermore, lesioning MRN-projecting IPN neurons "feminized" anxiety and ethanol-related behaviors in male mice.

Conclusions: We generated transcriptomic, connectomic, and neurophysiology data that comprehensively characterized the sex-dependent differences in the functioning of IPN neurons in the context of alcohol use and anxiety. Our results identify that the IPN is a major hub mediating sex-dependent differences in these behaviors.

Keywords: Alcohol and Substance Use Disorders, Cholinergic System, Habenula, Sex-Differences, Interpeduncular Nucleus

Disclosure: Nothing to disclose.

P597. Heroin-Seeking Behavior and the Synaptic Proteome are Both Regulated by Phospholipase Cgamma1 in the Nucleus Accumbens

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Background: Chronic opioid use leads to long-lasting increases in drug-seeking behavior; however, the causal molecular and cellular mechanisms responsible are not fully understood. One mechanism may involve the brain-derived neurotrophic factor (BDNF) signaling pathway through its activation of phospholipase Cgamma1 (PLCgamma1) in the nucleus accumbens (NAc). Since opioids increase NAc PLCgamma1 signaling, we hypothesized that reducing PLCgamma1 levels in the NAc would increase heroin-seeking behavior. In addition, since both opioids and PLCgamma1 signaling regulate drug-induced dendritic spine density and morphology, we hypothesized that a reduction of NAc PLCgamma1 levels would modulate the synaptic proteosomal changes that occur following heroin self-administration.

Methods: We first infused a shRNA expression viral vector that reduces PLCgamma1 levels (AAV-shPLCgamma1) or a control virus bilaterally into the NAc of both male and female rats using stereotaxic surgery. Three weeks later we allowed the rats to self-administer heroin for at least 12 days. Following a 7-day abstinence period, we measured context-associated heroin seeking during extinction conditions. In a separate experiment, we again infused AAV-shPLCgamma1 or a control virus into the NAc, then we allowed rats to self-administer either heroin or saline for 12 days in a 2x2 design (shPLCgamma1 vs control virus and saline vs heroin self-administration). After 7 days of abstinence, rats were placed into their self-administration chambers during extinction conditions for 30mins. Rats were then immediately euthanized and NAc tissue was harvested. Next, tissue was enriched for synaptosomes using Syn-PER reagents and proteins were quantified using high resolution multiplexed liquid chromatography mass-spectrometry according to methods of Carlyle et al., 2021 in an attempt to pinpoint the signaling events that may drive increased drug-seeking behavior. All experimental protocols in animal studies were approved by the Medical University of South Carolina's 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: We first found that reducing PLCgamma1 in the NAc led to an increase in context-associated heroin seeking during extinction conditions. We tested equivalent numbers of male and female rats and did not detect any obvious sex differences. We then examined NAc synaptic proteomal changes using mass-spectrometry following heroin or saline self-administration and the PLCgamma1 knockdown condition vs a control virus using a 2x2 design. We found significant changes in the levels of 223 proteins in these enriched synaptosomal NAc samples. Interestingly, a subset of these proteins were decreased in synaptosomes by heroin, then further decreased in the PLCgamma1 knockdown group, suggesting potential causal proteomic changes that could explain how PLCgamma1 reduces drug-seeking behavior and alters the synaptic changes that occur during drug-seeking.

Conclusions: These results show that endogenous NAc PLCgamma1 limits heroin-seeking behavior. These findings suggest that therapeutics targeting PLCgamma1 function might be helpful for treating relapse vulnerability in individuals suffering from opioid use disorder. In addition, PLCgamma1 either alone, or in combination with heroin, is capable of altering the synaptic proteome of the NAc. These results suggest that these NAc synaptic changes could be causal to the increased opioid-seeking behavior observed when PLCgamma1 is experimentally reduced in the NAc. Determining if these synaptic changes are causal to the behavior might reveal new therapeutic targets for treating substance use disorders.

Keywords: Proteomics, Opioid Use Disorder, Nucleus Accumbens

Disclosure: NeuroEpigenix, LLC: Founder (Self).

P598. Cannabis Use Changes Conditioned Stress Responses by Altering the Morphological Plasticity of Astrocytes and the Activity of Matrix Metalloproteinases in the Nucleus Accumbens Core

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Background: The increasing legal status of cannabis and the high comorbidity between cannabis use disorder and post-traumatic stress disorder (PTSD) creates a need to understand how stress and cannabis interact in the brain. Although the use of cannabis has been suggested to self-medicate PTSD, the literature is mixed on whether cannabis improves or aggravates PTSD symptoms. Using acute restraint stress combined with a rat cannabis self-administration paradigm, I recently found that cannabis use promotes two primary PTSD-like symptoms, avoidance coping behaviors and the generalization of stress-coping responses to a neutral stimulus not previously associated with stress exposure. These changes were accompanied by a reduction in spine density in the nucleus accumbency core (NAcore) and a further decrease in spine head diameter after exposure to the stress-conditioned stimulus (stress-CS). Here we sought to determine whether stress and cannabis exposure also affects matrix metalloproteinases (MMPs), and the perisynaptic astroglial processes.

Methods: Rats were restraint stressed for 2h and simultaneously exposed to an odor that became the stress-CS. Control rats were exposed to the same odor in the home cage. Three weeks after acute stress, rats self-administered cannabinoids (delta9-tetrahydrocannabinol+cannabidiol; THC+CBD) or vehicle for 10 days. After 10 days of extinction, we evaluated the effect of the stress-CS on MMP-2,9 activity, astrocyte, and coping strategies in a defensive burying task (DBT). To this end, rats were microinjected with FITC-quenched gelatin into the NAcore immediately before 15 min of DBT. We then used confocal microscopy to quantify astrocyte association with synapses and MMP-2,9 activity.

Results: In vehicle-trained rats, the stress-CS induced active coping (burying), while the THC+CBD prevented this effect by increasing avoidant coping strategies (immobility and self-grooming). Withdrawal from THC+CBD reduced synaptic insulation by astroglia in the NAcore. This effect is partially reversed by the stress-CS. Additionally, the synaptic proximity by astroglia is correlated with the active coping. Moreover, the stress-CS reduced the astrocyte morphology (surface and volume) by increasing the MMP-2,9 activity only in THC+CBD-trained rats. These effects are correlated with avoidant coping strategies.

Conclusions: Taken together, these data suggest that THC +CBD potentially exacerbates stress responses by increasing the MMP-2,9 activity and reducing the astrocyte coverage in NAcore. These findings will help to identify new targets for regulating PTSD and cannabis use disorder comorbidity.

Keywords: Cannabis Use Disorder, Post Traumatic Stress Disorder, Astrocytes, Matrix Metalloproteinases, Nucleus Accumbens

Disclosure: Nothing to disclose.

P599. Cortical Astrocyte Calcium Imaging During Heroin Taking and Seeking

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Background: Findings from rodent models of heroin self-administration (SA) and heroin seeking demonstrate that chronic opioid exposure leads to corticostriatal plasticity mechanistically linked to drug craving and drug seeking. Recent data from our labs and others support a critical role for drug-induced astrocyte dysfunction in the mechanistic underpinnings of this relapse vulnerability. Similar to neurons, elevated intracellular astrocytic Ca2+ levels serve as a reliable biomarker of astrocyte activity, and also like neurons can be measured using genetically encoded Ca2+ indicators. In recent years, it has been established that astrocytes decode afferent information via internal Ca2+ oscillations, responding to bouts of neurotransmission and/or local neuron activity with elevations of intracellular Ca2+ and subsequent release of neuroactive molecules often deemed gliotransmitters. Interestingly, gliotransmitter release can either elevate or suppress synaptic strength or neuronal activity depending on which neuroactive molecule is released. Several studies now demonstrate that astrocyte Ca2+ signaling can orchestrate neuronal synchronization, direct circuit integration, and control neuronal gain and coincidence detection across circuits. Given the complex nature of astrocytic Ca2+ dynamics, data describing the spatiotemporal activity patterns of astrocytes within neural networks in awake, animals active taking or seeking heroin has only just begun to emerge.

Methods: Using virally mediated astrocytic expression of a genetic Ca2+ indicator, two-photon Ca2+ imaging, and head-fixed operant heroin self-administration, we have analyzed activity of astrocytes, longitudinally, in the prelimbic prefrontal cortex (PrL) in animals undergoing heroin SA, extinction training and context-, cue- and drug-induced heroin seeking. Using a custom python pipeline, PrL astrocyte Ca2+ recordings are motion corrected and individual astrocytes are identified and analyzed for Ca2+ activity. These data are are aligned to lever responding, cue presentation and reward delivery at each stage of the heroin taking and seeking paradigm and expressed as astrocyte deltaF/F responses, akin to the field standard for neuronal Ca2+ imaging.

Results: Importantly, we observe significant lever discrimination during head-fixed heroin SA [$F(1,12) = 43.18$ $p < 0.0001$], and significant context- (paired t-test, $p < 0.05$), cue- (paired t-test, $p < 0.05$), and drug-induced reinstatement (paired t-test, $p < 0.05$). During the first few sessions of heroin SA, average astrocyte Ca2+ deltaF/F responses in the 6 second interval following active lever responding are significantly greater than background levels when there is no behavioral output, (unpaired t-test, $p < 0.05$, $n = 7$ mice). This is not the case as heroin SA progresses onward to late stages (sessions 10-11). Interestingly, drug seeking reinvigorates active lever press time locked astrocyte Ca2+ responses in the PrL. Specifically, PrL Astrocyte activity rebounds significantly in the 6 second interval following active lever responding, when reward would normally be delivered, during context (unpaired t-test, $p < 0.05$, $n = 6$ mice), cue (unpaired t-test, $p < 0.01$, $n = 6$ mice) and heroin-primed seeking (unpaired t-test, $p < 0.05$, $n = 5$ mice). Within cued heroin seeking sessions, time locked astrocyte Ca2+ responses following the active lever press and cue presentation were significantly greater when isolating the first 10 cue-reinforced active lever responses compared to the last 10 cue-reinforced active lever responses (unpaired t-test, $p < 0.05$, $n = 6$).

Conclusions: PrL astrocyte activity evolves during heroin SA, gradually being refined downward as heroin SA task is acquired. Time locked astrocyte Ca2+ responses reemerge during context-, cued- and drug-induced heroin seeking. Even within cued heroin seeking sessions, astrocyte activity is dynamically refined, reflecting a potential role for cortical astrocytes in updating contingencies related to availability of drug, which parallels our earlier work in the nucleus accumbens. We predict that these dynamic cortical astrocyte Ca2+ responses are indicative of ongoing astroglial regulation of neuronal activity as heroin taking progresses to heroin seeking. Our ongoing studies have expanded to similar analyses of nucleus accumbens astrocytes using the same model. In both the cortex and nucleus accumbens, we are currently focusing on means for astrocyte manipulation in order to gain a functional understanding of the role that astrocytes play in directing the formation of the neuronal ensembles required for cued heroin seeking.

Keywords: Astrocyte, Heroin, Two-Photon Calcium Imaging

Disclosure: Nothing to disclose.

P600. Unraveling the Role of Central Amygdala PKCδ Neurons: Insights From a Transgenic Rat Model

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Background: The central nucleus of the amygdala (CeA) is a brain region characterized by a high level of cellular heterogeneity, being composed of several functionally and molecularly distinguishable neuronal types, whose role in controlling behavior is highly diverse. It is therefore crucial to investigate the specific contributions of these different cell types to shaping animal behavior. The development of a transgenic mouse line expressing the recombinase Cre on PKCδ-positive neurons (Prkcd-cre mice) has previously allowed to widely scrutinize the role of CeA PKCδ neurons on several relevant behaviors, including fear-conditioning, anxiety, pain processing, and feeding. Importantly, we recently found a contribution of PKCδ-mediated signaling on compulsive alcohol intake in rats. To better define the functional role of CeA PKCδ microcircuits and their output connections in alcohol-related behaviors we developed and validated a novel transgenic rat line expressing Cre under the control of the PKCδ promoter (Prkcd-Cre rat line).

Methods: Prkcd-Cre rat line was developed by genOway applying CRISPR nuclease technology in Wistar genetic background. The presence of the knock-in construct in the offspring was confirmed by PCR and DNA sequencing and further breeding with Wistar wild-type rats confirmed germline transmission. Furthermore, male Prkcd-Cre rats and their wild-type counterparts ($N = 10$-17/group) were evaluated for control behaviors and physiological responses. To confirm specific Cre and PKCδ neuronal expression and further validate CeA PKCδ projection output targets we injected an AAV5-hSyn-DIO-mCherry virus into the CeA of Prkcd-Cre rats ($N = 5$/group). We also examined local and long-range functional connectivity by combining patch-clamp recording with optogenetic stimulation of CeA PKCδ neurons in acute amygdala slices ($N = 6$). The behavioral role of CeA PKCδ neurons was established by optogenetically stimulating these cells during a battery of behaviors ($N = 8$-10/group).

Results: We found a high rate of co-expression between PKCδ and Cre. In the CeA, Cre expression was limited to PKCδ neurons and detected in approximately 62% of all PKCδ cells. Compared to their wild-type counterpart, Prkcd-Cre rats did not display

deficiencies when tested in a range of control behaviors and basal physiological responses. We used anterograde anatomical tracers and electrophysiological recordings to assess local and long-range projection patterns of these neurons. The most prominent CeA PKCδ output projection targets included the bed nucleus of the stria terminalis, the substantia innominata, the substantia nigra pars compact, and the ventrolateral periaqueductal gray area. Finally, we optogenetically stimulated these cells and observed that their activation reduced palatable food intake, promoted aversion, and increased footshock responsivity, but did not alter locomotor activity.

Conclusions: Altogether, our results recapitulate data previously obtained in Prkcd-cre mice suggesting that this novel Prkcd-Cre rat represents a valid tool to investigate the role of CeA PKCδ neurons in a range of behaviors in rats.

Keywords: Central Amygdala, PKCdelta, Transgenic Rat Model

Disclosure: Nothing to disclose.

P601. Biased Allosteric Modulator of the Neurotensin Receptor 1 Confers G Protein Selectivity

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Background: The neurotensin receptor 1 (NTSR1) regulates the mesolimbic dopaminergic system and is a promising anti-addiction therapeutic target. NTSR1 is a neuropeptide G protein-coupled receptor (GPCR) that signals through G protein- and β-arrestin-mediated pathways. Clinical development of balanced NTSR1 agonists that activate both pathways is precluded by their on-target side effects. Biased allosteric modulators, typified by the compound SBI-553, may be the path forward for NTSR1 drug development efforts. SBI-553 activates β-arrestin but not canonical Gq protein signaling. SBI-553 also confers β-arrestin bias to neurotensin (NT), the endogenous ligand, by selectivity antagonizing its Gq signaling. SBI-553 attenuates cocaine self-administration and methamphetamine-induced conditioned place preference and hyperlocomotion in rodent models but does not produce the thermal and hemodynamic side effects characteristic of balanced NTSR1 agonism. The mechanism by which SBI-553 exerts its behavioral and physiological effects has yet to be elucidated. Neuronal GPCRs signal through one or more of 16 Gα proteins (e.g., Gq, Gi, Go, Gs) as well as through β-arrestins. While NTSR1's calcium-dependent physiological effects implicate Gq/11 as preferred signaling mediators, NTSR1 has been shown to activate at least 12 different G proteins in model cell systems. SBI-553 does not activate Gq and blocks NT-induced Gq activation, but its effect on other G proteins have not been thoroughly explored. Here, we present the most comprehensive assessment of SBI-553's ability to modulate NTSR1 signaling to date and provide new insights into its mechanism of action.

Methods: We assessed SBI-553-induced NTSR1 signaling in HEK293T cells transiently expressing the receptor using an effector panel that included 14 G proteins and β-arrestin1 and β-arrestin2. NT and the competitive antagonist SR142948A served as positive and negative controls, respectively. Cells were treated with NT (10 pM-10 μM), SR142948A (100 pM-100 μM) and SBI-553 (100 nM-100 μM) alone and in combination. G protein activation was monitored using a BRET2-based platform consisting of an Rluc8-tagged Gα and a GFP2-tagged Gγ subunit. β-arrestin recruitment was monitored using a BRET1-based assay using an Rluc8-tagged receptor and mVenus-tagged human β-arrestin1 or β-arrestin2. 96-well plates containing cells expressing NTSR1 and effector activation sensors were treated and read every 5 min for 20 – 40 min. BRET ratios were computed as the ratio of GFP2 or mVenus emission to RLuc8 emission. The net BRET ratio was calculated by subtracting stimulated GFP2 or mVenus/Rluc8 ratios from control ratios. The maximum change in net BRET ratio over time was averaged within treatments and combined between experiments. Mean net BRET ratios from at least 3 independent experiments were fit using a 4-parameter sigmoidal model in GraphPad Prism version 9.5.1. Curve fits were compared using the extra sum-of-squares F test. A p-value of < 0.05 was accepted as statistically significant.

Results: In line with previous results, both NT- and SBI-553-induced NTSR1 β-arrestin1/2 recruitment, and, in combination studies, SBI-553 was permissive of NT-induced β-arrestin translocation. In G protein activation assays, NT activated 12 of the 14 G proteins assessed. Notably, SBI-553 exhibited previously unappreciated G protein activity. SBI-553 acted as a weak partial agonist of G15, Go, and G12/13. In combination with NT, SBI-553 exhibited complex allosteric interactions that were highly G protein-dependent. SBI-553 noncompetitively antagonized NT-NTSR1 Gq-family G protein activation but permitted NT-NTSR1 Gi/o- and G12/13-family G protein activation. SBI-553 fully or partially reduced NT-induced activation (i.e., reduced NT concentration-response curve Emax) of Gq [F (DFn, DFd), p value] = [30.29 (4, 225), $p < 0.001$], G11 [42.25 (4, 225), $p < 0.001$], G15 [15.73 (4, 225), $p < 0.001$], Gi1 [5.849 (4, 225), $p < 0.001$], Gi2 [6.212 (4, 225), $p < 0.001$], Gi3 [152.0 (4, 225), $p < 0.001$], Gz [61.57 (4, 225), $p < 0.001$], and Gg [4.394 (4, 305), $p < 0.01$]. SBI-553 did not reduce NT-induced activation of GoA [1.765 (4, 225), $p = 0.1369$], GoB [0.1702 (4, 225), $p = 0.9534$], G12 [0.1168 (4, 273), $p = 0.659$] or G13 [1.735 (4, 225), $p = 0.143$]. SBI-553's promotion of NT G protein selectivity contrasted with the action of SR142948A, which uniformly and competitively antagonized NT effector activation.

Conclusions: These findings suggest that SBI-553 biases NTSR1 toward both β-arrestin recruitment and noncanonical G protein signaling. The role of this noncanonical G protein signaling in SBI-553's effects on stimulant locomotion, conditioned place preference, and intravenous self-administration is an ongoing area of investigation. These findings reveal uniquely complex allosteric interactions and suggest the ability to generate small molecule compounds with exquisite effector selectivity, laying the foundation for pathway-selective drug discovery efforts.

Keywords: Substance Use Disorder, Neurotensin, G Protein-Coupled Receptors, Biased Signaling, Allosteric Modulator

Disclosure: Nothing to disclose.

P602. Somatostatin Peptide Signaling in the Cortex During Healthy and Dysregulated Brain States

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Background: Somatostatin neurons play a role in a range of behaviors, and clinical evidence points to a pro-resiliency, protective role of this peptide. However, the neuromodulatory role of the peptide itself, particularly within the prefrontal cortex or its dysregulation in diseases and therapeutic potential is less characterized.

Methods: SST peptide effects were established using whole-cell patch clamp electrophysiology in naïve C57Bl/6J male and female mice. Similar approaches were used to explore pathway-specific effects, using combinations of ChR2 and retrograde AAVs in SST-IRES-Cre mice. For alcohol exposure studies, we employed the 'drinking in the dark' binge drinking model, where mice reach clinically significant blood alcohol concentrations (above 0.08%). Behavioral data was analyzed by 2 way ANOVA were applicable

(sex x drug). All electrophysiology had a range of 5-10 cells per group (maximum of 2 cells per animal) and was analyzed by t-tests, 2-way ANOVA, or generalized linear modeling.

Results: SST dampens cortical microcircuits through inhibition of both local GABA neurons and projection neurons. We found specificity of this long-range modulation, with greater inhibition of NAC-projecting circuits as compared to BNST-projecting circuits, and a broad dysregulation of this system following a mouse model of voluntary binge alcohol consumption.

Binge alcohol consumption reduced ChR2-evoked SST release from PFC SST neurons. Binge drinking leads to a significant reduction in SST release and post hoc analysis revealed a significant reduction in ChR2-evoked (10 Hz) SST release in the binge drinking group only.

We further explored bath application of SST onto PFC pyramidal neurons, and found that while SST led to a significant decrease in membrane potential in control females and control males. SST did not significantly decrease membrane potential in binge drinking females or males – suggesting compensatory downregulation in SST receptor function. Interestingly, this was not driven by changes in PFC-->NAC circuits, as SST effects were similar in control and binge drinking mice.

Conclusions: SST pharmacologically modulates cortical circuits, and is modified by, and potential treatment of, binge alcohol consumption.

Keywords: Somatostatin, Medial Prefrontal Cortex, Binge Drinking, Pharmacology

Disclosure: Nothing to disclose.

P603. Cellular and Transcriptional Correlates of Drug-Associated Memories in the Nucleus Accumbens

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Background: Substance use disorders exemplify a maladaptive imbalance wherein drug seeking and taking persists despite negative consequences of drug use. Such imbalance is orchestrated by neurobiological adaptions linked to faulty cellular, epigenetic, and transcriptional modifications in brain reward regions such as the nucleus accumbens (NAc). However, while these events are commonly assigned to withdrawal, extinction, or renewal/relapse phases, there is a pressing need to characterize these alterations in a sex-, subregion-, and cell-specific manner.

Methods: Here, we used cocaine self-administration (SA) in rats combined with RNA-sequencing (RNAseq) of NAc subregions (core and shell) to transcriptionally profile the impact of extinction learning on countering withdrawal- and renewal-associated drives.

Results: As expected, rats receiving extinction training in the original SA context (levers/cues) significantly reduced their seeking when compared with rats receiving forced abstinence in either their home cages or the original SA context. Further analysis showed that undergoing withdrawal in the original drug context promotes incubation of drug seeking. Consistent with this observation, subsequent bioinformatic analyses revealed distinct transcriptional patterns of this group when compared with home cage withdrawal or extinction training. Additional studies extend these findings by identifying the cellular and transcriptional basis of transferring extinction memories across contexts. These experiments involve rats acquiring and extinguishing in different contexts followed by transcriptomic analyses and patterns of opposing phenotypes (i.e., extinction vs. renewal). Complementary to these datasets, and with the goal of cell-specific characterizations, we are using chemogenetics, fiber photometry, and slice electrophysiology of NAc subregions and cell types (D1 vs. D2 medium spiny neurons) in a sex-dependent manner.

Conclusions: Together, these approaches are providing unprecedented evidence of how extinction, withdrawal, and renewal reprogram cellular activity and transcriptomics of the NAc, insight which will guide identification of new ways of preventing relapse.

Keywords: Extinction, Dopamine, Medium Spiny Neuron, Renewal

Disclosure: Nothing to disclose.

P604. Acute Morphine and Glucocorticoid Signaling Regulate Heat Shock Gene Expression in Astrocytes

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Background: Opioid use disorder affects millions of individuals worldwide, and overdose-related deaths remain a public health crisis in the United States, highlighting the need for continued development of targeted intervention strategies. Opiates, including morphine, are often prescribed in a clinical setting for the treatment of chronic pain. Both pain and opioid experience induce transcriptional responses in brain regions that mediate responses to reward and contribute to the development of substance use disorders, like the ventral tegmental area (VTA).

Methods: To better understand cell-type specific transcriptional alterations engaged by initial morphine exposure, and how this interacts with pre-existing pain state, we performed single-nucleus RNA sequencing (snRNA-seq) on 54,954 nuclei from the VTA of adult rats that received acute morphine in the presence or absence of chronic pain.

Results: We found that heat shock-associated genes, including Fkbp5, were selectively upregulated in glial populations after morphine exposure regardless of pain state. Interestingly, heat shock gene expression was elevated in astrocytes despite sparse expression of classical opioid receptors. Previous literature has implicated a role for glucocorticoid signaling in regulating both molecular adaptations to morphine and the expression of heat shock genes. Further, the glucocorticoid receptor Nr3c1 was highly enriched in VTA astrocytes. To examine glucocorticoid contribution to the transcriptional responses we observed in vivo, we next employed a human-derived astrocyte cell culture system to investigate the mechanisms behind opioid and glucocorticoid-induced gene regulation. We found that treatment with the endogenous glucocorticoid cortisol, and the selective glucocorticoid receptor (GR) agonist dexamethasone, were both sufficient to drive FKBP5 expression in human astrocytes. Further, cortisol and dexamethasone induced-induced changes in FKBP5 were blocked by pre-treatment with the GR antagonist mifepristone.

Conclusions: These results suggest GR activation is required for the induction of FKBP5 in human astrocytes. Ongoing experiments will continue to explore the role of glucocorticoid signaling in mediating transcriptional and behavioral responses to morphine using a CRISPR inhibition (CRISPRi) approach to selectively target GRs in astrocytes in vitro and in vivo.

Keywords: Opioid Addiction, Ventral Tegmental Area (VTA), Single Nucleus RNA Sequencing, Glucocorticoids

Disclosure: Nothing to disclose.

P605. Neuropharmacological Evidence Implicating Drug-Induced Glutamate Receptor Dysfunction in the Affective and Cognitive Sequelae of Subchronic Methampehtamine Self-Administration

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Background: Anxiety is one of the most common comorbid neuropsychiatric conditions with methamphetamine (MA) use disorder and the abrupt discontinuance of MA following chronic exposure induces withdrawal symptoms in humans consisting of depression and anxiety that can drive continued drug use and relapse to drug use in MA-abstinent individuals. In addition to affective symptoms, methamphetamine (MA) use disorder is also characterized by cognitive impairments that impede treatment prognosis and recovery. In humans with MA use disorder, cognitive dysfunction is theorized to reflect glutamate-related neurotoxicity within frontal cortical regions. However, relatively little is known regarding the psychobiological consequences of subchronic MA exposure, more typical of recreational MA use. To that end, we worked to develop a mouse model of high-dose oral MA self-administration under operant-conditioning procedures to examine the effects of a relatively brief history of oral MA upon brain and behavior.

Methods: For this, adult male and female C57BL/6J (B6) mice were trained to orally self-administer water or one of three MA concentrations (0.8, 1.6 and 3.2 g/L) under a fixed ratio 1 reinforcement schedule for 1 h/day for 7 consecutive days. Then, negative affect was assayed using a behavioral test battery consisting of the light-dark shuttle-box, elevated plus maze, novel object reactivity test, marble-burying, acoustic startle and forced swim tests. Following affective testing, spatial learning and memory were determined in a Morris water maze, followed by testing for reference and working memory in a water version of the radial arm maze. Immunoblotting was then conducted on tissue from ventral and dorsal prefrontal cortex (vPFC and dPFC) to examine for glutamate receptor correlates of abnormal affective and cognitive behavior. Follow-up pilot studies were then conducted to determine the relationship between a MA-induced reduction in NMDA receptor expression and reversal learning deficits in the Morris water maze and a MA-induced increase in mGlu1 expression in the manifestation of anxiety. For this, male and female mice were pretreated systemically with 0.2 mg/kg of the NMDA receptor antagonist MK-801 prior to testing for reversal learning in the Morris water maze, while a different cohort of mice were pretreated with 5 mg/kg of the mGlu1 antagonist JNJ16259685 prior to testing for negative affect. The data were analyzed using analyses of variance, with alpha set to 0.05, followed by Tukey's post-hoc tests, when appropriate.

Results: Females consumed more MA than and males [Sex: $F(1,78) = 4.255$, $p = 0.043$]. MA-3.2 increased the number of marbles buried [Dose: $F(3,109) = 5.950$, $p = 0.001$]. While a MA effect was also detected for the number of contacts with the novel object [Dose: $F(3,109) = 3.309$, $p = 0.023$], no post-hoc group differences were observed. Both the 0.8 and 3.2 g/L MA doses reduced the number of investigatory dips over the edge of the elevated plus maze [Dose: $F(1,108) = 23.862$, p0.05).

Conclusions: Taken together, these data indicate that a relatively brief history of oral MA is sufficient to induce some signs of anxiety-like behavior and cognitive dysfunction during early withdrawal that may reflect, at least in part, a MA-induced changes in the expression of certain glutamate receptor subtypes within the vPFC.

Keywords: Methamphetamine Self-Administration, Anxiety, Cognitive Dysfunction, NMDA Glutamate Receptors, Metabotropic Glutamate Receptor

Disclosure: Nothing to disclose.

P606. Relapse to Cocaine Self-Administration is Regulated by Medial Habenula Nr4a2

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Background: Recent studies have implicated the medial habenula in cocaine-associated behaviors, yet the role of the medial habenula in regulating reinstatement of cocaine self-administration remains unknown. The lab recently identified the histone deacetylase 3 (HDAC3; a powerful epigenetic regulator of gene expression) target gene, nuclear orphan receptor subfamily4 groupA member2 (Nr4a2), as an important for reinstatement of cocaine conditioned place preference. NR4A2 is a transcription factor that regulates aspects of dopamine signaling during development, and is densely expressed in the medial habenula. Further, Nr4a2 expression is altered by cocaine exposure. We hypothesized that reducing medial habenula NR4A2 function would reduce reinstatement of cocaine self-administration.

Methods: The studies below were approved by the UCI 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. Using cre-dependent AAV, the transcriptionally inactive dominant negative form of NR4A2 (NURR2C, $n = 14$) or GFP (GFP, $n = 8$) was expressed in medial habenula cholinergic neurons of ChAT-Cre mice. Mice were then trained to self-administer cocaine. During self-administration active lever presses resulted in a cocaine reward (8.5µl/kg/infusion) and a cue presentation (light/tone). To facilitate reinstatement behavior, an incubation of craving model was used in which animals were given 30 days of homecage withdrawal after 12 days of self-administration. After the withdrawal period, mice were extinguished for 5 hours, wherein pressing on the previous active lever was inconsequential. Immediately after extinction, mice were re-exposed to drug-associated cues to drive cued reinstatement.

To develop a more in-depth understanding of the medial habenula response to reinstatement of cocaine-seeking, and to observe the network of genes impacted by Nr4a2 during reinstatement, single nucleus RNA sequencing (snRNAseq) was performed on habenula tissue collected one hour after reinstatement.

Sex as a Biological Variable: The data described in this abstract come from male mice only. Because there are documented instances of estrous cycle effects and sex differences in aspects of drug-seeking behaviors, ongoing work for this project examines both males and females. We have consulted the NIH's guidelines on sex as biological variable and have begun to incorporate routine monitoring of the estrous cycle in our ongoing work.

Results: During self-administration, presses on the active lever were greater than presses on the inactive lever in both groups (GFP: $F(1,14) = 38.60$, $p < 0.0001$; NURR2C $F(1,26) = 21.52$, $p < 0.0001$). While there were no differences between groups in rates of self-administration (SA) or extinction (EXT) (SA $F(1,20) = 0.356$, $p = 0.556$; EXT $F(1,20) = 0.421$, $p = 0.524$), NURR2C mice had dramatically reduced reinstatement compared to GFP controls ($t(11) = 2.70$, $p = 0.014$).

After reinstatement, transcriptome perturbation analysis revealed that clusters of both medial and lateral habenula neurons are highly perturbed by the medial habenula NURR2C manipulation, suggesting a novel interaction between these regions during relapse-like behavior. We were also able to identify hundreds of differentially expressed genes in medial habenula neurons. We used weighted gene coexpression network analysis to identify groups of genes (modules) that go up or down together, that are thought to function together. To identify associated biological mechanism, we examined the gene ontology enrichment in these modules, and found that these modules are associated with nicotine, alcohol, and opioid addiction; as well as GABAergic and glutamatergic signaling. Of our 8 modules, modules 2 and 7 were highly enriched for addiction related gene ontology, inviting deeper consideration. When we looked for genes within these modules that were highly connected to other genes (hub genes, candidate network regulators), we found several overlapping hub genes between modules 2 and 7, and these have become areas of active investigation in the lab.

Conclusions: These findings necessitate consideration of the medial habenula and Nr4a2 as pivotal contributor to relapse behavior, and identify the nuclear orphan receptor NR4A2 (with recently identified exogenous ligands) as a therapeutic target for medicinal chemistry to develop agonists/antagonists that may be relevant for addiction treatment. Lastly, transcriptomic changes in lateral habenula nuclei indicate more interplay between the medial and lateral habenula than previously thought, which needs further study.

Keywords: Alcohol and Substance Use Disorders, Drug Relapse, Epigenetic Regulation

Disclosure: Nothing to disclose.

P607. Dorsal Raphe and Orbitofrontal Cortical Serotonin Signaling in Excessive Alcohol Intake

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Background: Alcohol induces plasticity in dorsal raphe (DR) serotonin systems, resulting in dysregulated output and modifications in downstream regions. The orbitofrontal cortex (OFC) is a site of DR 5-HT input that may contribute to alcohol use disorder by driving excessive and compulsive intake. Here, we assessed alcohol-induced changes in OFC 5-HT signaling in mice and nonhuman primates (NHP) and examined their role in promoting excessive alcohol intake.

Methods: Patch clamp recordings were performed in OFC slices from mice following a drinking in the dark (DID) assay and rhesus macaques after 1 year of open access to alcohol. Quinine adulterated alcohol was used to measure aversion-resistant drinking. In situ hybridization was used to quantify Htr1a and Htr2a mRNA expression. Htr1a was deleted in OFC pyramidal neurons (PN) by injecting AAV5-CaMKIIa-GFP-Cre in Htr1a floxed mice and Htr2a was deleted by co-injecting AAV5-CAMKIIa-Cre and AAV1-FLEX-saCas9-sgHTR2A in C57BL/6J mice. The DR 5-HT-OFC circuit was chemogenetically modulated by injecting the OFC of ePet1-cre mice with retrograde AAVs encoding for hM3 and hM4.

Results: Bath application of 5-HT hyperpolarized OFC PN in low-drinking (LD) NHP and H2O control mice. This effect was absent in binge-drinking NHP ($n = 2$-4 NHP/sex, $p < 0.05$ vs LD) and mice after DID ($p < 0.01$ vs H2O; $n = 4$-6 mice/grp/sex). Quantification of OFC Htr1a and Htr2a mRNA showed that Htr1a was reduced after DID ($p < 0.01$), suggesting the loss of 5-HT inhibition was mediated by reduced 5-HT1a. We next tested the role of 5-HT1a and 5-HT2a in binge alcohol intake by deleting these receptors in OFC PN. Htr1a deletion increased binge alcohol intake in males ($p < 0.05$ vs GFP; $n = 10$/grp) but not females ($n = 7$-8/grp) and promoted aversion-resistant alcohol drinking in both sexes ($p < 0.05$). Htr2a deletion had no effect ($n = 7$-8/grp/sex). In males ($n = 8$-10/grp/sex) hM3 activation in DR 5-HT-OFC neurons decreased alcohol intake ($p = 0.01$ vs GFP) and preference ($p < 0.001$) while inhibition decreased intake only ($p = 0.028$).

Conclusions: Binge alcohol alters OFC 5-HT signaling in mice and rhesus macaques. This effect is mediated by a loss of Htr1a, which drives excessive and aversion-resistant alcohol intake. Our findings also establish that DR 5-HT input to the OFC and 5-HT1a receptors in this cortical region sex-specifically regulate binge-like alcohol intake and aversion-resistant drinking in mice. Overall, this work identifies a conserved mechanism across species that may underly dysregulated cortical serotonin signaling following binge alcohol drinking. Thus, we suggest that there may be therapeutic potential in targeting OFC 5-HT1a to treating excessive alcohol drinking.

Keywords: Serotonin, 5-HT1A Receptors, Binge Alcohol Use, Nonhuman Primate Models, Mouse Models

Disclosure: Nothing to disclose.

P608. PCSK9 Inhibition Attenuates Alcohol-Associated Neuronal Oxidative Stress and Neuroinflammation

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Background: Alcohol use disorder (AUD) is a chronic condition associated with neuroinflammation, neuronal oxidative stress, and neurodegenerative processes. Although proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition (PCSK9i) has shown promise in reducing alcohol-associated liver inflammation, its effects on the brain remain largely unexplored. This study aimed to evaluate the effects of alirocumab, a monoclonal antibody that reduces systemic low-density lipoprotein cholesterol (LDL-C) via PCSK9i, on central nervous system (CNS) pathology in a rat model of chronic alcohol exposure.

Methods: Alirocumab (50 mg/kg) or vehicle was administered weekly for 6 weeks in 32 rats receiving either a 35% alcohol liquid diet or a control liquid diet ($n = 8$ per group). The expression of PCSK9, LDL receptor (LDLR), oxidative stress, and neuroinflammatory markers was assessed in brain tissues.

Results: Chronic alcohol exposure upregulated PCSK9 expression in brain while alirocumab treatment significantly attenuated PCSK9 levels, upregulated neuronal LDLR, and reduced oxidative stress in neurons and brain vasculature (3-NT, oxLDL, p22phox). Furthermore, PCSK9i lowered alcohol-induced recruitment of microglia in cortex and hippocampus (Iba1). The treatment also decreased the expression of pro-inflammatory cytokines and chemokines (CCL2, CXCL3, TNF) in the whole brain tissue and attenuated the upregulation of adhesion molecules in brain vasculature (ICAM1, VCAM1, e-selectin).

Conclusions: This study provides new evidence that PCSK9i decreases oxidative stress and neuroinflammation in the brain produced by chronic alcohol exposure. Further research is warranted to elucidate the underlying mechanisms by which PCSK9 signaling affects the brain under chronic alcohol exposure.

Keywords: Alcohol Use Disorder - Treatment, Oxidative Stress, Neuroinflammation, Monoclonal Antibodies

Disclosure: Nothing to disclose.

P609. Changes in Stress Neuropeptide Regulation of Dopamine Release Following Long-Term Ethanol Consumption in Rhesus Macaques

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Background: Alcohol use disorders (AUD) affect over 16 million individuals each year in the US. AUDs are complex and multifaceted brain disorders that affect several brain signaling systems including dopamine and corticotropin-releasing factor (CRF), each of which has been studied extensively individually. However, little work has examined the interaction of CRF and dopamine, particularly in models of AUD. Thus, the current study examined the effects of CRF on dopamine signaling in the caudate nucleus and putamen, two brain regions implicated in AUD, of long-term alcohol consuming rhesus macaques.

Methods: We used fast-scan cyclic voltammetry to assess the effects of CRF (200nM) on electrically evoked dopamine release in ex vivo caudate nucleus or putamen brain slices from eight control or eight long-term drinking male rhesus macaques. We also performed real-time PCR to examine the expression of several CRF-related genes including CRF, CRF Receptor 1 (CRF1), CRF Receptor 2 (CRF2), and the CRF binding protein (CRFBP).

Results: We found that bath application of CRF decreased evoked dopamine release in brain slices from control subject in both caudate nucleus and putamen. Interestingly, in putamen brain slices from long-term ethanol consuming subjects, application of CRF had no effect on dopamine release, suggesting a downregulation of CRF signaling. We followed up by examining the gene expression of CRF-related genes. We found several changes in long-term ethanol consuming subjects including a significant decrease in CRF1 and CRFBP in caudate and trends towards decreases in caudate CRF, putamen CRF2, and putamen CRFBP.

Conclusions: Altogether, these data support the idea that long-term alcohol consumption can alter brain reward signaling to be resistant to the effects of the stress neuropeptide CRF in the putamen, a brain region associated with the development and expression of habitual or inflexible behaviors.

Keywords: Corticotropin Releasing Factor, Striatum, Dopamine, Voltammetry

Disclosure: Nothing to disclose.

P610. Unraveling the Epigenetic Landscape of Compulsive-Like Alcohol Drinking

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Background: Alcohol use disorder (AUD) afflicts close to 15 million individuals in the US, and effective interventions remain limited. The major hallmark of AUD is the persistent use of alcohol despite experiencing negative consequences, often leading to relapse even after conscientious and persistent attempts to achieve long-term abstinence. While there have been several genetic variants linked to AUD, they account for only a small fraction of overall phenotypic variance, and, moreover, most of these variants are located within non-coding regulatory regions rather than protein-coding genes. These findings suggest that potent epigenetic mechanisms can play a critical role in AUD vulnerability, but, currently, specific epigenetic factors and their exact influence on the transition from hedonic to persistent alcohol use have not been elucidated. Delineation of these epigenetic parameters has the potential to identify new therapeutic targets that can improve outcomes for AUD.

Methods: Our laboratory has developed a unique and novel mouse model of AUD that can be used to study epigenetic influences associated with the disease. We trained C57BL6/J mice ($n = 96$) in an operant alcohol self-administration task, where a 20% alcohol solution in tap water was made available for 60 seconds following a successful response on a lever. After establishing a stable level of alcohol consumption, alcohol drinking despite adverse consequences was assessed by measuring consumption after quinine adulteration or pairing alcohol delivery with a mild foot shock.

Results: Mice were classified using unsupervised cluster analysis into three distinct categories based on their alcohol consumption patterns: non-drinkers (~19%: completely abstain from alcohol during SA experiments; low drinkers (~24%; consume minimal amounts of alcohol that do not lead to intoxication levels; high drinkers (~57%; consume alcohol to the point of intoxication). Among high drinkers, two subgroups could be identified: punishment-sensitive mice (~26%: exhibit a notable decrease in alcohol consumption during punishment sessions; and punishment-resistant mice (~31%; continue to consume alcohol despite punishment, reflective of AUD. Single-nucleus RNA sequencing was used to conduct a comparative transcriptomic analysis between punishment-resistant, punishment-sensitive, and water-control mice, focusing on the dorsomedial striatum (DMS) - a brain region that plays a critical role in goal-directed behaviors, habits, and compulsions. We have sequenced a total of 700,000 nuclei and are currently conducting a comprehensive and unbiased analysis to characterize the diverse range of differentially expressed genes in each one of DMS' cell types. A concurrent follow-up analysis is being conducted to investigate the correlation between histone markers, chromatin accessibility, and behavioral responses in mice categorized as punishment-resistant, punishment-sensitive, and water control groups.

Conclusions: This comprehensive exploration will not only deepen our comprehension of the epigenetic determinants implicated in AUD susceptibility but also hold the potential to inspire the emergence of innovative therapeutic interventions for this debilitating condition.

Keywords: Alcohol Epigenetic Marks, Alcohol Abuse, Single Cell RNA-Seq, Compulsive Drug Intake

Disclosure: Nothing to disclose.

P611. An EEG Signature of MCH Neuron Activities Predicts Cocaine Seeking in Rats

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Background: Identifying sleep biomarkers that may reveal pathophysiology or the effectiveness of therapeutics will greatly advance our health care for psychiatric diseases including substance use disorders (SUDs). We demonstrated a close relationship between rapid eye movement sleep (REMS) and cocaine seeking after withdrawal, using a rat model of cocaine

self-administration (SA). Furthermore, we found that the melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus (LH) are an important contributor to REMS-induced anti-relapse effects. Recently, we discovered a sleep electroencephalogram (EEG) derivative which was strongly correlated with the real-time MCH neuron calcium activities in vivo. Potential biomarkers from this EEG feature will have important implications for SUD research and therapeutic development.

Methods: Sprague Dawley rats (Male and Female) at postnatal ~8 wk received unilateral intra-LH injection of AAV5-PMCH-GCaMP6f and implantation of optic fiber and EEG/EMG electrodes. Dual-color fiber photometry (465 nm for Ca2+ and 405 nm for isosbestic control) and EEG were recorded ~2-6 wk later, and analyzed using MatLab and Somnivore®. For cocaine SA, rats underwent jugular surgery at postnatal ~ 6-7 wk and started training at ~ 8 wk (0.75 mg/kg/ infusion, fixed ratio 1, 1 overnight + 2 h/d x 5 d). Rats then underwent withdrawal (WD) in home cages. 24 h EEG recordings were performed both before cocaine exposure and after WD. EEG Ratio was calculated using optimized empirical formula and compared to machine learning-based predictions. Correlation analysis was used to assess EEG Ratio features in predicting future cocaine seeking in rats.

Results: Ca2+(MCH) activities during REMS were accompanied by changes in EEG theta and delta magnitudes and a shift in theta peak frequency. EEG Ratio formula was optimized with cross-validations to best correlate EEG Ratio with sec-by-sec Ca2+(MCH) amplitude across sleep and wake states. The EEG Ratio – Ca2+(MCH) correlation was robust across sex, circadian phase, REMS manipulations, and cocaine experience. Furthermore, cocaine SA and WD led to changes in the 24-h EEG Ratio distributions, consistent with the changes in REMS. Finally, features of EEG Ratio at both baseline sleep and after long-term WD showed correlations with cocaine seeking during SA training or after WD.

Stats:

1. There is a positive correlation between EEG Ratio and Ca2+(MCH) activity in vivo in both male and female rats (correlation r values: pilot male, $r = 0.72 \pm 0.02$, $n = 9$; pilot female, $r = 0.72 \pm 0.03$, $n = 5$; total $n = 42$)
2. Ca2+(MCH) activity co-varies with the EEG Ratio under different treatments including environmental warming and DREADDsGq-activation of MCH neurons. (Linear regression: $r = 0.966$, $p < 0.0001$, total $n = 20$)
3. After cocaine SA and 21-d WD, there was a shift of EEG Ratio events from dark phase to light phase (total time: phase x WD d, $F1,54 = 9.957$, $p < 0.01$; dark phase $p < 0.05$, light phase $p = 0.07$; two-way RM ANOVA). REMS time showed a similar shift (total time: phase x WD d, $F1,54 = 12.97$, $p < 0.001$; dark phase $p < 0.001$, light phase $p = 0.06$; two-way RM ANOVA).
4. The dark-light distribution of EEG Ratio events before cocaine exposure was positively correlated with future cocaine intake during SA training (total event duration dark/light: $r = 0.657$, $n = 31$, $p < 0.001$; total event AUC dark/light: $r = 0.589$, $n = 31$, $p < 0.001$).
5. EEG Ratio cluster analysis revealed that the EEG Ratio 24-h total # of long clusters after long-term WD was inversely correlated with cocaine seeking on WD d45 ($r = -0.596$, $n = 16$, $p < 0.05$).

Conclusions: There is a strong positive correlation between EEG Ratio and Ca2+ activities in LH MCH neurons in rats. The EEG Ratio provides novel functional measures of REMS, a potential means to assess MCH neuron activities in vivo, and various quantifiable measures to relate to behavioral outcomes.

Keywords: Sleep, EEG, MCH Neurons, Biomarker, Cocaine

Disclosure: Nothing to disclose.

P612. Increased GFAP Expression in the Rostromedial Tegmental Nucleus During Acute Withdrawal From Chronic Ethanol Exposure

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Background: Alcohol use disorder (AUD) is a complex brain disease characterized by excessive drinking and the inability to stop despite adverse consequences. Approximately 50% of individuals with AUD experience withdrawal symptoms upon abstaining from alcohol, including anxiety, which can trigger alcohol relapse. The rostromedial tegmental nucleus (RMTg) is a brain region involved in processing aversive stimuli and negative affect. Previous work from our laboratory suggests that the RMTg is also involved in the mechanisms underlying symptoms of withdrawal. In support of this, we observed significant cFos induction in the RMTg during acute withdrawal and reduced anxiety-like behavior when the RMTg was pharmacologically inactivated. However, the precise mechanism driving this heightened RMTg activity during withdrawal remains unclear. Emerging data suggests that chronic ethanol exposure significantly alters astrocyte morphology in a number of brain regions. Given that astrocytes modulate neuronal activity at tripartite synapses it is likely that structural changes in these cells lead to alterations in synaptic transmission.

Methods: To explore the effect of withdrawal from chronic ethanol exposure on RMTg astrocytes, Long-Evans rats underwent a chronic intermittent ethanol (CIE) vapor exposure paradigm, where they were exposed to ethanol vapor or room air for 14 hours per day for 14 consecutive days. Twelve hours after their final vapor session, rats were transcardially perfused with 4% paraformaldehyde and the brain was extracted. Slices containing the RMTg were double-labeled for glial fibrillary acidic protein (GFAP), an astrocyte marker, and FoxP1, a transcription factor whose expression is enriched in the RMTg, using standard immunofluorescence. Labeling was analyzed across the rostrocaudal extent of the RMTg by measuring percent area stained and integrated density using ImageJ. Statistical analyses were conducted to compare RMTg GFAP expression between AIR- and CIE-exposed rats.

Results: GFAP expression was consistent across the rostrocaudal extent of the RMTg in AIR controls. A significant increase in GFAP expression was observed in CIE-exposed rats compared to AIR controls when data from the entire rostrocaudal extent of the RMTg were analyzed together (*$p < 0.05$; nested t-test). Closer inspection of these data at each rostrocaudal level analyzed revealed that that this effect was driven primarily by group differences apparent toward the rostral extent of the RMTg.

Conclusions: These data suggest that withdrawal increases astrocytic complexity in the RMTg and provide insight into how chronic ethanol exposure affects astrocyte morphology and distribution within the RMTg. Overall, these findings point to a possible mechanism by which astrocytic glutamate drives heightened RMTg activity as a result of greater astrocyte-neuron interactions in this region during acute withdrawal. Future work will explore this possibility directly using in vivo fiber photometry and chemogenetics. This work was supported by NIH R01 AA029130, NIH P50 AA022538 and NIAAA T32 AA02657.

Keywords: Astrocyte, GABA Neuron, Alcohol Withdrawal

Disclosure: Nothing to disclose.

P613. Disrupted Brain State Dynamics in Opioid and Alcohol Use Disorder: Attenuation by Nicotine Use

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Background: Substance use disorder (SUD) is a chronic relapsing disorder with long-lasting changes in brain intrinsic networks. While most research to date has focused on static functional connectivity, less is known about the effect of chronic drug use on dynamics of brain networks.

Methods: Here we investigated brain state dynamics in individuals with opioid use (OUD) and alcohol use disorder (AUD) and assessed how concomitant nicotine use, which is very common among individuals with OUD and AUD, affects brain dynamics. Resting-state functional magnetic resonance imaging data of 27 OUD, 107 AUD and 137 healthy participants were included in the analyses. To identify recurrent brain states and their dynamics, we applied a data-driven clustering approach that determines brain states at a single time frame.

Results: We found that OUD and AUD non-smokers displayed similar changes in brain state dynamics including decreased fractional occupancy or dwell time in default mode network (DMN)-dominated brain states and increased appearance rate in visual network (VIS)-dominated brain states, which were also reflected in transition probabilities of related brain states. Interestingly, co-use of nicotine affected brain states in an opposite manner by lowering VIS-dominated and enhancing DMN-dominated brain states in both OUD and AUD participants. Our finding revealed a similar pattern of brain state dynamics in OUD and AUD participants that differed from controls, with an opposite effect of nicotine use suggesting distinct effects of various drugs on brain state dynamics.

Conclusions: Different strategies for treating SUD may need to be implemented based on patterns of co-morbid drug use.

Keywords: Opioid Use Disorder, Alcohol Use Disorder, Nicotine/ Substance Use Disorder, Resting-State fMRI

Disclosure: Nothing to disclose.

P614. An fMRI Investigation of the Effects of a Novel GHSR Inverse Agonist on Alcohol- Related Cue Reactivity in People With Alcohol Use Disorder: A Pilot Study

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Background: Clinical studies have demonstrated the role of ghrelin in alcohol use behaviors by showing that ghrelin levels are suppressed by both oral and intravenous acute alcohol administration and that baseline ghrelin levels are positively correlated with craving for alcohol. In human laboratory studies, intravenous ghrelin administration significantly increased cue alcohol craving, and self-administration of alcohol and influenced increases in neural activation of the amygdala and medial orbitofrontal cortex during an alcohol related incentive delay task in non-treatment seeking individuals with AUD. Moreover, preclinical investigations have revealed the effects of ghrelin antagonism on alcohol consuming behaviors and neural mechanisms that modulate the reinforcing properties of alcohol.

Recently, we investigated the safety and tolerability of a novel ghrelin receptor inverse agonist/competitive antagonist, PF-5190457, when it is co-administered with alcohol. While our focus was on safety, we conducted a preliminary investigation on the drug's effect on alcohol cue elicited craving in a small subset of the study and found it reduced alcohol cue elicited craving in a bar like laboratory setting. Based on these preliminary findings, we expanded our investigations on PF-5190457 to assess the drug's effects on neural activation during an emotion processing fMRI task in a small inpatient sample of treatment-seeking individuals with AUD.

Methods: The study was a within subjects, double blinded, placebo controlled clinical trial, comparing the effects of PF-5190457 100 mg b.i.d. up to steady state cue reactivity. Eleven treatment-seeking, detoxified individuals with AUD (M=, F=) participated in an optional fMRI portion of a larger clinical trial to assess the effects of PF-5190457 on cue reactivity during an emotional processing task. Participants viewed alcohol-containing beverages, food, and sexually explicit images during an fMRI scan, and we assessed changes in neural activation under the drug and placebo conditions.

We performed general linear modeling to estimate individual effects of each condition (contrasts) of interest (Alcohol- Control, Food- Control, Sexually Erotic- Control, Non-Erotic- Control) and compared drug conditions (PF-5190457 vs. placebo) to determine the mean condition specific group- level regional responses, using paired t-tests. We focused our analysis on the following a priori regions of interests (ROIs): amygdala, ventral striatum, insula, nucleus accumbens, medial orbitofrontal cortex, and dorsal anterior cingulate.

Results: The drug significantly increased neural activation in the left amygdala ($t = 3.91$, $p = .003$) and right nucleus accumbens ($t = 3.45$, $p = .006$) during in Alcohol - Control contrast. There were no other significant effects of the drug during our other conditions or within our regions of interest.

Conclusions: We found that the novel ghrelin receptor inverse agonist PF-5190457 had a significant effect on neural activity when participants viewed alcohol related images during an emotional processing task. Contrary to our hypothesis, PF-5190457 appeared to increase neural activation in these regions after exposure to alcohol related cues, while having no effect on food or sexually appetitive cue related neural activation. These preliminary findings add to the growing body of evidence that ghrelin signaling plays a role in reward- and emotional- related processing. Larger, future investigations should explore the effects of ghrelin antagonism on current AUD and treatment status, ghrelin levels, and behavioral craving measures.

Keywords: Ghrelin, Clinical Trial, Alcohol Use Disorder - Treatment, Cue Reactivity, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P615. Phase 1b/2a Drug-Drug Interaction Study of Lemborexant as an Adjunctive Treatment to Buprenorphine-Naloxone for Opioid Use Disorder

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Background: Medications for opioid use disorder (OUD), like buprenorphine, save lives; yet treatment outcomes remain suboptimal. Evidence supports the potential role of the orexin system in OUD medication development. This study evaluated the initial safety and tolerability of lemborexant, a novel dual orexin antagonist, as an adjunctive treatment with buprenorphine among patients with OUD.

Methods: Patients with OUD were recruited from outpatient clinics who were: 18-65 years old, receiving a total buprenorphine

daily dose 8-24 mg, screening positive for poor sleep (Pittsburgh Sleep Quality Index total score > 6). Exclusion criteria included: pregnancy, taking CYP3A4 inhibitors/inducers, current severe alcohol use disorder. An intended sample size of 18 completers (goal 9 male/female) was determined by primary safety outcomes. Participants completed two days on the inpatient clinical research unit. Participants were randomized (double blinded) to receive placebo or lemborexant (5 mg on day one and 10 mg on day two) at 7 AM followed by their prescribed buprenorphine dose at 8 AM. Serial assessments were captured through 4 PM; participants were discharged after a safety assessment. Primary outcomes included adverse events, physiologic measures (pulse oximetry, end tidal CO2, heart rate, blood pressure) and the Richmond Agitation Sedation Scale (RASS; range −3, +3). Generalized linear mixed model analysis compared study drug and time on outcomes.

Results: Of the 48 consented participants who completed screening, 18 were randomized to lemborexant (n = 11) or placebo (n = 7). Lemborexant and placebo participants did not differ by age (37.1 + 7.7; 43.6 + 12.9 years), male/female sex (8/3; 6/1), Black/white race (3/8; 4/3), weight (189.8 + 36.8; 210.1 + 61.4 pounds), nor Pittsburgh Sleep Quality Index total score (9.6 + 2.1; 8.5 + 1.9; range 0-21). Lemborexant participants were receiving a slightly lower total daily buprenorphine dose (14.8 + 6 vs. 19.4 + 3.6 mg; $p = 0.05$). No unanticipated problems occurred; five adverse events occurred in the placebo group and three in the lemborexant group after study drug administration with no serious events. Accounting for baseline differences and multiplicity adjustment, none of the physiologic measures showed a significant interaction of time and placebo vs. lemborexant (5 or 10 mg): Pulse oximetry (t = 0.5; $p = 0.87$), End tidal CO2 (t = 0.4; $p = 0.92$), Heart rate (t = 0.8; $p = 0.59$), Systolic blood pressure (t = 0.5; $p = 0.87$), Diastolic blood pressure (t = 1.0; $p = 0.48$). Consistent with lemborexant's intended effects, there were significant differences in RASS score changes (more sedation) by lemborexant vs. placebo, between baseline and 1.5 hours ($p = 0.02$), 3 hours ($p = 0.03$) and 5 hours ($p = 0.02$). However, there was no significant interaction between group and time (t = 1.2; df = 10,40; $p = 0.34$), and there was no significant difference in change from baseline to end of study day ($p = 0.48$) between study drug groups. Notably, at 9 hours after receiving lemborexant/placebo, all participants returned to baseline sedation levels and were discharged.

Conclusions: Findings support the initial safety and tolerability of lemborexant as an adjunctive treatment to buprenorphine in humans. Future longitudinal work with larger samples and balanced by sex should assess the efficacy of lemborexant to improve OUD treatment outcomes.

Keywords: Buprenorphine-Naloxone, Dual Orexin Receptor Antagonist (DORA), Clinical Trial

Disclosure: Nothing to disclose.

P616. Post-Treatment Efficacy of Extended Release Naltrexone on Abstinence From Opioids and Illicit Substances in Opioid Dependent Patients

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Background: Although Extended Release Naltrexon (XR-NTX) is effective, safe and feasible in short-term treatment, studies evaluating post-treatment effects are scarce. Some studies have found that relapse prevention, regarding drug use waned with time. This study investigated any sustained abstinence from opioids and other illicit substances up to 12 months after longer term XR-NTX treatment.

Methods: This is a naturalistic, multicenter, open-label, clinical trial on six to 12 months treatment and 12 months post-treatment efficacy of XR-NTX. The study took place at five urban, outpatient addiction clinics in Norway, The intervention were XR-NTX administered as intramuscular injections (380 mg) every 4 weeks. Data was collected every four weeks throughout the treatment period and at 3,6 and 12 months during the post treatment period.

Both men and women (n = 162) were recruited from community services and hospitals in designated urban areas.

Results: Among the 162 participants entering the study, 100 participants reported post-treatment data. The majority of participants (78%) reported no relapse to weekly or daily opioid use during post treatment follow up. A small subgroup (15%) reported weekly or daily opioid use, and 10% reported at least one overdose. The use of other illicit substances was also low, only 9 % reported use of amphetamine and none used cocaine, but 36% reported weekly or daily cannabis use. Weekly alcohol use for intoxication was reported by 9 %. The mean opioid craving score was less than two on a scale ranging from 0 to 10.

Conclusions: This study showed a substantial longer term post treatment efficacy of XR-NTX both on opioid use, opioid craving and use of other illicit substances. Individual follow up after fulfilled XR-NTX treatment in clinical practice should be established to further explore facilitating factors promoting post treatment efficacy.

Keywords: Opioid Dependence, Extended-Release Naltrexone, Post Treatment Efficacy, Sustained Remission

Disclosure: Nothing to disclose.

P617. Dual Neuromodulation Targets for Treatment of Substance Use Disorders: Unraveling the Interacting Role of DLPFC and Frontopolar Cortex During Drug Cue Reactivity

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Background: Substance Use Disorder (SUD) presents a significant global health challenge, necessitating innovative interventions for its treatment, including noninvasive brain stimulation (NIBS) methods. However, target selection for SUD treatment, particularly for transcranial stimulation methods, like TMS, remains challenging. Emerging evidence underscores the crucial role of mainly fronto-limbic cortico-subcortical connections in driving addictive behaviors. These connections can be targeted with brain stimulation interventions. Previous NIBS studies for SUDs have mainly targeted the DLPFC, but recent evidence suggests that the frontopolar cortex might play a mediating role in the observed clinical effects of NIBS protocols, even when not directly stimulated. However, the distinct and interacting roles of DLPFC and frontopolar cortex have not been thoroughly explored as dual targets. In this study, we investigated the interactions between frontopolar, DLPFC, and amygdala during drug cue exposure, at both individual and group levels, indicating that targeting DLPFC and frontopolar cortex separately may have opposite or augmenting effects on subcortical areas and cue-induced craving.

Methods: fMRI drug cue reactivity data were collected from 60 male participants (mean ± SD = 35.86 ± 8.47 years) with methamphetamine use disorder as part of a pre-registered trial (NCT03382379). Prior to and immediately after drug cue exposure, cue-induced cravings were assessed using VAS scores. Averaged cue reactivity was extracted from all brain sub-regions using

Brainnetome atlas parcellation. The subcortical brain region exhibiting the highest functional reactivity to drug cues was selected as the seed region (amygdala) for further seed-to-whole brain psychophysiological interaction (PPI) analysis. To demonstrate inter-individual variability in fronto-limbic connections, Brodmann's masks that showed overlap with seed-to-whole brain clusters in prefrontal cortex were extracted. The location and strength of the most positive connection between the subcortical seed region (amygdala) and the mask that showed increased PPI connectivity, as well as the most negative connection between the subcortical seed region and the cluster that showed decreased PPI connectivity during drug cue exposure, were calculated. Correlations between ROI-to-ROI PPI connectivity and changes in craving scores were also assessed.

Results: After drug cue exposure, craving scores exhibited a significant increase ($P = 0.002$). Among the subcortical regions, the left medial amygdala was chosen as the seed region due to its highest fMRI drug cue reactivity (mean ± SD:0.31 ± 0.29) ranging from −0.47 to 1.14. Left medial amygdala-to-whole brain PPI revealed two clusters, one in the frontopolar area with increased PPI connection (size:863, center:[20,44,30],P-FDR:0.0007, Hedge's $g = 0.1$, overlap with Brodmann area 10), the other one in DLPFC (size: 42, center:[44,38,6],P-FDR:0.001, Hedge's $g = 0.15$, overlap with Brodmann area 9,46). Amygdala-to-DLPFC mask PPI analysis revealed notable inter-individual variability in terms of both the location ([29.5,49.9,22.1] ± [16.2,10.1,15.7]) and strength (-1.2 ± 0.6) of the most negative PPI connections. Regarding the amygdala-to-frontopolar mask PPI connectivity, inter-individual variabilities were found in both location ([0.2,61.8,3.9] ± [25.7,6.1,10.2]) and strength (1.05 ± 0.45). We also found variations in terms of connectivity strength between individualized frontopolar and DLPFC locations ranging from −0.98 to 1.2. Furthermore, a significant positive correlation ($R = 0.27$,$P = 0.03$) was found between craving scores following drug cue exposure and frontopolar-amygdala PPI connectivity, while the correlation with DLPFC-amygdala connectivity was negative ($R = -0.15$, $P = 0.2$).

Conclusions: Our study highlights distinct brain connectivity patterns between the frontopolar-amygdala and DLPFC-amygdala during drug cue exposure. The frontopolar-amygdala connection shows significantly higher functional coupling during drug cue exposure, suggesting a stronger interaction between drug cue-related value processing and the amygdala's arousal response. In contrast, the DLPFC-amygdala connection shows significantly lower functional coupling during drug cue exposure, possibly indicating DLPFC's top-down regulatory role in suppressing reactivity to drug cues in the limbic system. These findings suggest that effective control of cue-induced craving may involve inhibiting the frontopolar-limbic connection and exciting top-down regulatory role of the DLPFC, as supported by prior TMS studies. Our results underscore the importance of personalized target selection in brain stimulation studies, considering the significant inter-individual variability in cortico-subcortical connections. Our pipeline offers dual fMRI-informed targets for personalized stimulation.

Keywords: Substance Use Disorders, Noninvasive Brain Stimulation, Dorsolateral Prefrontal Cortex (DLPFC), Frontopolar Cortex, Personalized Medicine

Disclosure: Nothing to disclose.

P618. Intranasal Oxytocin as a Pharmacological Intervention for Stress-Induced Opioid Withdrawal: A Human Laboratory Study

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Background: Although stress has long been linked to substance use, craving and relapse, there are no available medications that target stress-induced substance use disorder (SUD). In particular, with the rise in opioid use, there is still a crucial need for developing effective pharmacological treatments that target and integrate the complexity of this disease. Here we investigated the safety, tolerability and opioid withdrawal after administration of intranasal oxytocin for one week in a human laboratory paradigm comprised of administration of yohimbine, paired to a cue-reactivity procedure.

Methods: This is a double-blind, placebo controlled, randomized trial, individuals with patients with OUD ($N = 20$) who are currently receiving treatment with buprenorphine/naloxone or methadone and randomized to intranasal oxytocin (40 international units, IU) and oxytocin-matched placebo, administered twice/day for 7 days with 7 days washout period. On days 5 and 7 of period 1, and on days 19 and 21 of period 2, patients completed two counter-balanced sessions in which they received yohimbine (32.4 mg) or yohimbine-matched placebo.

Results: Safety outcomes were excellent with no serious adverse events, nor adverse events of severe grade. As expected, after noradrenergic activation induced by yohimbine, systolic ($B1 = 15.77$, $p = .001$) and diastolic ($B1 = 6.06$, $p < .05$) blood pressure significantly increased in both conditions. Intranasal oxytocin, compared to placebo, significantly reduced opioid withdrawal after noradrenergic activation ($B1 = 6.96$, $p = .029$).

Conclusions: The study confirms the safety and tolerability of intranasal oxytocin when co-administered with yohimbine in a human laboratory paradigm. Under stress-induce condition, intranasal oxytocin, compared to placebo reduced the cue-induced opioid withdrawal.

Keywords: Opioid Use Disorder, Noradrenergic System, Human Laboratory Study

Disclosure: Nothing to disclose.

P619. Distinct Responses in Plasma Gonadal Hormones and Immune Markers are Evident and Associated With Neuroimmune Changes in the Prefrontal Cortex During Stages of Alcohol Dependence in Female Rats

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Background: The prefrontal cortex (PFC) and hippocampus are important for the development of alcohol addiction. Neuronal activity in these regions are regulated by immune responses, and inflammatory responses are assisted by disruption of the blood-brain barrier (BBB). The detrimental effects of chronic alcohol consumption, abstinence and relapse to alcohol drinking on gonadal hormones, inflammatory markers and BBB integrity proteins in these regions have been minimally explored. Moreover, the female specific effects on these markers in the context of alcohol consumption and relapse are unknown. Here we seek to answer these questions.

Methods: Adult female rats were made ethanol dependent by chronic intermittent ethanol vapor (CIE) and ethanol drinking (ED) procedure. Rats were euthanized during acute withdrawal (6-8h after CIE), protracted abstinence (2 weeks) or after relapse session. Plasma isolated from trunk blood and brain tissue homogenate of the PFC were analyzed for estrogen, progesterone, cytokines and chemokines using a 9-plex panel from Meso Scale Discovery. BBB disruption was analyzed with tight junction and adherens junction proteins claudin-5 and cadherin5 (Cdh5, VE-cadherin) via Western

blotting and VEGF by ELISA. This allowed us to associate the levels of gonadal hormones, individual cytokines to neuroimmune responses in the PFC and hippocampus.

Results: CIE increased ED, with rats having higher ethanol consumption during CIE and relapse to ethanol drinking sessions compared with pre-CIE sessions. In the PFC, Cdh5 was increased during acute withdrawal and reduced during abstinence compared with ethanol naïve controls and subsequently increased with relapse albeit to a lower extent compared with acute withdrawal, suggesting tolerance to this effect. Concurrently, in the PFC, interleukin-β (IL-β) and VEGF were enhanced during abstinence. These changes were specific to the PFC and were not evident in the hippocampus. Analysis of Iba-1-labeled microglia (ramification as total process length, area of cell soma) in the PFC showed reduced processes length and increased area of soma during abstinence, indicating an activated state. Lastly, estrogen levels were enhanced during acute withdrawal and abstinence, indicating a positive correlation between peripheral estrogen levels and neuroimmune activity in the PFC. Progesterone levels were unaltered.

Conclusions: These results reveal significant effects of ethanol dependence in peripheral estrogen and pro-inflammatory cytokine levels in the PFC, which were associated with BBB disruption during abstinence, and emphasize their possible role in relapse to ethanol seeking in ethanol dependent female rats.

Keywords: Gonadal Hormones, Prefrontal Cortex, Hippocampus

Disclosure: Nothing to disclose.

P620. Neural Mechanisms Underlying the Prospective Estimation of Self-Control Costs: A Preliminary Investigation

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Background: Failures of self-control continue to be a major challenge across a number of economic and health domains. Converging work across cognitive and decision neuroscience has shown that exerting control is registered as cognitively costly. We previously used a neuroeconomic approach to demonstrate that the subjective cost of self-control can be measured behaviorally using a willingness-to-pay mechanism, and further, that stress exposure increases the perceived cost of exercising control. Here, we sought to acquire a preliminary characterization of the neural circuits underlying how these costs are estimated. We tested the hypothesis that prospective control cost estimates will be encoded in more anterior prefrontal regions consistent with past precommitment work [e.g. frontopolar cortex (FPC), orbitofrontal cortex (OFC)] as well as regions that have been implicated in encoding the cognitive cost of control [dorsal anterior cingulate cortex (dACC)], rather than traditional control regions that are known to actively deploy control [e.g., dorsolateral prefrontal cortex (dlPFC)].

Methods: Healthy dieters ($n = 23$) first rated a snack-foods on health, taste and temptation level in order to select a low, medium and high-tempting food for each individual. Participants then underwent fMRI scanning while completing a self-control choice task. On each trial, participants viewed a food image that varied on temptation level (low, medium, high), quantity (small, medium, large) and duration of time with the food (1-60 min). They reported trial-by-trial willingness-to-pay (from a $10 study endowment) to avoid the food depicted on each trial. A realization phase followed the scanning session, during which one trial (bid) was randomly selected and entered into a standard economic auction procedure, which determined whether the food was successfully avoided or not.

Results: We first contrasted BOLD responses from all high vs. low tempt trials, collapsing across quantity and time, to examine activation for trials that required the highest vs. lowest self-control demands. Consistent with our hypothesis, this contrast revealed significant increases in FPC and OFC activation (threshold at $p < 0.005$, uncorrected). These preliminary data lend support that hypothesized brain regions are involved in estimating self-control costs. Brain activity was then modeled with a parametric modulator of (raw) bid value during the 4s decision period when participants evaluated how much to pay to avoid control. Higher bids yielded increased activation in mOFC and dACC ($p < 0.01$, uncorrected), pointing to a central role in these brain regions in prospectively estimating the perceived cost of self-control. Activation extracted from the dorsolateral prefrontal cortex did not differ from zero during bid decisions ($p = 0.38$).

Conclusions: Our preliminary data suggest that estimating the subjective cost of exercising self-control engage a distinct neural circuit than those traditionally involved in implementing control. Acquiring a better understanding the neural basis of these cost estimates may provide potential neural targets to help improve the success of prospective self-control strategies.

Keywords: Self-Control, Human Neuroimaging, Effort-Cost Decision-Making

Disclosure: Nothing to disclose.

P621. Neural Correlates of Inhibitory Control and Alcohol Cue Reactivity Predict Change in Alcohol Consumption over Time in Women

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Background: Poor inhibitory control and alcohol cue reactivity are well-established risk factors for Alcohol Use Disorder (AUD), and there is some evidence to suggest they interact to promote risky drinking. However, little is known about potential biologically-based sex differences underlying these risk factors, or how they may interact to predict drinking specifically in women. Here we examine the degree to which neural correlates of inhibitory control and alcohol cue reactivity are related to each other and influence changes in drinking behavior over time in young adult female drinkers.

Methods: Female drinkers performed the stop signal task to assess inhibitory control and an alcohol cue reactivity task while undergoing fMRI. Women were scanned during the early follicular phase of the menstrual cycle, when ovarian hormones are low. Participants completed the Timeline Follow Back (TLFB) calendar to assess drinking habits at the time of the scan and again nine months later.

Results: Data collection is currently ongoing, and to date 9 women have completed the fMRI portion of the study and the 9 month follow-up. Preliminary analyses show less brain activity in the right insula during response inhibition is associated with greater brain activity in bilateral nucleus accumbens during cue reactivity ($r = -0.71$; $p = 0.05$). Further, increases in binge drinking 9 months later are associated with less insula activity during inhibition ($r = -0.75$; $p = 0.02$) and greater accumbens activity during while viewing alcohol cues ($r = 0.74$; $p = 0.03$).

Conclusions: These findings extend previous findings showing an inverse relationship between inhibitory and reward circuitry. Further, they show that less activity in inhibitory circuitry and greater activity in reward circuitry predict escalation in drinking over time in young adult women. As data collection progresses we

will test the degree to which hormonal influences on neural correlates of inhibition and cue reactivity are related to changes in drinking over time. Taken together, these findings will provide important information regarding biologically-based sex-specific risk for excessive alcohol consumption in women.

Keywords: Alcohol, Women, Inhibitory Control, Cue-Reactivity, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P622. Effects of Sex and Anxiety on Bnst-Anxiety Network Activation and Connectivity in Adults in Early Abstinence From Alcohol Use Disorder

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Background: Alcohol use disorder (AUD) is a debilitating, chronic disorder that occurs in 30% of the US population. Recovery from AUD (abstinence) is possible; however, 50% of individuals relapse within the first year. Long-term recovery is hindered by anxiety and stress that emerge during early abstinence, likely driven by neurobehavioral changes in the stress system responding to chronic alcohol use. Understanding how these neurobehavioral changes confer risk for anxiety and subsequent relapse is essential for developing successful treatments for AUD.

Methods: To determine effects of sex and anxiety on the brain during alcohol abstinence, we used a translational model of unpredictable threat to investigate an anxiety network containing the bed nucleus of the stria terminalis (BNST). Controls (HC = 20) and adults with AUD in early abstinence (EA = 19) underwent functional imaging to assess neural activity to unpredictable and predictable threat in a cued anticipation task. Participants were trained to associate three different cues (colored shapes) with three different events: (1) a predictable neutral cue, always followed by a neutral face; (2) a predictable threat cue, always followed by a fear face; and (3) an unpredictable threat cue, followed either by a neutral face or a fear face. An anxiety composite score was created from measures of trait anxiety, social anxiety, and worry. Whole-brain analyses were performed in SPM12 to investigate brain activation and BNST functional connectivity during anticipation (cues) and image viewing; significance was considered at cluster correction $\alpha = 0.05$ with a threshold of $k = 90$. For all analyses, predictor variables were group (EA/HC), sex, anxiety score, and all interactions.

Results: Unpredictable vs. Predictable Threat Cues: We found no main effects or interactions during cues in whole-brain activation. However, we detected a group x anxiety x sex interaction in BNST functional connectivity with clusters containing the rostral anterior cingulate cortex (ACC), dorsomedial prefrontal cortex (dmPFC), and thalamus during unpredictable vs. predictable threat cues (rostral ACC/dmPFC: $k = 2381$, $t = 5.66$; thalamus: $k = 978$, $t = 5.61$). Post-hoc analyses by sex revealed that in men there was a group x anxiety interaction in BNST-thalamus functional connectivity ($p = 0.001$), such that greater levels of anxiety in HC negatively correlated with connectivity; there was no significant correlation in EA group. Post-hoc analyses in women revealed a group x anxiety interaction in BNST-rostral ACC ($p = 0.007$) and BNST-dmPFC ($p = 0.004$) functional connectivity, such that greater levels of anxiety were negatively correlated with connectivity in EA, but positively correlated in HC.

Unpredictable vs. Predictable Threat Images: We found a group x anxiety interaction during unpredictable vs. predictable threat images in whole brain activation in clusters that contain the dorsal ACC, insula, and BNST (dorsal ACC: $k = 133$, $t = 4.57$; insula: $k = 1308$, $t = 4.89$, $k = 734$, $t = 3.98$; BNST: $k = 283$, $t = 4.28$). Specifically, greater levels of anxiety in the HC group negatively correlated with activation of the dorsal ACC ($p = 0.03$), insula ($p = 0.005$), and BNST ($p < 0.001$); there was no significant correlation in EA group. We did not detect main effects or interactions in BNST functional connectivity during unpredictable vs. predictable threat images.

Conclusions: These data suggest that sex and anxiety play unique roles in threat anticipation and image viewing. Specifically, anxiety in women plays a role in threat anticipation during early abstinence, while anxiety in healthy men contributes to threat anticipation and image viewing. These data support a growing body of translational work highlighting the BNST as a central hub for anxiety, and its role in anxiety during early abstinence. Importantly, these data further contribute to literature on sex differences that exist in BNST function and early abstinence from AUD.

Keywords: BNST, Anxiety, Alcohol Use Disorder, Human Neuroimaging, Sex Differences

Disclosure: Nothing to disclose.

P623. Preliminary Findings Linking Smoking Withdrawal to Decreased Lateral Prefrontal Cortex Activation in Response to Thermal Pain

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Background: Smoking is disproportionately prevalent among individuals with chronic pain. It is thought that smoking and pain influence each other through a positive feedback loop, in which smoking exacerbates pain conditions, and pain increases motivation to smoke. Evidence suggests that nicotine has mild analgesic properties, which may reinforce continued smoking. By contrast, smoking withdrawal is associated with increased sensitivity to pain, which is likely to make quitting particularly difficult among people who smoke with chronic pain. However, despite the growing behavioral evidence from both laboratory and real-world settings, and the importance of the central nervous system in pain perception, no studies have examined the neural mechanisms underlying smoking withdrawal-induced hyperalgesia. The present study is a preliminary investigation examining the effects of smoking withdrawal on brain activation in response to thermal pain stimulation. We also examined the relationship between withdrawal-induced changes in brain activation and subjective pain ratings. We hypothesized that withdrawal would be associated with increased activation throughout regions involved in affective responses to pain, including the insula, somatosensory cortex, and lateral prefrontal.

Methods: Twelve adults (both sexes included) who reported smoking at least 10 cigarettes per day completed screening and training sessions, followed by two fMRI sessions. One of the sessions followed 24-hours abstinence from smoking, and the other followed smoking as usual. Abstinence was biochemically verified with breath carbon monoxide level, and session order was counterbalanced across participants. During the initial training session, participants rated their pain in response to heat stimuli across a range of temperatures delivered to their lower leg in order to calibrate the temperature as thresholds for high pain ("high" temperature, typically 47 degrees Celsius, evoking pain ratings of approximately 7 or 8 out of 10) or minimal pain ("low" temperature, typically 46 degrees Celsius, evoking very low pain

ratings). Calibrations were verified again immediately prior to scanning at each session. During scanning, participants completed four 7-minute runs of a heat pain task, with 30-second blocks of high and low temperatures delivered through a thermode applied to the lower leg. After each block of thermal stimulation, participants provided their subjective ratings of pain; this was followed by a 20 second rest period before the onset of the next block. Analyses were conducted with SPM12. First-level contrasts were created for each temperature compared to rest (high > rest; low > rest) and between temperatures (high > low). For each first-level contrast, group level activation maps were created to analyze differences between withdrawal and satiation. Group-level contrasts were thresholded at $p < 0.05$ with family-wise error cluster corrections. Imaging data for one female participant was omitted from the final analysis due to poor registration.

Results: The sample included 7 males and 5 females, had a mean age of 44.4 years (SD = 10.5), and were predominantly white (92%). Four participants endorsed chronic pain. As expected, participants had significantly greater scores in the Minnesota Withdrawal Scale (p rest contrast as participants showed greater activation during satiation compared to abstinence in the left ventrolateral prefrontal cortex (VLPFC) extending into the anterior insula (178 voxels, $p = 0.03$). No significant effects were observed for the low > rest contrast. We then examined whether the magnitude of differences in pain ratings between abstinence and satiety was associated with the difference in activation extracted from the left VLPFC cluster. Those who experienced greater abstinence-induced increases in pain ratings in response to high vs low temperatures also exhibited greater abstinent-induced decreases in left VLPFC activation ($r(11) = -0.60$, $p = 0.0495$).

Conclusions: Compared to the satiated state, smoking abstinence was associated with reduced activation in the VLPFC in response to thermal pain. The observed cluster encompassed portions of the anterior insula, which is a region involved in the affective dimension of pain and pain unpleasantness, as well as the inferior frontal gyrus and orbitofrontal cortex, which have both been implicated in pain modulation. These findings are consistent with difficulties in pain tolerance or regulation in smokers experiencing withdrawal. Given the small sample size, future studies are needed to replicate these findings with large samples. Additional longitudinal studies investigating withdrawal-induced hyperalgesia among smokers with and without chronic pain are also needed to inform neurobehavioral mechanisms underlying negative reinforcement in poor smoking cessation outcomes.

Keywords: Nicotine Addiction, Pain, Affective Components of Pain, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P624. A Peri-Ceorulear Neuropeptidergic Pathway for Modulating OFC-Mediated Drug-Seeking Behavior

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Background: Research demonstrates that substance-use disorder, including opiate use disorder, affects one's decision making and reward processing resulting in impaired goal-directed behaviors. However, there remains a lack of understanding of the neurobiology that underlies persistent reward-seeking despite value changes that would normally alter goal-directed behavior. The neuropeptide S (NPS) system, made up of the peptide and its receptor (NPSR1), drives reward-seeking behaviors, however the underlying mechanism is not understood. We generated both NPS-Cre and NPSR1-Cre driver mouse lines for accessing and examining the key circuit components of NPS/NPSR1-mediated behaviors.

Methods: Whole cell patch clamp electrophysiology: NPSR1-Cre mice (n = 8 [4 female/4 male]) expressing AAV-CaMKIIA-ChR2 in the periLC were recorded in NPSR1-postive cells from the OFC. OFC-NPSR1 neurons that received input from the periLC were identified using blue light delivered through a 40× objective via a LED. In vivo Fiber Photometry: NPSR1-Cre (n = 16 [8 female/8 male]) mice received unilateral infusions of AAVDJ-DIO-GCaMP6s-eYFP to the OFC and a photometry implant (Doric) above the injection site. After recovery, mice underwent an oral fentanyl self-administration task. Mice underwent 120m sessions where they could nosepoke for extension of a sipper for 10ug/ml fentanyl solution access. In vivo 2-Photon Imaging: NPSR1-Cre mice (n = 8 [4 female/4 male]) received unilateral infusion of AAVDJ-DIO-GCaMP6s-eYFP to the OFC with a 1mm diameter GRIN lens positioned above. After recovery, mice underwent a head-fixed oral fentanyl self-administrations task where mice were required to turn a wheel for delivery of fentanyl reward. All experiments were carried out in accordance with the NIH Guide and approval of the University of Washington IACUC.

Results: We determined that the periLC sends excitatory projections to the orbitofrontal cortex (OFC) that connect directly with NPSR1-expressing neurons (n = 12/35). Bath application of NPS caused a significant increase in the peak amplitude of the optically evoked excitatory postsynaptic current (paired t-test, $p = 0.0131$). We also found that OFC-NPSR1 neuron activity was strongly associated with delivery of a conditioned stimulus that predicted delivery of a sucrose pellet (paired t-test, $p = 0.0044$). We also found during and FR1 task where mice could nosepoke for fentanyl access, NPSR1 activity was enhanced during drug cue delivery (paired t-test, $p = 0.0475$). In addition, the OFC-NPSR1 neuron activity dipped during consumption of fentanyl reward (paired t-test, $p = 0.0001$) which denotes a bidirectional response to delivery of the conditioned stimulus (enhanced activity), and fentanyl reward (quiescence). Therefore, we utilized two-photon imaging to further examine the OFC-NPSR1 calcium activity during fentanyl self-administration. We found heterogeneity in the response of OFC-NPSR1 neurons during self-administration of fentanyl reward.

Conclusions: We found that periLC-NPS neurons are connected with OFC-NPSR1 neurons. We also demonstrate that the OFC-NPSR1 population responds differently to delivery of drug-predictive cues as well as to consumption. These data indicate the heterogeneity of the OFC-NPSR1 population. From this we have gained new insights indicating OFC-NPSR1 signaling as an important new target for modulating drug-seeking behavior.

Keywords: Fentanyl, Two-photon Calcium Imaging, Neuropeptide S, NPSR1, Orbitofrontal Cortex (OFC)

Disclosure: Nothing to disclose.

P625. Longitudinal Changes in Cortico-Striatal-Limbic Responses to Stress and Alcohol Cues Associated With Reduction in Heavy Drinking During Treatment for Alcohol Use Disorder

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Background: Converging lines of evidence suggest that chronic alcohol use is associated with profound disruptions in neural stress and reward circuitry, which lead to increased stress- and alcohol cue-related craving and poor treatment outcomes in

patients with alcohol use disorder (AUD). The extent to which these neuroadaptations are restored and stabilized with standardized AUD treatment remains unclear. Therefore, the current study examined changes in neural stress, alcohol cue and neutral relaxed control cue responses post- versus pre-treatment, and whether such changes were associated with improvements in heavy drinking following 8-week behavioral AUD treatment. We also assessed changes in post versus pre-treatment subjective alcohol craving reported during functional magnetic resonance imaging (fMRI) scans.

Methods: Fifty-four treatment-entering adults with AUD (ages 18-60, 34 men and 20 women) underwent fMRI scanning during which they participated in a well-validated cue provocation task exposing them to standardized and matched stress (S), alcohol (A) and neutral control (N) cues over six successive runs in a randomized block design per condition with repeated subjective alcohol craving assessments. After the baseline fMRI scan, participants started once-weekly treatment sessions for eight weeks, during which period they also reported their daily alcohol intake using brief surveys administered in a smartphone application. Based on their drinking patterns during treatment, patients were grouped into either continued heavy drinkers (HD, $n = 28$) or non-heavy drinkers with zero heavy drinking days (NHD, $n = 26$). Following treatment, participants underwent a second fMRI scan during which they again completed cue provocation task with repeated subjective alcohol craving assessment in each block per condition using a new set of S, A and N images. The order of the blocks was consistent within-subject between the two scans. Linear mixed effects (LME) models with a random intercept were conducted to assess main and interaction effects for scan session (pre and post) and condition (S, A and N) on alcohol craving during fMRI. A whole brain, voxel-based second level 3dLME (AFNI) analysis ($p < .001$, whole brain cluster correction at $\alpha < .05$) with post-pre first level contrast maps was conducted to examine main and interactive effects during Condition (S, A, N) x Group (HD vs NHD), with participant as a random effect and age and sex as covariates.

Results: As hypothesized, we found no differences in baseline craving between pre and post scans ($p = .47$). However, during cue provocation, there was a significant decrease in alcohol craving ratings across conditions ($p < .001$). In fMRI, post- versus pre-treatment there were no functional response differences during N, but a significant increase in left anterior insula (AIC), putamen and ventral caudate response during S-N and greater right amygdala, AIC and ventral striatum, orbitofrontal cortex (OFC), ventromedial prefrontal cortex (vmPFC), hypothalamus, and rostral anterior cingulate cortex (rACC) activation during A-N. Following up a significant Condition x Group interaction showed post-pretreatment decrease in dorsal ACC (dACC) and increase in anteromedial thalamus during S-N and A-N as well as hypoactivation in OFC during N in heavy drinkers. These participants also exhibited hyperactive rACC during S-N and OFC, vmPFC, right amygdala, rACC, and bed nucleus of the stria terminalis (BNST) during A-N. In contrast, non-heavy drinkers evidenced a post-pretreatment decrease in OFC, vmPFC, bilateral dorsal caudate, and dACC during S-N, increase in vmPFC and dACC during A-N and greater anteromedial thalamus during N.

Conclusions: Taken together, these findings suggest specific altered neural responses to stress and alcohol cues in prefrontal and striatal-limbic regions during treatment. Among participants who did not engage in heavy drinking behaviors during treatment, we found stress cue-induced hypoactivation in several prefrontal areas, whereas continued heavy drinkers exhibited stress cue-induced limbic hyperactivation. Although both groups evidenced prefrontal hyperactivation during alcohol cue exposure post relative to pre treatment, heavy drinkers evidenced a more widespread increase in prefrontal and striatal-limbic functioning compared to non-heavy drinkers. Our findings suggest that standardized behavioral AUD treatment may help stabilize and restore altered neural brain functions and promote better treatment outcomes in some but not all AUD patients. To improve prognostic predictions, development of novel, more effective and mechanistically driven treatments is needed.

Keywords: Alcohol Use Disorder - Treatment, fMRI, Cue-Induced Craving

Disclosure: Nothing to disclose.

P626. Adolescent Social Isolation Increases Alcohol Drinking and Anxiety-Like Behavior: Role for Disrupted Endocannabinoid Signaling in the Central Amygdala

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Background: Reduced social interaction during adolescence increases susceptibility to many neuropsychiatric disorders in adulthood, including anxiety and substance use disorders, and adolescent females respond differently to stressors compared to males. Social isolation is a particularly profound stressor with high human relevance during the COVID-19 pandemic, when millions of adolescents faced prolonged periods of isolation. Both stress and cognitive neural systems undergo major developmental changes during adolescence, a critical period for maturation of the central nucleus of the amygdala (CeA). Dysfunction of GABAergic networks within the CeA during development increases vulnerability to adult affective disorders. However, the precise neurobiological mechanisms that underlie differential responses to adolescent social isolation stress are understudied. In this study, we investigated the impact of social isolation exposure during adolescence on alcohol drinking and adult susceptibility to anxiety in both sexes and the associated GABAergic synaptic changes in the CeA.

Methods: All experiments were performed in adolescent (from PND 28) male and female Wistar rats.

First, we assessed the impact of social isolation on voluntary alcohol drinking. Male and female rats were intermittently socially isolated for 24h prior to 2-bottle choice (2BC) access to alcohol (20% v/v, 2h/session) vs. water. After each drinking session, isolated rats were regrouped until the next day, when they were isolated again. This protocol was repeated 3 times/week across 4 weeks. Grouped-control rats were housed 3-4/cage for the entire adolescence and exposed individually to 2BC 3 times/week. Second, we tested all rats for irritability-like symptoms by assessing aggressive- and defensive-like behaviors in the bottle brush test, after two weeks of abstinence from alcohol (PND 70 ± 2). Third, we used liquid chromatography-mass spectrometry (LC-MS) to measure CeA endocannabinoid levels in abstinent male and female, isolated and grouped rats (PND 75 ± 2). Lastly, we used ex vivo slice electrophysiology to characterize the GABAergic synaptic activity of CeA in abstinent male and female, isolated and grouped rats (PND 75 ± 2).

Results were analyzed using unpaired t-tests or multifactorial ANOVAs and Tukey's post-hoc tests as appropriate.

All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the Scripps Research Institute.

Results: We found that social isolation during adolescence increases alcohol intake ($F1, 28 = 5.537$, $p = 0.0259$; $n = 8$/group) and preference ($F1, 28 = 19.47$, $p = 0.0001$; $n = 8$/group), where females show higher preference than males. Males also show an

overall higher irritability-like behavior than females during post-adolescence abstinence from alcohol (F1, 28 = 12.60, $p = 0.0014$; n = 8/group) and social isolation significantly increases the number of aggressive signs, i.e., biting, selectively in males ($p = 0.029$; n = 8/group). We then used a lipidomic approach to quantify CeA tissue levels of endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). Interestingly, we found i) significantly reduced 2-AG levels in socially isolated rats (F1, 19 = 5.315, $p = 0.0326$; n = 6/group); ii) lower levels of 2-AG in females compared to males (F1, 19 = 66.26, $p < 0.0001$; n = 6/group); and iii) no sex- or isolation-dependent effects on CeA AEA levels. To determine whether endocannabinoid changes affect inhibitory GABAergic signaling in CeA in socially isolated rats that had access to alcohol during adolescence, we used ex vivo patch-clamp slice electrophysiology and found that social isolation reduced spontaneous inhibitory postsynaptic currents (sIPSCs) frequency in males (t = 2.72, $p = 0.010$; n = 19-21 cells/group) but increased sIPSCs frequency in female rats (t = 2.207, $p = 0.034$; n = 17-18 cells/group).

Conclusions: Our findings indicate that social isolation during adolescence increases alcohol intake and preference across adolescence (PND 28-PND 56) in both males and females and aggressive-like behavior selectively in males. These behavioral changes were accompanied by dysregulation of GABAergic postsynaptic currents in the CeA, possibly through 2-AG mediated mechanism as CeA tissue content was reduced. A sex-dependent response to social isolation that alters inhibitory CeA synaptic activity during development may enhance susceptibility to emotional disturbance and alcohol use disorder in adulthood. Disrupted CeA 2-AG signaling might represent one of the drivers of the lasting effects of adolescent isolation and alcohol use, and we are currently investigating the endocannabinoid system as a therapeutic target.

Keywords: Adolescence, Social Isolation Stress, Alcohol, Sex Differences, Endocannabinoids

Disclosure: Nothing to disclose.

P627. Neural Bases of Impaired Avoidance Learning and Extinction Learning in Individuals With Alcohol Misuse

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Background: The escalation from social drinking to alcohol misuse is typically characterized by increased maladaptive pain avoidance and weakened cognitive control. These are the primary features of avoidance learning dysfunction. Chronic drinking is then maintained even when alcohol consumption no longer delivers the rewarding or pain-relieving effects in the long run. This further indicates impaired extinction learning in those with alcohol misuse. Despite such behavioral evidence for avoidance and extinction learning impairments, their neural bases remain unclear.

Methods: We acquired fMRI and behavioral data that assessed avoidance learning in 35 adult humans with alcohol misuse and 35 sex- and age-matched social drinkers. The subjects performed a probabilistic learning go/no-go task. The task involved learning to associate visual cues with outcomes to avoid painful electric shocks and optimize monetary reward. Crucially, after the acquisition period, the task included an extinction phase during which responses no longer resulted in reward or punishment. We built specific reinforcement learning models that optimally described behavioral performance during avoidance learning and extinction learning. We hypothesized that relative to social drinkers, those with alcohol misuse would exhibit (1) poorer avoidance learning, (2) impaired extinction learning; and (3) reduced involvement of prefrontal regions previously implicated in avoidance learning and extinction learning, particularly the anterior cingulate cortex (ACC).

Results: Behaviorally, individuals with alcohol misuse exhibited worse avoidance learning performance than social drinkers. The former group also maintained their response patterns during the extinction phase even though the responses were no longer rewarded or punished, suggesting poor extinction learning. Our reinforcement learning models confirmed lower learning rates in those with alcohol misuse in both acquisition and extinction phases. Neurally, we found decreased pregenual ACC activation during acquisition of avoidance learning across both groups in association with pain-avoidance drinking motive. During extinction, there was an increase in activity of the dorsal ACC across both groups though this activity was significantly lower in subjects with alcohol misuse compared to social drinkers. Across the two groups, the dorsal ACC activity was negatively correlated with drinking severity.

Conclusions: Our findings lend support to the notion that alcohol misuse is motivated by pain avoidance. Paradoxically, drinking is associated with both avoidance learning and extinction learning impairments. We further confirmed the disengagement of two distinct regions of the ACC during avoidance learning and extinction learning, thus identifying potential targets for treatment.

Keywords: Alcohol, Extinction Learning, Avoidance, Functional MRI (fMRI), Anterior Cingulate Cortex (ACC)

Disclosure: Nothing to disclose.

P628. Peripheral Endocannabinoid Concentrations in Chronic Non-Medical Prescription Opioid Users and its Relationship to Social Stress

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Background: The U.S. is suffering from a persistent opioid epidemic emphasizing the need for a better understanding of the neurobiological mechanisms underpinning opioid use disorder (OUD). Recent preclinical and clinical evidence suggests that the endocannabinoid system (ECS), including the main endocannabinoids 2-arachidonylglycerol (2-AG) and anandamide (AEA) and the structurally related N-acylethanolamines oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), plays a key modulatory role in stress response and reward behavior, both crucially involved in the development of substance use disorder (SUD). Specifically, animal models indicate a crosstalk between the ECS and the opioid system linked to opioid and cannabis tolerance, sensitization, and reward. Animal studies further indicate that administration of mu-opioid receptor (MOR) agonists such as morphine has stress-buffering effects on social stress (i.e., social isolation/exclusion), which was also found in human pharmacological studies, even though not consistently. While animal models postulate a link between the opioid and endocannabinoid system, human translational studies are missing so far. Therefore, we aimed to test previous preclinical findings by investigating basal endocannabinoid (eCB) plasma levels in individuals with chronic non-medical prescription opioid use (NMPOU) and their association with laboratory-induced social stress.

Methods: We compared basal 2-AG, AEA, PEA, and OEA plasma concentrations between chronic NMPOU (n = 21) and opioid-

naïve healthy controls (n = 29). Additional associations between endocannabinoids and social stress of exclusion were assessed by the Cyberball task, a virtual ball-tossing game, where the participant was first included for about one minute receiving the ball six times (10%) and then excluded from the game for the subsequent two minutes as controlled by the computer. After the task, self-reports of exclusion/inclusion were conducted by asking how often they believed they have received the ball (in percentage) as well as how included and excluded they felt.

Peripheral endocannabinoids in plasma were quantified by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Based on our preliminary results, we are currently assessing the activity of fatty acid amide hydrolase (FAAH), which is the main enzyme hydrolyzing AEA and related NEAs, in homogenized whole blood by using a [3H]AEA hydrolysis analysis.

Analyses of covariance (ANCOVAs) were used, with GROUP (NMPOU vs controls) as the fixed factor, to control for well-known confounding variables AGE and SEX. For our secondary analyses to assess associations between eCBs and social stress of exclusion, we used Linear Mixed Models (LMM) with self-reported inclusion/exclusion after the Cyberball as dependent variables and the fixed factors: eCB (AEA, 2-AG, OEA, PEA, respectively), GROUP, AGE, SEX, and their interactions. For significant eCB*GROUP interactions, we further checked for group specificity by using additional Spearman's correlation analyses within each group. The statistical comparisons were carried out with a significance level of p.

Results: ANCOVAs showed significant elevated eCB plasma levels of AEA ($F(1,46) = 6.17$, $p = .017$, partial eta2 = .12), OEA ($F(1,46) = 9.66$, $p = .003$, partial eta2 = .17), and PEA ($F(1,46) = 9.42$, $p = .004$, partial eta2 = .17) in NMPOU compared to controls. No group difference was found for 2-AG ($F(1,46) = .02$, $p = .894$). The additional FAAH analysis will crucially complement our eCB plasma results contributing to an in-depth understanding of the ECS mechanism in chronic opioid use, which will be reported and discussed at the ACNP meeting.

LMM showed significant AEA*GROUP interaction for percentage of received balls ($F(1,50) = 9.96$, $p = .003$) and feelings of inclusion ($F(1,50) = 7.06$, $p = .011$), as well as a trend effect for feelings of exclusion ($F(1,50) = 2.90$, $p = .095$). Follow-up correlation analysis indicate a positive correlation within NMPOU between AEA and percentage of received balls ($r(21) = .45$, $p = .041$), feelings of inclusion ($r(21) = .46$, $p = .034$), and on trend level a negative correlation between AEA and feelings of exclusion ($r(21) = -.41$, $p = .068$). No correlations were found within the controls (p's>.299).

Conclusions: Together with our recent findings of elevated basal 2-AG plasma levels in dependent cocaine users, present results indicate substance-specific alterations of the ECS. Elevated AEA basal plasma levels in NMPOU and its association with reduced experience of social stress (social exclusion) indicate a specific MOR/ECS interaction, which might contribute to stress-relieving effects. Moreover, elevated AEA in NMPOU might have protective effects against social stress, which may explain early results reporting stress relieving effects of opioids to social exclusion/isolation in animals and the inconsistent findings in human translational studies so far. Present findings contribute to a better understanding of the underlying mechanism of the ECS in OUD, specifically regarding social stress. Moreover, our present and previous results indicate substance-specific alterations of the ECS suggesting different pharmaco-therapeutic targets within the ECS as novel treatments of SUD.

Keywords: Endocannabinoids, Opioid Addiction, Emotional Stress

Disclosure: Nothing to disclose.

P629. Altered Effective Connectivity of Emotional Perception and Regulation Networks During an Emotional Face Perception Task in Adults With Alcohol Use Disorder

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Background: Impairments in emotional regulation and mood symptoms are interrelated and associated with alcohol use disorder (AUD) risk, but the underlying aberrant neural circuitry involved is not clearly defined. In the present study, we sought to extend the current knowledge of AUD pathophysiology by examining alterations in effective (directional) connectivity (EC) during social information processing in persons with and without AUD using functional MRI.

Methods: We utilized functional MRI data from the Human Connectome Project database obtained during performance of an emotional face perception task in 70 participants with either DSM-IV alcohol abuse or dependence (combined as AUD group) and 70 age- and sex-matched control (CON) participants. During the task, participants were visually presented with either pictures of fearful, angry, and neutral faces or shapes and asked to match them to a face picture or shape at the bottom of the screen (Hariri et al., 2001). Within a network consisting of several key nodes including ventromedial prefrontal cortex (vmPFC), bilateral ventrolateral prefrontal cortex (vlPFC), bilateral amygdala (AMY), bilateral fusiform gyrus (FG), and right hypothalamus, we performed dynamic causal modeling (DCM) analysis to test AUD vs. CON group-level differences in EC as modulated by facial-emotion content (contrast: ΔEC during emotional-face trials minus ΔEC neutral-shape trials). We used linear regression analyses to characterize relationships between each EC outcome and measures of cumulative alcohol exposure and anxiety/depression.

Results: For AUD vs. CON group-level analyses, differences in EC outcomes were observed in multiple node-node circuits. Highlighting thresholded EC findings (ECs ≥0.3 Hz, Bayesian posterior probabilities [PPs] = 1): AUD participants had lower EC from vmPFC to bilateral vlPFC, right AMY, and left FG and greater EC from left vlPFC to right vlPFC and bilateral FG than CON participants. For correlational analyses, again highlighting significant, thresholded findings (EC betas ≥0.1 Hz, PPs = 1): In the total sample, EC from vmPFC to bilateral vlPFC, right AMY, and left FG were negatively associated with cumulative alcohol use, and ECs from the left vlPFC to right vlPFC and bilateral FG were positively associated with cumulative alcohol use. EC from the left vlPFC to the left FG was negatively associated with anxiety/depression scores. The PP reported above is ≥ 0 and < 1. Larger PP reflects stronger confidence in the corresponding EC strength or linear regression beta.

Conclusions: Individuals with AUD have disrupted EC in cortical-cortical and cortical-amygdala circuits during processing of emotion-laden social information that are centered in medial and lateral PFC regions and relate to cumulative alcohol exposure and mood symptoms. These results point to disruptions in prefrontal-prefrontal and prefrontal-amygdala circuits that subserve emotion recognition and "top-down" emotion regulation among individuals with AUD. Our findings may carry clinical implications for the treatment of AUD and comorbid mood disorders.

Keywords: Alcohol Use Disorder, Facial Emotion Processing, fMRI Effective Connectivity

Disclosure: Nothing to disclose.

P630. Sex Differences in Neural Response to Social-Evaluative Threat in Individuals With an Alcohol Use Disorder

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Background: Stress is a major cause of relapse in individuals with an alcohol use disorder (AUD) and is a common motivator for alcohol consumption. AUD and stress influence overlapping neural circuits in the brain, which result in maladaptive changes that serve to promote continued alcohol use and alter an individual's ability to appropriately respond to stressful events. There have been dramatic increases in alcohol consumption, binge drinking, AUD, and alcohol-related harms in women. Despite men having significantly higher alcohol consumption levels, women surpass men in the number of psychiatric problems associated with their alcohol use. Women also have higher rates of stress-related psychopathology, and show a heightened stress-reactivity response. These phenomena and their associated neurobiology may interact as unique risk factors in alcohol drinking women to lead to the development and AUD. Sex differences in the neural response to stress have been understudied in the AUD field; therefore, the present study explored sex differences in neural responses to social evaluative threat.

Methods: Twenty-five treatment-seeking individuals with a moderate-to-severe alcohol use disorder (15M/10F) were recruited to participate in the neuroimaging study from a larger clinical trial of a neuroimmune medication (NCT03594435). To assess acute social-evaluative stress, participants completed the Montreal Imaging Stress Task. The task is composed of a series of mental arithmetic problems with an induced failure algorithm. Social evaluative threat is induced in two ways; first through the comparison of the individual's performance with that of an "average" participant, and second, through negative feedback delivered by the investigator between scan runs. The amygdala was selected as an a priori region of interest. Whole-brain analyses were also conducted. Subjective ratings of anxiety and distress were collected pre- and post-scan. To examine sex differences in the response to stress on subjective ratings, repeated measures ANCOVAs were run. In these models, time was included as a within-subjects' factors, and sex (male vs. female) and the interaction between time and sex were included as between-subjects' factors. For the fMRI data, group analyses were conducted comparing whole brain activation to the experimental and control conditions in males vs. females. Sex differences in amygdala activation to stress were modeled using a repeated measures ANCOVA design, as described above. For all analyses, medication, age, smoking status, and THC use status were included as covariates.

Results: For anxiety, there was a trend-level interaction between time and sex ($F = 3.68$, $p = 0.07$), such that males had lower anxiety at baseline than females ($p = 0.01$), and anxiety ratings for males rose significantly following stress exposure ($p < 0.001$), while ratings for females did not significantly increase ($p = 0.30$). For distress, there was also a trend toward an interaction between time and sex ($F = 3.84$, $p = 0.07$), which followed a similar pattern. Males had lower baseline ratings than females ($p = 0.07$), which significantly increased following stress exposure ($p = 0.002$); whereas distress ratings for females did not significantly increase following stress exposure ($p = 0.75$). There was a significant interaction between task condition (stress vs. control) and sex on amygdala activation ($F = 5.49$, $p = 0.02$), such that females had higher amygdala activation in response to stress relative to control conditions ($p = 0.006$); whereas males had no significant difference in amygdala activation between the stress and control conditions ($p = 0.73$). The whole brain analysis also revealed an effect of sex on activation to stress vs. control conditions. Specifically, females had greater activation compared to males in the precuneus, posterior cingulate cortex, right inferior frontal gyrus, and the lateral occipital cortex during stress vs. control ($Z's > 3.56$, $p's > 0.03$).

Conclusions: Sex differences in subjective and neural responses to social evaluative stress were identified. Females had greater baseline (pre-scan) levels of stress and anxiety, but did not report increases in stress ratings after exposure to the stressor; whereas males had lower levels of baseline stress and anxiety which rose after exposure to the stressor. On a neural level, females recruited circuitry involved in affective regulation (inferior frontal gyrus) and self-referential processing (posterior cingulate and precuneus). This pattern of activation may reflect thoughts about performance, comparison of self-performance to others, and regulating emotions related to poor performance. Higher activation of this network may explain why females did not have an increase in stress and anxiety ratings following the stress-induction task. Conversely, males, who had less activation related to self-referential processing and affective regulation, reported higher stress following the scan, which was positively associated with amygdala activation. Therefore, males may not have recruited higher order regulatory circuitry to the same extent as females, resulting in a greater subjective experience of stress.

Keywords: Alcohol Use Disorder, Acute Stress, Sex Differences, Social-Evaluative Stress, Amygdala

Disclosure: Nothing to disclose.

P631. Neural Correlates of Risky Drinking in Adults With Social Anxiety

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Background: Social anxiety disorder (SAD) is characterized by excessive fear of negative evaluation, which results in avoidance of, or intense distress during, social interactions. Many people with social anxiety use alcohol to cope with social stress, which can lead to risky drinking behaviors and comorbid alcohol use disorders. While the comorbidity between SAD and risky drinking is well documented, the neural mechanisms underlying this relationship have not been well established. We present findings examining the neural correlates of risky drinking in adults with SAD during affective face processing and modulation.

Methods: 28 adults with SAD ranging in age from 18-45 ($M = 25$ years, 71% women) completed the Shifted Attention Emotion Appraisal Task during MRI scanning. We established brain regions involved in the modulation of emotional reactivity to affective faces during attention shifting and emotional appraisal (dorsolateral prefrontal cortex [dlPFC], anterior cingulate cortex [ACC], amygdala, insula; $p < .05$ FWE corrected after thresholding at $p < .001$ uncorrected). Clinical interview questions identified participants who endorsed past year risky drinking (consuming 3 or more alcoholic drinks within 3 hours on 3 or more occasions).

Results: Participants with SAD who endorsed risky drinking ($N = 9$) demonstrated less deactivation in the ACC than the non-risky drinking group ($N = 19$) during emotion modulation by attention shifting, $t(22) = -2.1$, $p = .047$. Participants with SAD who endorsed risky drinking demonstrated less activation in the dlPFC than the non-risky drinking group during emotion modulation by appraisal, $t(24) = 2.9$, $p = .01$.

Conclusions: These findings suggest that risky drinking is associated with difficulty down regulating salience processing regions (ACC) and less recruitment of attentional control regions (dlPFC) in people with SAD when modulating reactivity to

affective faces. This pattern of results may point to exaggerated affective processing and reduced capacity to regulate emotions in people with risky drinking and SAD. Future studies are needed to determine whether these mechanisms could be risk factors or consequences of this common comorbidity.

Keywords: Social Anxiety, Alcohol, BOLD fMRI Signal

Disclosure: Nothing to disclose.

P632. Neural and Biopsychosocial Markers of Transitioning to Hazardous Alcohol Use in Healthy Young Adults

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Background: Young adult hazardous alcohol use is associated with risk for developing an alcohol use disorder. We aimed to identify neural and biopsychosocial markers of transitioning from low-risk to hazardous alcohol use on the Alcohol Use Disorder Identification Test (AUDIT) over a 12-month follow-up period.

Methods: 78 individuals enrolled in the study; 64 met criteria for longitudinal analysis. Young adults with low-risk alcohol use at baseline completed self-report measures of alcohol use and biopsychosocial variables, as well as reward-based and emotion regulation tasks during MRI. We assessed the effects of baseline neural activation during reward anticipation, emotional reactivity, cognitive reappraisal, and threat anticipation (in the nucleus accumbens, amygdala, superior frontal gyrus, and insula, respectively) on odds of transitioning to hazardous use. Participants completed self-report measures at 3-, 6-, 9-, and 12-month follow-up timepoints.

Results: 16 participants transitioned to hazardous alcohol use. Neural regions of interest were not significantly associated with odds of transitioning (p>.09). Higher baseline AUDIT scores were associated with greater odds of transitioning to hazardous use (versus not transitioning; OR = 1.70, 95%CI: 1.15-2.52). While not statistically significant, emotional reactivity (amygdala) had a large odds ratio (OR = 44.44, 95%CI: 0.55-3611.69) and was higher in those who transitioned to hazardous alcohol use (g = −0.42).

Conclusions: Emotional reactivity in the amygdala may be explored as a neural marker of transitioning to hazardous alcohol use in a larger replication study. Higher levels of alcohol use, even below established thresholds, appear to be associated with greater risk of transitioning to hazardous use in the coming year.

Keywords: Amygdala, Negative Affect, Adolescent Alcohol

Disclosure: Nothing to disclose.

P633. Individual Differences in Baseline Positive Affect is Related to Neural Reward Reactivity to Δ9-Tetrahydrocannabinol among Young Adult Cannabis Users

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Background: Accumulating evidence indicates that the main psychoactive component of cannabis, Δ9-Tetrahydrocannabinol (THC), activates brain reward circuitry. One factor that may contribute to acute response to THC is baseline affect. Several studies find positive affect is linked to cannabis use among young adult cannabis users. It is not clear how THC impacts brain reward circuitry and if this is modulated by individual differences in baseline positive affect. In this study, we examined if an acute dose of THC altered neural reward reactivity young adult cannabis users and if this moderated by baseline positive affect. We hypothesized that THC would increase neural reward reactivity and that individuals with higher positive affect at baseline would demonstrate greater neural reward reactivity.

Methods: In a within-subject, randomized, double-blind, counterbalanced, placebo-controlled design, 24 young adult cannabis users completed the Doors monetary reward task during functional MRI (fMRI) approximately 120 minutes after ingestion of placebo or 7.5mg oral THC (dronabinol). Participants completed a measure of positive affect (PANAS) at baseline. Bilateral striatal anatomical regions related to reward processing (nucleus accumbens, caudate, putamen) were defined via the AAL3 atlas for a priori ROI analyses and submitted as separate dependent variables with drug as a time-varying independent variable and baseline positive affect as a fixed independent variable in linear mixed models.

Results: There were no significant effects of THC (vs. placebo) on neural reward reactivity (p-values > .05). There were significant interactions between baseline positive affect and neural reward reactivity for all striatal ROIs (p-values < .05). Follow up analyses found that individuals with higher positive affect at baseline demonstrated greater neural reward reactivity to THC for all striatal ROIs (p-values < .05). Positive affect at baseline was not related to neural reward reactivity to placebo (p-values > .05).

Conclusions: This is one of the first studies to examine how THC may alter neural reward reactivity and how that may be related to individual differences in positive affect in young adult cannabis users. We found that there was no effect of THC (vs. placebo) on neural reward reactivity. However, there were important individual differences in how baseline positive affect was related to neural reward reactivity to THC. Specifically, individuals with higher baseline positive affect demonstrated greater neural reward reactivity to THC. Positive affect at baseline was not related to neural reward reactivity during the placebo session. Overall, our findings expand upon previous studies linking positive affect to cannabis use among young adults, suggesting individual differences in positive affect contributes to how THC impacts brain reward circuitry.

Keywords: Cannabis, Functional MRI (fMRI), Reward, Positive Affect, Young Adults

Disclosure: Nothing to disclose.

P634. Drug-Biased Whole-Brain Responses to a Naturalistic Stimulus in Heroin Use Disorder and Recovery With Treatment

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Background: In drug addiction, brain circuits show enhanced response to drug cues at the expense of other reinforcers, such as food or social cues. This biased processing is thought to underlie craving for drugs, which contributes to drug seeking, perpetuating the addiction cycle. However, the neural correlates of this drug-biased processing, especially of naturalistic stimuli in heroin addiction, and its relationship to drug-cue induced craving are poorly understood. Moreover, it remains unclear whether typical treatments for heroin addiction can affect this biased processing. Unlike previous picture-based drug-cue reactivity studies, here we quantified the degree of drug-biased neural processing in heroin-

addicted individuals by measuring shared responses to an engaging drug-related movie. We further test whether this biased processing changes with 15-weeks of inpatient treatment as correlated with concomitant changes in cue-induced craving.

Methods: Thirty treatment seeking inpatients with heroin-use disorder (HUD) on medication-assisted therapy (40.00 ± 8.41 years, 24 M) and 25 healthy controls (HC, 39.97 ± 10.76 years, 16 M) watched the first 17 minutes of the heroin-related movie "Trainspotting" during fMRI. After the movie, subjects provided cue-induced craving ratings (prompted by 3 second video clips sampled every 30 seconds from the movie). All subjects then repeated the same procedure an average of 15 weeks later (the HUD group remained in inpatient treatment throughout this time). The whole brain was parcellated into 450 regions and a reverse correlation approach was applied within each region to identify synchronized brain responses within each group and the movie content that elicited these responses. Drug bias was quantified as the fraction of group-synchronized brain responses that followed drug (vs. non-drug) content in the movie (accounting for hemodynamic lag), compared between groups and sessions via permutation testing and FDR-corrected over the 450 regions of interest (ROIs).

Results: We found significantly greater drug-biased responses in HUD compared to HC in 28 ROIs, most notably in multiple regions in the orbitofrontal cortex (OFC) and ventromedial prefrontal cortex (vmPFC). A separate whole brain analysis also showed a significantly greater reduction in this drug bias in 12 ROIs in HUD compared to HC between both sessions. Notably 10 ROIs, 5 of which in the OFC, exhibited both significant drug bias in HUD at session 1 and a significant reduction in drug bias with treatment. There was also a significant reduction in cue-induced craving in HUD after treatment, which correlated with inter-subject correlation scores in the OFC of the HUD group.

Conclusions: Here for the first time we report a drug-biased neural signal shared by inpatients with HUD in response to a naturalistic stimulus. Notably, multiple OFC regions exhibited this initial drug-bias, which was reduced with treatment and tracked treatment-induced reductions in cue-induced craving. The OFC is thought to play a major role in the addiction cycle, underlying aberrant assignment of value and dysfunctional inhibitory control and decision making. Our results point to the potential normalization of these functions with treatment and shared OFC activity as a potential marker of recovery in HUD.

Keywords: Drug Addiction, Orbitofrontal Cortex (OFC), Naturalistic Drug Cues, Cue-Induced Craving, Longitudinal Study

Disclosure: Nothing to disclose.

P635. A Multi-Symptomatic Model of Heroin Use Disorder in Rats Revealed Distinct Behavioral Profiles and Neuronal Correlates of Heroin Vulnerability Versus Resiliency

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Background: Opioid use disorder (OUD) has become increasing prevalent world-wide. However, the behavioral and neurobiological mechanisms underlying OUD vulnerability versus resiliency remain underexplored. Furthermore, the behavioral and diagnostic heterogeneity within human OUD diagnosis are not readily captured in current animal models, posing a limitation on translational relevance.

Methods: In the current study, over 900 male and female heterogeneous stock rats, a line capturing genetic and behavioral heterogeneity present in humans, were assessed at two geographically distinct sites for several measures of heroin taking, refraining and seeking behaviors. Using a non-linear stochastic block model clustering analysis, a novel approach to assessing the complexity of substance use disorder, rats were assigned to OUD vulnerable, intermediate and resilient clusters. Following initial analysis, subsets of animals were further assessed compulsive heroin-taking behavior following forced abstinence, withdrawal-induced ultrasonic vocalizations and heroin-taking in the presence of an adverse stimuli. Hierarchical clustering analysis was also employed to discern the presence of subpopulations within the established OUD clusters. Lastly, differences in correlated neuronal activation patterns between OUD vulnerable and resilient clusters were assessed following a test for heroin cued reinstatement.

Results: Females exhibited a more vulnerable OUD behavioral phenotype relative to males, with estrous cycle phase contributing to within sex variability for cued reinstatement ($p = 0.01$). Overall, OUD vulnerable rats exhibited greater heroin taking, refraining and seeking behaviors relative to those in the intermediate and resilient cluster ($p < 0.01$ for all). Behavioral heterogeneity in traits conferring vulnerability were also observed, with males biased toward potentiated heroin taking behavior and females toward heroin seeking traits ($p = 0.001$). Vulnerable rats exhibited several behaviors associated with DSM-V criteria, with substantial sex differences present, akin to the human population. Lastly, heroin cue-induced neuronal activity patterns differed between resilient and vulnerable OUD phenotypes, supporting OUD resiliency as an active neurobiological mechanism. Further analyses showed profound sex differences in neuronal connectivity in the vulnerable cluster, with males preferentially engaging extended amygdala stress circuitry, and females the more classic cortico-striatal circuitry (all correlations $p < 0.05$ with $q = 0.1$).

Conclusions: Through the use of a non-linear clustering analysis, we captured the behavioral diagnostic heterogeneity associated with OUD in the human population. OUD vulnerability and resiliency engage distinct neuronal activation patterns, furthering the necessity to explore the neurobiological mechanisms associated with OUD propensity, specifically in a sex-dependent manner.

Keywords: Individual Variation, Sex Differences, Opioid Addiction, Resilience, Vulnerability

Disclosure: Nothing to disclose.

P636. The Brain Connectome During Rest Shows Interaction Between Cocaine Use Disorder and Childhood Maltreatment

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Background: Childhood maltreatment is known to alter social stress- and reward-related brain regions, which are also highly implicated in substance use disorders. Despite the significant clinical relevance, little is known regarding the neural effects of childhood maltreatment in addiction. We therefore studied the interaction between childhood maltreatment and cocaine use disorder (CUD) on neural function using resting-state functional connectivity (rsFC).

Methods: Individuals with moderate/severe CUD with 21.9 (SD = 12) lifetime years of cocaine use and healthy controls (HC) were grouped into those with low and high childhood trauma

(−/+) using the Childhood Trauma Questionnaire: 20 HC-; 20 HC +; 12 CUD; and 17 CUD+. Network-based statistic, a group analysis method controlling for family-wise error rate, was conducted on rsFC estimated from 44 brain regions clustered into 4 subnetworks: 1) high-level subnetwork (frontal pole, dorsomedial prefrontal cortex, posterior cingulate cortex, temporo-parietal junction, temporal pole, middle temporal gyrus, Precuneus), implicated in theory-of-mind, attention, executive, memory, and spatial processes; 2) intermediate-level subnetwork (anterior insula, anterior mid-cingulate cortex, inferior frontal gyrus, supramarginal gyrus, supplementary motor area, cerebellum, posterior superior temporal sulcus), implicated in empathy and pain tasks, sensory input and motor response preparation; 3) limbic subnetwork (ventromedial prefrontal cortex, rostral anterior cingulate cortex, amygdala, hippocampus, nucleus accumbens) implicated in emotion regulation; and 4) visual-sensory subnetwork (frontal gyrus, posterior superior temporal sulcus, middle temporal V5 area) implicated in perception-action and integration of visual information.

Results: We observed a trend drug effect (CUD < HC, pcorrected = .06) in a cluster of 54 connectivity edges with high-degree nodes (i.e., node with high number of significant edges) including limbic [nucleus accumbens (NAcc), ventromedial prefrontal cortex (vmPFC), amygdala], dorsomedial PFC (dmPFC), and precuneus nodes. Trauma effect (High < Low Trauma, 63 edges, pcorrected = .03) was most evident in limbic nodes (vmPFC, amygdala) and precuneus. Importantly, Drug x Trauma interaction (74 edges, pcorrected = .002) was most evident in nodes of the drug/trauma main effects (NAcc, amygdala, dmPFC), as well as insula, inferior frontal, and visuosensory nodes (V5, fusiform).

Conclusions: While the main effects of drugs and trauma were most evident in limbic and executive subnetworks, we observed wide-spread alterations with high-degree nodes showing significant interaction in multiple networks that are core to addiction and psychiatric symptomatology. Altogether, the results suggests that childhood trauma has protracted effects on intrinsic neural connectivity despite the recency and extent of cocaine use disorder.

Keywords: Cocaine Use Disorder, Childhood Maltreatment, Resting State Functional Connectivity, Substance Use Disorder, Network Based Statistic (NBS)

Disclosure: Nothing to disclose.

P637. How Biased-Beliefs About Drug Use are Maintained

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Background: Media outlets consistently report the personal, societal, and economic impacts of illicit drug use. Opioids, especially heroin and fentanyl, dominate these conversations given high rates of overdose and relapse among people in recovery for opioid use disorder. Despite this widespread information about the risks of opioid use, drug use remains common, indicating a need for additional understanding about how undesirable information is incorporated into beliefs about personal risk. One mechanism may be a person's tendency to believe that good outcomes are more likely than bad outcomes, termed 'optimism bias'. This biased belief updating process is thought to be maintained by how information about risk is processed in the inferior frontal gyrus, dorsomedial prefrontal cortex, and ventral striatum. However, whether undesirable information about drug use is neurally encoded and perceived differently from other personally relevant, and undesired outcomes remains unclear. To address this, we used cognitive tasks and models to evaluate how people with opioid addiction and healthy individuals think about their chances of various negative outcomes due to drug use or other reasons.

Methods: During fMRI, treatment-engaged individuals with opioid use disorder ($N = 33$; n = 11 women, mean age = 42.91 years; median treatment duration = 12.52 mos.) and matched healthy controls (n = 29; n = 13 women, mean age = 41.30 years) estimated their chances of 60 drug- and nondrug-related negative life events (e.g., overdose, bone fracture). They then saw the actual likelihood of each event before re-estimating its probability. Subjects were incentivized to be as accurate as possible in their estimates. Trials were partitioned based on whether subjects received desirable or undesirable information about their chances of each event and the subjective drug- and nondrug-relatedness of that event. We used a modified learning model to assess how much likelihood estimates changed as a function of having received desirable vs. undesirable information and drug-relatedness. A Bayesian belief updating model was also used to capture how prior and new information is integrated into biased posterior beliefs. fMRI analyses modeled estimation errors (first probability estimate vs. actual event likelihood by trial type) and predicted Bayesian posterior beliefs as parametric regressors during presentation of the actual likelihood of each event. Activity correlating with these regressors was extracted from a priori ROIs (inferior frontal gyrus, ventral striatum, and dorsomedial prefrontal cortex). We used a linear mixed-effects model with random intercepts and slopes per subject to test differences in belief updating weights and neural activity between groups, event type, and information valence.

Results: We found that both patients with opioid user disorder and controls held an optimism bias, updating their beliefs about personal risks more after receiving desirable information vs, undesirable information about their actual likelihood of experiencing different negative drug and nondrug-related events ($p < 0.001$). In addition, patients held a greater optimism bias than controls specifically for drug-related events ($p = 0.03$), although they were not more optimistic about drug vs. nondrug events. Our computational model further supported that biased belief updating was maintained by subjects giving disproportionate weight to desirable evidence about personal risk ($p < 0.01$). At a neural level, optimistic beliefs about negative drug-related events in patients were found to be modulated by biased error encoding in inferior frontal gyrus ($p = 0.047$) and ventral striatum ($p = 0.03$), with opposite patterns of neural responses to receiving desirable compared to undesirable information. Integration of this biased response into subsequent beliefs were reflected by activity in the ventral striatum ($p < 0.001$) and dorsomedial prefrontal cortex ($p < 0.001$).

Conclusions: By leveraging the cognitive neuroscience of optimism and belief updating, our findings reveal a novel mechanism for improving psychoeducational interventions for opioid use and provide potential targets for neurobiological treatments for opioid use disorder.

Keywords: Human Neuroimaging, Optimism, Belief Updating, Opioid Addiction

Disclosure: Nothing to disclose.

P638. The Relationship Between mPFC Ca2+ Activity and Behavioral Responding to Appetitive and Aversive Stimuli is Altered After Chronic Ethanol Exposure

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Background: Reward prediction error (RPE) is a computational neural process that compares outcomes with expectations to guide decision making and adaptive responding. Impaired decision making is one of the most consistently observed consequences of alcohol use disorder (AUD). While some evidence suggests that disrupted RPE signaling may underlie these impairments, this idea has not been thoroughly explored. The medial prefrontal cortex (mPFC) plays a critical role in decision making and emerging data suggests that distinct mPFC cell populations encode RPE. However, the effect of chronic ethanol exposure on RPE processing in the mPFC is unknown.

Methods: Adult male and female Long-Evans rats were classically conditioned to associate an audiovisual cue with delivery of either sucrose pellet or foot shock. In vivo fiber photometry was used to measure GCaMP7s signal, an indicator of intracellular Ca2+ activity, in the prelimbic (PL) subregion of the mPFC during learning and in response to expected and unexpected outcomes. Rats were subsequently rendered dependent using a standard 14-d chronic intermittent ethanol (CIE) vapor exposure paradigm. Controls were exposed to room air (AIR). One week after their last vapor exposure session, rats resumed behavioral testing to determine the effect of CIE on changes in PL mPFC Ca2+ activity in response to expected and unexpected outcomes.

Results: PL mPFC Ca2+ signal increased significantly from baseline in response to both appetitive and aversive cues ($p < 0.0001$). Ca2+ signal significantly decreased from baseline in response to appetitive outcomes ($p < 0.01$), whereas it was significantly increased in response to aversive outcomes ($p < 0.0001$). Ca2+ signal did not reflect trial-by-trial changes in expected outcome for appetitive or aversive stimuli across learning or RPE probe sessions. However, Ca2+ signal was significantly negatively modulated by reward seeking in the appetitive task ($p < 0.001$) and positively modulated by active threat responses in the aversive task ($p < 0.0001$). Prior to CIE exposure, rats adaptively reduced reward seeking during appetitive negative RPE probe sessions. However, after CIE reward seeking rates were similar between positive and negative RPE sessions ($p > 0.05$). A similar inability to distinguish between positive and negative RPE sessions was observed after CIE in rats that underwent the aversive task and this was associated with a disruption in the relationship between threat responding and PL mPFC Ca2+ signal.

Conclusions: These results indicate that bulk PL mPFC Ca2+ activity does not encode RPE. Instead, Ca2+ activity reflects a combined signal corresponding to both salience of environmental stimuli and behavioral responding to those stimuli. Our data further suggest that CIE exposure promotes changes in PL mPFC activity and behavioral responding that impairs cognitive flexibility. Supported by NIH NIAAA grants R01 AA029130, P50 AA022538.

Keywords: Reward and Aversion, Decision Making, Alcohol and Substance Use Disorders, Reward Prediction Error, In Vivo Fiber Photometry

Disclosure: Nothing to disclose.

P639. Early Social Isolation Potentiates Heroin Seeking Via Disrupting the Function of PFC-VTA Circuit

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Background: Opioid use disorder (OUD) is a chronic life-long disease characterized by compulsive drug taking and persistent vulnerability to relapse. Stress has been reported to induce vulnerability to opioid relapse by affecting the gene expression in key brain regions within the reward pathway. However, the specific brain circuits contributing to stress-induced relapse vulnerability as well as the correspondence molecular changes within key brain circuits remain unclear.

Adolescence is a critical period when behavioral and brain circuitry maturation occurs. Stress during this key period results in higher rates of psychiatric disorders including drug addiction. Social interaction during early life plays an essential role in cognitive development. Preclinical studies reported that social isolation during adolescence is associated with increased drug taking and drug seeking. Concomitantly, our lab previously reported that early social isolation (ESI) stress potentiates heroin-seeking behavior after forced abstinence. Yet, the underlying mechanisms remain elusive.

Stress and substance abuse are associated with neuroplasticity in the mesocorticolimbic pathway. For example, the maladaptation of glutamatergic projecting neurons from the prefrontal cortex (PFC) and its projecting subcortical regions (e.g., ventral tegmental area [VTA]) are implicated in both stress and addiction. Therefore, we hypothesized that ESI stress may increase addiction vulnerability via exerting pathological impairments in these key brain regions. To test our hypothesis, we used a mouse heroin self-administration model to examine how chronic early social isolation stress affects the behavioral and neural responses to heroin during adulthood.

Methods: C57BL/6J mice and B6;129S4-Gt(ROSA)26Sortm9(EGFP/Rpl10a)Amc/J (GFP-L10a) mice were purchased from Jackson lab. Both male and female mice were used in this study. Based on previous publications, early social isolation (ESI) stress was carried out after weaning from postnatal day 21 (P21) to P60 (about 5 weeks). Control mice are group-housed (GH, 4-5 mice per cage). All the procedures are approved by the Institutional Animal Care and Use Committee at the University of Kansas. All animals were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities.

At the age of P60, GH and ESI C57 mice were subjected to 10 days of heroin SA (50 ug/kg/infusion, 3 h/session), followed by 14 days of abstinence. During abstinence, mice received an injection of retrograde tracer CTB488 injection into VTA. On the last day of abstinence, mice were sacrificed for generating brain slices and then the projecting pyramidal neurons in PFC were visualized under a microscope for ex vivo recording of action potential.

To manipulate PFC-VTA circuit activity, retrograde double-floxed Gq (hM3Dq) AAV was injected bilaterally into the VTA and Cre-AAV was injected into the PFC of C57BL/6J mice during abstinence following heroin self-administration. After virus expression, mice received vehicle or C21 (1 mg/kg, i.p.) injection and were subjected to a 1 hr cue- and context-induced heroin-seeking test.

To profile the transcriptional changes within PFC-VTA projection, GFP-L10a mice received AAVrg-Cre injection in the VTA during abstinence following saline or heroin self-administration. Then PFC-VTA projection-specific mRNA was isolated via translating ribosome affinity purification and then used for RNA sequencing (TRAP-seq).

Results: The frequency of spontaneous action potential (sAP) was significantly lower in the PrL-VTA projecting neurons from mice who underwent heroin abstinence compared to mice from the saline group ($N = 12$-18 cells/group, F1, 57 (drug) = 17.7, $P < 0.0001$, two-way ANOVA). In addition, sAP frequency was notably lower in the PrL-VTA projecting neurons from ESI mice compared to GH mice (F1, 57 (stress) = 14.0, $P = 0.0004$, two-way ANOVA). Moreover, the inhibition of sAP frequency was

potentiated by the interaction of heroin abstinence and ESI stress. Interestingly, the number of total active responses during the heroin-seeking test was significantly higher in ESI group compared to GH group ($N = 9$-19 mice/group, F1, 47 (stress) = 6.8, $P = 0.0119$, two-way ANOVA). Recovering the PFC-VTA projection hypoactivity by chemogenetic activation via C21 injection was able to lower the total active responses in EIS mice ($N = 9$-19 mice/group, F1, 47 (drug) = 13.1, $P = 0.0004$, two-way ANOVA), without altering total inactive responses and the general locomotion activity. Next, we further explored the genome-wide gene expression changes in PrL-VTA projecting neurons induced by ESI stress, heroin abstinence and their interaction (GH SAL, $n = 3$; ESI SAL, $n = 3$; GH HER, $n = 3$; ESI HER, $n = 3$). We found that ESI stress and heroin abstinence convergently affected the expression of genes enriched in signaling pathways involved in synaptic function, while the interaction of ESI stress and heroin abstinence altered the expression of genes related to DNA damage response and cell cycle.

Conclusions: These data indicate that ESI stress-induced susceptibility to heroin relapse is associated with the ESI-potentiated neuronal dysfunction in PFC-VTA projections; and that this neuronal dysfunction is accompanied by gene transcriptional changes within PFC-VTA circuits. Our studies provide novel insight into the neurobiology underlying heroin addiction.

Keywords: Heroin Seeking, Heroin Self-Administration, Prefrontal Cortex, Ventral Tegmental Area (VTA), Cell- and Circuit-Selectivity

Disclosure: Nothing to disclose.

P640. Rapid Appearance of Negative Emotion During Oral Fentanyl Self-Administration in Male and Female Rats

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Background: Opioid use disorder is an epidemic in the United States, where fentanyl has caused a dramatic spike in overdose deaths (>70,000/year). Fentanyl's low price, ready availability, and extreme potency contribute to its profound impacts and difficult treatment. It produces powerful but fleeting euphoria, while withdrawal causes severe physical and emotional turmoil. In abstinence, strongly conditioned drug-cues and hypersensitivity to emotional distress motivate persistent relapse. Careful preclinical modeling of emotional components of volitional fentanyl intake is essential to understanding both the adaptations in neurobiology caused by chronic drug exposure and the individual differences in neurobiology that could predict addiction susceptibility.

Methods: Male ($n = 10$) and female ($n = 12$) rats (Long Evans) were trained to self-administer (SA) liquid fentanyl, (70 µg/mL) for 5 days a week (3 hr) over three weeks on an FR1 schedule. Whole-brain levels of fentanyl were estimated using a two-compartment model for rats which was used to isolate the "loading" and "maintenance" phases of SA. Ultrasonic vocalizations (USVs) were recorded during oral fentanyl SA, extinction, and reinstatement. USVs were automatically detected using DeepSqueak v3 and clustered using a combination of variational autoencoder embeddings and contour frequency parameters. Calls were also classified as either positive affective (>38kHz) or negative affective (<38kHz) and were visualized using UMAP. A subset of animals was used for fiber photometry and miniscope (UCLA V4) recordings of the lateral habenula (LHb), to determine if LHb processing of fentanyl cues and consumption was impacted by the shift from loading to maintenance.

Results: Animals showed escalating fentanyl intake, strong association of conditioned cues, and reinstatement, along with key individual and sex differences. They also displayed inelastic demand curves, maintaining stable intake across a wide range of doses. Oral fentanyl SA produced patterned SA similar to IV cocaine, with distinct "loading" and "maintenance" phases. During loading, animals responded rapidly to increase their drug level, then switched to slower maintenance responding to hold a stable drug-level. Positive-affective 50 kHz USVs were produced during the loading phase, while negative-affective 22 kHz USVs were produced during maintenance (session type*affect interaction; $F(1,67) = 11.24$, $p < 0.01$). The LHb, a region known to process both aversion and the motivational value of reward related cues, responded to drug-cues and consumption differentially depending on the phase of SA. Changes in LHb activity were potentiated during the negative affective maintenance phase compared to loading.

Conclusions: Together, the USVs and LHb activity reveal a rapid shift from positive reinforcement during the loading phase to a negative reinforcement during the maintenance phase, occurring much earlier than generally thought and revealing a within-session opponent process. Furthermore, oral fentanyl SA provides substantial translational and technical benefits, enables the study of neurobiological, motivational, and emotional aspects of fentanyl use, and captures valuable individual and sex differences related to substance use risk propensity. This model also provides a level of convenience that could democratize access to fentanyl SA procedures and accelerate neurobiological discoveries.

Keywords: Fentanyl, Ultrasonic Vocalization (USV) Model, Oral Self-Administration, Miniscope

Disclosure: Nothing to disclose.

P641. Perceptual Generalization of Alcohol-Related Value in Risky Drinking

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Background: Stimulus generalization, or the ability to spread value and associated responses to perceptually similar stimuli, is a fundamental learning process that is conserved across species and contributes to both adaptive (allowing past experience to facilitate actions in new situations) and maladaptive behavior (overgeneralizing fear responses to safe situations). This tendency to overgeneralize fear associations is a hallmark of anxiety disorders. Notably, there is also evidence for overgeneralized memories in chronic alcohol use, and more widely generalized memories for drinking may cause a person to seek alcohol in situations that even vaguely resemble past experiences. However, the precision of memory for alcohol-related associations in heavy drinkers has not yet been quantified. In a set of online experiments, we assessed generalization of alcohol-related rewards (Study 1) and losses (Study 2) among light and heavy drinkers using a novel translational paradigm, hypothesizing that riskier drinkers would generalize alcohol-related associations more broadly.

Methods: We conducted two online experiments assessing generalization of alcohol-related gains (Study 1) and losses (Study 2) among individuals who engaged in light or risky patterns of drinking (Study 1: $N = 88$ (aged 24-44); Study 2: $N = 87$ (aged 21-44)). After completing a screening questionnaire to assess their drinking behavior, participants were told that they would be playing a card game. In the conditioning phase, participants formed conditioned associations between cards portraying shapes (CS) and photographs (US) portraying alcoholic beverages or

neutral objects in naturalistic contexts. This created three CS types: CSalc (paired with alcoholic beverages), CSobj (paired with objects), and CSnull (not paired with photographs). In Study 1 (preregistered), US images were "tokens" (added to participants' bonuses at the end of the study), whereas in Study 2, US images were "penalty tokens" (deducted from the bonus). Next, participants completed the generalization phase, in which they were presented with a series of cards. On each trial, they could choose to play with that card or select a random draw from a deck. Critically, the presented cards varied in perceptual similarity to the original CSs, allowing us to compute generalization gradients. Finally, participants completed a surprise recognition memory test for the US photographs.

Results: We found comparably conditioning effects in both drinking groups, such that all participants learned to prefer (Study 1) or avoid (Study 2) CS shapes paired with alcohol-related or neutral US images. Consistent with our hypothesis, we found differences in how light vs risky drinkers generalized these associations. In Study 1, higher AUDIT scores were associated with greater CSalc generalization, such that they were more likely to pick cards that were increasingly dissimilar to the original CSalc, but not CSobj (AUDIT x CS type x dissimilarity: $\chi2(2) = 9.75$, $p = .008$). We found a similar pattern in Study 2, with riskier drinking associated with greater CSalc generalization, such that they were more likely to avoid cards that were increasingly dissimilar to the original CSalc ($\chi2(2) = 8.99$, $p = .01$). These tendencies to generalize were modulated by episodic memory for light drinkers, with more precise memory for alcohol US images associated with attenuated generalization of CSalc.

Conclusions: These results demonstrate a novel real-world correlate of stimulus generalization, highlighting the potential clinical importance of this memory process in the context of addiction. In ongoing work, we are bringing participants into the laboratory to perform this experiment to assess (1) whether proxies for dopaminergic and noradrenergic signaling (obtained using eyetracking) are associated with differences in memory generalization and (2) whether healthy aging, which is associated with decreases in memory specificity, also contributes to broader generalization, highlighting a potentially important cognitive distortion associated with risk for risky drinking in older age.

Keywords: Learning Generalization, Alcohol, Binge Drinking, Pavlovian Conditioning, Memory Bias

Disclosure: Nothing to disclose.

P642. The Effects of Adolescent or Adult Acute Stress on Adulthood Cocaine-Seeking Behavior

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Background: Adolescent or adult traumatic stress, such as violence and physical or sexual assault, has emerged as a substantial risk factor for substance use disorder (SUD). Neurobiological alterations that mediate this correlation, on the other hand, are poorly known. Preclinical studies suggest that stressful experiences during adolescence have long-term behavioral outputs and neurophysiological consequences, including stress, anxiety, and altered efficacy of synaptic transmission in adulthood. Additionally, studies investigating stress prior to cocaine self-administration in rats have reported an increase in drug acquisition and reinstatement. To identify groups at risk of developing SUD, researchers must first elucidate the neurobiological mechanisms behind the stress/drug-addiction comorbidity. We hypothesized that stressful experiences, either during adolescence, or adulthood, or both, will lead to higher cocaine-seeking behavior.

Methods: To test this, we used the fear-conditioning (FC) paradigm as a stressful experience in both, adolescent (P30) or adult (P60) Sprague Dawley rats. In adolescent rats, 30 days after stress induction, rats were exposed to 12 days of short-access (2hr) cocaine self-administration, followed by 15 days of extinction, and two reinstatement sessions (cue- and cocaine-primed). In adult rats the exact same protocol was used, except it was started five days (not 30 days) after FC.

Results: Contrary to our hypothesis, the stressed adolescent group showed no differences in term of cocaine consumption, extinction, and cue-primed reinstatement, relative to the controls. Moreover, cocaine-primed reinstatement significantly decreased compared to the non-stressed adolescent group. On the other hand, stressed adult male rats showed seemingly higher cocaine acquisition (not statistically significant), no difference in extinction, and statistically significant difference in both, cue- and cocaine-primed reinstatements, compared to non-stressed adult rats.

Conclusions: Our findings show that the effects of acute stress on cocaine seeking behavior are dependent on the timing of the stressful event, with stressed adults showing higher reinstatement than adolescent. We are currently conducting experiments with female rats to determine if there are any gender-related differences in the results.

Keywords: Cocaine, Fear Conditioning, Acute Stress

Disclosure: Nothing to disclose.

P643. Neurobiological Subtypes with Distinct Comorbidity Profiles in Alcohol Use Disorder

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Background: Previous studies have demonstrated considerable heterogeneity in the neurobehavioral mechanisms underlying Alcohol Use Disorder (AUD), defining distinct behaviorally defined AUD subtypes. However, neurobiologically based AUD subtypes have not been investigated. We chose to investigate neurobiological subtypes derived from resting-state fMRI (rs-fMRI) that provides an unbiased assessment of whole-brain function without task demands.

Methods: We used rs-fMRI data from the Human Connectome Project (HCP) dataset ($N = 668$, mean age: 28.9 years, 58% female, 22% with AUD). We generated five graph theory measures per brain region (379 regions). We employed a semi-supervised clustering algorithm, HYDRA, with 10-fold cross-validation and assessed a range of subtyping solutions ($K = 2$-10) per graph theory measure with the Adjusted Rand Index (ARI). We assessed reproducibility through repeated stratified subsampling and permutation testing. We compared resting-state fMRI connectivity of each subtype to controls, co-varying for age and gender and correcting for multiple-comparisons (p-FDR < 0.05). To describe the phenotypic profiles per subtype, we extracted phenotypic latent factors via Exploratory Factor Analysis from all the HCP measures (100 input variables). We identified 18 latent factors with good model fit (RMSEA = 0.03; TLI = 0.86) in the Exploratory Factor Analysis. We compared the phenotypic profiles of each subtype to controls, co-varying for age and gender and correcting for multiple-comparisons ($p < 0.05$).

Results: We identified two neurobiological AUD subtypes as the most stable and robust solution. The optimal number of subtypes, displaying the highest overall ARI values for all assessed graph theory metrics, was a cluster solution of $K = 2$. Based on repeated

stratified subsampling, the solution using the 'participation coefficient' metric was most stable and robust (ARI = 0.74 for K = 2). Permutation tests showed that this ARI at K = 2 was significantly higher than the null distribution ($p < 0.05$).

The two identified neurobiolgical AUD subtypes differed by their neurobiological and psychiatric profiles. Subtype 1 had increased connectivity in the somatosensory and motor system, with decreased connectivity in prefrontal brain networks and subcortical areas (p-FDR <0.05). In contrast, Subtype 2 exhibited the opposite pattern with increased prefrontal and subcortical connectivity but decreased somatosensory and motor connectivity (p-FDR < 0.05). Behaviorally, the 'high motor connectivity' type showed high somaticism and the highest level of externalizing behaviors, whereas the 'high prefrontal connectivity' type had high negative affect, inattention and internalizing symptoms ($p < 0.05$ corrected).

Conclusions: We identified two robust resting-state-based subtypes with distinct profiles of psychiatric comorbidity, confirming neurobehavioral heterogeneity in AUD. These two subtypes evidenced opposite connectivity patterns in brain networks (e.g., in the frontoparietal network) that are commonly targeted in current AUD neuromodulation protocols. The results demonstrate the need to develop personalized treatment approaches (e.g., neuromodulation interventions and beyond) for AUD.

Keywords: Biotypes, Personalized Medicine, Alcohol Use Disorder - Treatment, Computational Neuroscience, Addiction

Disclosure: Nothing to disclose.

P644. Effects of Cocaine Cues on Neurobehavioral Choice Dynamics in People With Cocaine Use Disorder

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Background: Prior research on reward driven decision-making has shown that choices between options are based on the relative values of the associated rewards. Real-world decision-making occurs in dynamic contexts where both the individual and reward attributes change over time, requiring that option values update continuously. Drug-associated cues promote continued drug use, but the neurobehavioral mechanisms that underlie this phenomenon are not well understood. This study seeks to use a reinforcement learning (RL) framework to determine the effects of cocaine cues on choice dynamics in people with cocaine use disorder (CUD). We hypothesized that cocaine cues would impact observed choice and RL modeling parameters, and this effect would be associated with unique neural signatures related to the model-derived subjective value of cocaine cues.

Methods: Participants with CUD ($N = 17$, 8f) performed two versions of a probabilistic choice task (neutral vs. neutral cue ["neutral" choice task] and cocaine vs. neutral cue ["cued" choice task]) in an fMRI scanner. In both tasks, money reinforcers ($0.25/win) were scheduled probabilistically and alternated unpredictably between rich and lean payout blocks (6 blocks, 50 trials/block) for each cue type. Initially, five different choice models that described behavior were compared. The best fitting model including an exchange rate parameter that captured the substitutability of cues in each task. Exchange rate parameters were used to calculate the subjective value of choice options. Paired statistical tests were used to analyze behavioral data ($p < 0.01$). General linear models were used to isolate participant and task specific neural activity during each task as well as model-derived subjective value associations throughout the brain ($p < 0.001$, corrected).

Results: Significantly more (MEAN ± SEM) cocaine cue selections were made on the cued choice task, compared to the equivalent neutral cue option on the neutral choice task, when cocaine cues were both the rich (cocaine = 106.0 ± 3.0 vs. neutral = 93.2 ± 3.2) and the lean (cocaine = 74.8 ± 3.9 vs. neutral = 55.1 ± 2.7) payout options. The amount of money earned did not differ between cue types. The modeled probability of choosing the rich cue following a lean-to-rich payout switch was less for the cued choice task, compared to the neutral choice task. The calculated subjective value for cocaine cues on the cued choice task was significantly greater than that of neutral cues on the neutral choice task (cocaine cue = $0.35 ± 0.8 vs. neutral cue = $0.25 ± 0.1). Finally, greater cocaine cue subjective values on the cued choice task were associated with increased bilateral activity in the rostral medial prefrontal cortex (rmPFC), which was not observed for neutral cue subjective values on the neutral choice task.

Conclusions: This study shows that the value of rewards associated with cocaine-cues are increased relative to other options, which involves a rmPFC-related mechanism. It also demonstrates that the combination of dynamic choice tasks, reinforcement learning modeling and functional neuroimaging is well suited to capture neurobehavioral mechanisms of decision-making that could help to explain the disordered use of drugs such as cocaine. These findings also support the continued application of reward-based decision-making theory to the study of substance use disorder.

Keywords: Cocaine Use Disorder, Reinforcement-Based Decision-Making, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P645. Imaging Sex Differences in the Neuroimmune System in Alcohol Use Disorder

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Background: Alcohol use is one of the leading causes of disability in the United States. Women tend to drink for stress regulation and are more vulnerable than men to the consequences of alcohol use. Initially, acute alcohol stimulates microglia, the brain's resident immune cells, to carry out repair functions. However, excessive activation leads to the release of substances that cause neuronal dysfunction and death, such as inflammatory cytokines, chemokines, reactive oxygen species and nitric oxide. Importantly, these neurobiological mechanisms contribute to alcohol-induced neurodegeneration making microglia an important molecular target. Neurodegeneration impairs neurocognition and precipitates drinking. Stress further disrupts microglia function and neurocognition. Women with alcohol use disorder (AUD) report higher stress and have greater neurocognitive impairments than men with AUD, which predict poorer treatment outcomes. We previously showed lower microglia marker levels in AUD vs. controls. The current study aimed to investigate sex differences in the neuroimmune system of individuals with AUD in an expanded sample. Based on prior work, we hypothesized that women with AUD would show lower microglia marker levels relative to sex-matched healthy controls. We also hypothesized that lower

microglia marker levels would be related to worse cognitive performance and drinking outcomes.

Methods: To date, twenty-nine individuals with AUD (mean 42 drinks/month for 24 years; 10 women) and 28 age-, sex-, smoking status- and rs6971 single-nucleotide polymorphism genotype-matched control subjects participated in positron emission tomography (PET) scans with [11C]PBR28 to measure 18-kDa translocator protein (TSPO), a marker of microglia. The outcome measure, volume of distribution (VT), was estimated regionally in the hippocampus, frontal cortex, cerebellum, and putamen with multilinear analysis as a measure of TSPO availability. Univariate analysis of variance models (one per brain region of interest – hippocampus, frontal cortex, cerebellum, putamen) were conducted with VT as the dependent variable, diagnostic group (AUD vs. healthy control) and sex (male vs. female) as between-subject factors, and genotype ('high' vs. 'medium' affinity binders) as a fixed-factor. A priori post-hoc analyses were conducted to compare VT values between women with AUD and sex-matched controls. A subset of subjects completed a cognitive battery and the Alcohol Use Disorder Identification Test (AUDIT) to assess hazardous drinking. Performance on a verbal learning and memory cognitive task (International Shop List; immediate and delated recall) was compared between the AUD and healthy control group using independent-samples t-tests. Preliminary analyses of relationships between VT and cognitive task performance were conducted using linear regressions. Preliminary analyses of relationships between VT and AUDIT performance were conducted using linear regressions.

Results: We found a main effect of diagnostic group such that individuals with AUD had significantly lower VT than their control counterparts in the cerebellum ($p = 0.04$, effect size: $\eta p2 = 0.07$). The interaction of diagnostic group and sex was significant ($p = 0.02$, $\eta p2 = 0.09$) in striatum. Women (but not men) with AUD had significantly lower VT in all four regions ($0.01 \leq p > 0.05$, effect sizes:$0.07 \leq \eta p2 \geq 0.11$) compared to sex-matched controls. Preliminary analyses revealed that the AUD group performed worse than the control group on verbal learning and memory tasks ($p \leq 0.04$, Cohen's $d \geq 2.2$) but VT values were not related to cognitive performance ($p \geq 0.59$). Preliminary analyses in the AUD group revealed that lower VT values were significantly related to higher AUDIT scores in cerebellum, hippocampus, and putamen ($0.54 \geq R2 \geq 0.59$, $0.03 \geq p \geq 0.04$). No significant sex differences in these relationships were found.

Conclusions: We show evidence for sex differences in levels of a microglia marker in people with AUD vs. controls. These data extend our previous report of lower microglia marker levels in people with vs. without AUD to demonstrate greater impairment in women than men with AUD. We also show that this greater impairment is related to higher levels of hazardous drinking. This suggests that neuroimmune suppression may be more pronounced in women with AUD compared to sex-matched controls and this may underlie reports of greater neurodegeneration in AUD women than men.

Keywords: Alcohol Use Disorder, Sex Differences, Microglia, Translocator Protein (TSPO), Positron Emission Tomography (PET)

Disclosure: Nothing to disclose.

P646. Impaired Arbitration Between Reward-Related Decision-Making Strategies in Alcohol Users: A Computational Modeling Study

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Background: Decision making is a deliberate computational process aiming to balance the optimal allocation of brain resources to achieve efficiency. Such balance during decision-making is posited to be achieved by the arbitration between model-based (slow, deliberative, and predicated on goal-planning) and model-free (fast, reflexive, and habit-based) control, and are proposed to reflect distinct trade-offs between accuracy (i.e., value-maximization) and demand (i.e., computational load). Based on the reinforcement learning (RL) framework for low reward stakes, a greater influence of model-free decision-making is observed, whereas model-based control appears to positively correlate with reward opportunity. Thus, there is an arbitration between these two decision-making processes, which depends partly on the value of the anticipated reward. Impaired reward valuation, and reward related decision making are hallmarks of substance use disorders. Individuals with problematic substance use have shown to rely more on less taxing model-free control. Apart from substance users, impaired decision making and risky behaviors were observed in long-term alcohol users as well. Thus, while it is known that even less alcohol use may impact decision-making but it is unclear how alcohol users arbitrate between model-free and model-based decision-making under different reward stakes, and whether they engage in more model-based decision-making with higher compared to lower reward stakes, as similar to do non-alcohol users. We hypothesized that unlike Non-Users, Alcohol Users will not employ model-based decision-making in higher compared to lower reward conditions. We also explored whether such arbitration between the two control systems was associated with impulsivity and risk sensitivity.

Methods: In this study, 81 participants (22.15 years old; 47 females) who reported no alcohol use and those reported consuming alcohol rarely (less than once a month) were grouped as Non-Users ($n = 34$), and those who reported consuming alcohol multiple times a month to more than once a day were grouped as Alcohol-Users ($n = 47$). Participants completed a modified 2-step learning task, which is a sequential decision-making task designed to test whether choice behavior shows increased model-based control in the face of increased incentives—that is, when people stand to gain the most from superior accuracy in performance, offsetting the putative subjective cost of executive control.

A dual system RL model was used to calculate a weighing parameter separately for low and high stakes, which ranged between 0 (fully model-free decision making) to 1 (fully model-based decision making). Additionally, a utility function was used to estimate of the trade-off between the value preference and the risk preference separately for low and high stakes. The risk sensitivity parameter has been related to functions of serotonin in the basal ganglia. Model outcomes were analyzed using 2 (Stakes: High, Low) x 2 (Groups: Non-Users, Alcohol Users) analysis of variance (ANOVA). Spearman correlations were also used to examine the association between model outcomes, and false discovery rate (FDR) was used to correct for multiple correlations.

Results: The 2x2 ANOVA showed a significant main effect of Stake ($F = 5.469$, $p = .022$) and Stakes x Groups interaction ($F = 6.463$, $p = .013$). Follow-up within-subject tests revealed that whereas in Non-Users the weighting factor for the high stakes was significantly higher than that for the low stakes ($Z = -2.676$, $p = .011$), they were not different in Alcohol-Users ($Z = .189$, $p = .851$). Between-group follow-up tests did not reveal any significant differences ($Z < -1.864$, $p > .067$). Similarly, the 2x2 ANOVA of risks sensitivity parameter showed significant main effect stakes ($F = 36.08$, $p < 0.001$) and significant Stakes x Groups interaction ($F = 4.524$, $p = .037$). Follow-up tests revealed that the interaction stemmed from significantly higher risk sensitivity for

high stakes conditions in non-users compared to users ($t < 2.280$, $p < .030$), whereas the risk sensitivity was lower stakes was comparable ($t < -1.461$, $p < .152$). Correlational analyses revealed that weighting parameter was negatively correlated with risk sensitivity in high stakes condition across the entire sample ($r = -0.280$, $p = .013$), which was driven specifically by Alcohol-Users ($r = -0.349$, $p = .018$) and not by Non-Users ($r = -0.229$, $p = .207$).

Conclusions: This study examines the arbitration between model-based and model-free control when reward value is manipulated. Our results show that unlike Non-Users, Alcohol-Users did not employ greater model-based control during higher compared to lower stakes conditions. Additionally, while both groups were significantly more risk averse in high compared to low stakes condition, Non-Users were profoundly more risk averse than alcohol users in the higher stakes condition. Lastly, our results showed that greater risk aversiveness in alcohol users in high stakes was associated with greater model-free control. Thus, the results indicate that a lack of greater model-based control under higher compared to lower stakes conditions, relatively less risk aversiveness in high stakes condition (compared to Non-Users) and the association between greater model-free control and higher risk aversiveness may underpin maladaptive decision-making in alcohol users, especially in high stakes situation.

Keywords: Computational Modeling, Reward-Based Decision-Making, Risk, Alcohol

Disclosure: Nothing to disclose.

P647. Adult BNST Stimulation Promotes Sex-Specific Negative Affect-Like Behavior and Activation of the Parabrachial Nucleus in Mice With Adolescent Alcohol History

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Background: Adolescent alcohol use is a strong predictor for the subsequent development of alcohol disorders later in life. Additionally, adolescence is a critical period for the onset of affective disorders, which can contribute to problematic drinking behaviors and relapse, particularly in females. Although definitive causal relationships have not been fully elucidated, it is widely accepted that binge patterns of alcohol consumption during adolescence may lead to altered behavioral responses to stressors in adulthood, potentially contributing to the development of stress-related disorders. Previous research conducted in our laboratory has demonstrated that exposure to adolescent intermittent ethanol (AIE) vapor leads to alterations in glutamatergic transmission within the bed nucleus of the stria terminalis (BNST). Furthermore, when combined with adult restraint stress, AIE elicits sex-specific changes in glutamatergic plasticity and negative affect-like behaviors in mice. Building upon these findings, the present study aims to investigate whether BNST stimulation using designer receptors activated only by designer drugs (DREADDs) could substitute for adult stress to promote negative affect-like behaviors in adult male and female mice with a history of AIE. Given the dense reciprocal connections between the BNST and the parabrachial nucleus (PBN), a brain region involved in mediating threat assessment and anxiety-like behaviors, we hypothesized that increased negative affect-like behaviors following BNST stimulation would be associated with PBN activation.

Methods: A total of 90 three-week-old male and female C57BL/6J mice were used in this study ($n = 7$-10 mice/group). The AIE protocol consisted of exposing mice to two 4-day cycles of 16-hour ethanol vapor exposure per day, separated by 3 days of no exposure, from postnatal day (PND) 27 to 38. AIE results in blood ethanol concentrations in the range of 200-250 mg/dL. Approximately ten days after the final ethanol exposure, viral vectors (AAV5-CaMKIIa-hM3D(Gq)-mCherry or AAV5-CaMKIIa-mCherry) were administered into the dorsolateral BNST and mice were left undisturbed until reaching adulthood. For behavioral assays, mice received an intraperitoneal injection of CNO (3 mg/kg) one hour prior each test: the novelty-induced hypophagia (NIH) task at PND 80 and the social interaction test at PND 83. Following behavioral tests mice received another CNO injection and were euthanized one hour later. Brains were collected for RNAscope in situ hybridization in sections containing the PBN. The following probes were used: Fos, as a marker of cell activation; Calca, as a marker of calcitonin gene-related peptide (CGRP) neurons; Pdyn, as a marker of dynorphin neurons. Images were acquired using a ZEISS AxioScan.Z1 slide scanner and the subsequent analysis was performed using QuPath software. Individual cells were identified based on DAPI staining of the nucleus. To determine the percentage of cells expressing a specific mRNA, the number of positive cells was divided by the total number of DAPI-labeled nuclei. Data were analyzed using two-way ANOVAs followed by Tukey's post-hoc comparisons. The statistical significance threshold was set at $P < 0.05$.

Results: The stimulation of the dorsolateral BNST using DREADDs increased the latency to consume an appetitive reinforcer only in female mice with a history of AIE ($P = 0.0111$ vs. air control females that received the hM3D(Gq) virus; $P = 0.0006$ vs. AIE females that received the mCherry virus), but not in female controls or males of either group, mirroring the restraint stress-induced phenotype. Importantly, these changes in latency to consume were not influenced by alterations in body weight, as both male and female mice with AIE history recovered weight gain during adulthood before the NIH task. This behavioral phenotype was associated with an increase in activity in CGRP neurons in both the medial PBN ($P = 0.0003$ vs. air control females that received the hM3D(Gq) virus; $P < 0.0001$ vs. AIE females that received the mCherry virus) and lateral PBN ($P = 0.0168$ vs. AIE females that received the mCherry virus), as well as dynorphin neurons in the medial PBN ($P = 0.0223$ vs. air control females that received the hM3D(Gq); $P = 0.0295$ vs. AIE females that received the mCherry virus). BNST stimulation did not induce social avoidance in male or female mice, highlighting the involvement of the BNST in certain aspects of alcohol-induced negative affect-like behavior, particularly in females.

Conclusions: Our findings align with human literature, indicating that women who consume alcohol tend to experience greater negative affect following stress induction compared to men. These findings suggest that a history of adolescent alcohol exposure may lead to sex-dependent changes in BNST-PBN circuitry, which are further enhanced with later challenges in life. CGRP-expressing neurons in the PBN appear to play a role in mediating threat assessment in adult females with AIE history. Additionally, dynorphin-expressing cell in the medial PBN may also be involved in mediating these behaviors. These insights contribute to advancing our understanding of the neurobiological mechanisms underlying alcohol-related negative affect and provide a foundation for further exploration of sex-specific effects in addiction research.

Keywords: Adolescent Alcohol, Bed Nucleus of Stria Terminalis, Parabrachial Nucleus, Negative Affect, Social Behaviors

Disclosure: Nothing to disclose.

P648. Prenatal Exposure to Alcohol and its Impact on Reward Processing and Substance Use in Young Adulthood: Findings From a Prenatal Birth Cohort Study

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Background: Despite the recommended abstinence from alcohol during pregnancy, alcohol use during this critical period for child's brain development remains common. Some alcohol drinking during pregnancy has been reported by 30% of women, decreasing to 22% after the first month and to 8% during the third trimester (Ethen et al., 2009). The impact of chronic alcohol drinking during pregnancy on child's neurodevelopment has been reported by numerous studies, but the effects of low-dose alcohol drinking are less clear, and any potential safe level of alcohol use during pregnancy is not known. It is also not clear whether the relationship between exposure to alcohol during pregnancy and higher alcohol use in the offspring might generalize to other substances such as cannabis and other reward-related behaviors such as monetary reward processing and whether these effects might differ between men and women.

Methods: We have conducted a neuroimaging follow-up of the European Longitudinal Study of Pregnancy and Childhood (ELSPAC) prenatal birth cohort in young adulthood and assessed the relationships between maternal alcohol use during pre-conception and mid-pregnancy and substance use and monetary reward processing in the offspring in their late 20s. Alcohol drinking during preconception and mid-pregnancy were assessed during the 20th week of pregnancy using a self-report questionnaire. Reward processing in the young adult offspring (age 28 – 30 years, all of European ancestry was assessed using functional magnetic resonance imaging (fMRI) during a Monetary Incentive Delay (MID) task at a 3T Siemens Prisma Scanner. Substance use was assessed on the same day using a self-report questionnaire. A total of 191 participants (49% women, 51% men) had complete data including prenatal exposure to alcohol as well as fMRI and substance use data from young adulthood. Voxelwise general linear model (GLM) tested the effect of prenatal exposure to alcohol, sex, and their interaction on brain response to 4 conditions: anticipation of reward, anticipation of loss, reward feedback, and loss feedback. Clusters surviving the voxelwise threshold of FWEp < 0.05 were reported as significant. The effect size was calculated using Hedges'g for the main effect and using ΔR^2 for interaction terms. Finally, posthoc analyses evaluated the relationship between brain response to reward in the significant clusters and cannabis use in the late 20s. Multiple comparisons were corrected using a false discovery rate (FDR).

Results: Alcohol drinking was reported in 85% of women pre-conception and in 16% of women mid-pregnancy. Exposure to alcohol during mid-pregnancy was associated with reward feedback but not reward anticipation in the young adult offspring. Greater exposure to alcohol prenatally predicted greater brain response to reward feedback in 9 clusters including six frontal (left inferior frontal: 85 voxels, peak at −42, 20, 26; t = 5.21, FWEp < 0.001, Hedges' g = 0.95; left mid-frontal: 140 voxels, peak at −48, 8, 41; t = 4.73, FWEp < 0.001, Hedges' g = 0.85; left frontal mid orbital: 48 voxels, peak at −48, 38, −1; t = 4.02, FWEp = 0.011, Hedges' g = 0.80; left frontal superior orbital: 103 voxels, peak at −3, 50, −19; t = 4.65, FWEp < 0.001, Hedges' g = 0.87; left frontal superior medial: 64 voxels, peak at −6, 26, 56; t = 4.45, FWEp < 0.002, Hedges' g = 0.82; right mid-frontal: 46 voxels, peak at 39,5,56; t = 4.39, FWEp = 0.014; Hedges' g = 0.82), one parietal (left parietal inferior: 56 voxels, peak at −51, −49, 44; t = 4.18, FWEp = 0.005, Hedges' g = 0.73), one temporal (left inferior temporal: 49 voxels, peak at −57, −55, −16; t = 4.53, FWEp < 0.010, Hedges' g = 0.73), and one occipital (right lingual: 63 voxels, peak at 15, −94, −10; t = 4.79, FWEp < 0.002, Hedges' g = 0.88) region and these effects were independent of sex. Sex differences emerged in the effects of prenatal alcohol exposure on brain function during loss feedback, where men exposed to prenatal alcohol had the greatest response to loss feedback in the putamen (cluster of 41 voxels, peak at −27, −4, 8; t = 4.17, FWEp = 0.025, ΔR^2 = 0.122) and occipital region (cluster of 40 voxels, peak at −15, −73, 17; t = 4.05, FWEp = 0.028, ΔR^2 = 0.126). Moreover, prenatal exposure to alcohol also predicted greater cannabis (but not alcohol) use in the offspring 30 years later (t = 2.16, p = 0.03). Consistently, greater reward response in three (right lingual: B = 0.58, FDRp = 0.028, left mid-frontal: B = 0.30, FDRp = 0.050, and left frontal superior orbital: B = 0.73, FDRp = 0.028) out of the nine significant clusters was associated with greater cannabis use in the late 20s. No similar results were found for pre-conception exposure to alcohol, suggesting the effects of prenatal exposure to alcohol are pregnancy-specific.

Conclusions: We showed that even moderate exposure to alcohol in mid-pregnancy but not pre-conception is associated with altered brain response to reward and greater cannabis use in both men and women 30 years later. These results suggest that prenatal exposure to alcohol might alter the brain dopamine system in the offspring and contribute to intergenerational transmission of risk for substance use disorders.

Keywords: Prenatal Alcohol Exposure, Substance Abuse, Reward Processing, Longitudinal Study, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P649. Association of Non-Drug Reinforcement With Treatment Outcomes During Early Substance Use Recovery

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Background: Behavioral economic research consistently demonstrates that although humans and non-human animals will often consume drugs at a high rate when this is the only available option, that drug use is also inversely associated with availability and engagement with non-drug reinforcers. Increasing the access to and engagement with robust, non-drug alternative reinforcers can effectively compete with drug reinforcers and reduce drug use. Deficits in substance-free reinforcement are therefore a likely key risk factor for substance use. The purpose of this analysis was to use large scale clinical data from individuals in early substance use recovery to establish the direct clinic impact of relative frequency, access, and enjoyability of non-drug reinforcement on key addiction treatment outcomes.

Methods: Respondents enrolled in substance use treatment in clinics across the United States (N = 5,481; 62.5% male; 87 unique treatment programs) completed standardized assessments of non-drug reinforcement and treatment outcomes one-month post-treatment discharge. Data included patients admitted to residential treatment programs (n = 3,755), intensive outpatient programs (n = 1,191), and medically managed withdrawal (a.k.a. detoxification; n = 535). Respondents completed a novel battery of measures for non-drug reinforcement quantifying three elements of reinforcement: relative frequency, access, and enjoyability. Treatment outcomes included return to substance use (i.e., relapse) and life satisfaction. Non-drug reinforcement measures were used as predictors of return to use and life satisfaction with generalized linear models (logit link and identity link, respectively). Models were built such that unadjusted tests were first conducted followed by tests controlling for demographic covariates, other relevant biopsychosocial variables (i.e.,

sleep disturbance, anhedonia, and stress), primary drug of use, and level of care.

Results: Non-drug reinforcement indices robustly predicted return to use and life satisfaction in unadjusted models and models adjusted for sociodemographic variables and biopsychosocial variables. For example, 12.4% of patients reporting very easy access to non-drug reinforcement showed a return to use, but 58.3% of patients reporting very difficult access showed return to use in the month following treatment (model coefficients; OR = 1.72 [1.64, 1.80], p < .001). Non-drug enjoyability was also related to return to use such that high enjoyability of non-drug reinforcers was associated with a 12.6% likelihood of return to use, but low enjoyability associated with a 70.3% likelihood of return to use (model coefficients; OR = 1.81 [1.72, 1.90], p < .001). Results remained significant in omnibus covariate-adjusted models indicating the unique associations of each non-drug reinforcement measure. Non-drug reinforcement measures were similarly associated with life satisfaction with a single model including the three non-drug reinforcement measures and covariates having an adjusted R2 of 0.53 with significant associations for the relative frequency (b = 0.01, p < .001, β = 0.08), access (b = 0.08, p < .001, β = 0.05), and enjoyability (b = 0.40, p < .001, β = 0.22). Gender differences were explored with no significant moderating role on study outcomes.

Conclusions: All three non-drug reinforcement indices (relative frequency, access, and enjoyability) robustly predicted return to use a month after discharge from treatment as well as life satisfaction during this time. These results are consistent with research across the translational spectrum establishing an inverse relation between non-drug reinforcement and drug use and lend support for the continued preclinical and clinical evaluation of treatments that identify and bolster accessibility and engagement with valued life activities that are alternative to substance use.

Keywords: Behavioral Economics, Addiction, Anhedonia, Social Reward

Disclosure: Nothing to disclose.

P650. Disproportionate Increase in Cannabis Use Among Individuals With Serious Psychological Distress and Association With Psychiatric Hospitalization 2009-2019

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Background: Cannabis can be used by many individuals without apparent harm, but individuals with serious psychological distress (SPD) are disproportionately likely to both use cannabis and experience its negative effects. Cannabis use has been increasing in the United States alongside legalization of recreational use, but it is not known if these trends are differentially affecting individuals with SPD compared to the general population. Furthermore, while the impact of cannabis on poor outcomes in psychotic disorders is well known, impacts on other disorders has been poorly characterized. This study used a nationally representative data set to: 1) Investigate whether rates of cannabis use were changing more rapidly in individuals with SPD compared to the general population and 2) Whether patterns of use among individuals with SPD were associated with changes in rates of psychiatric hospitalization.

Methods: Data were obtained from the 2009-2019 National Survey on Drug Use and Health (NSDUH), a nationally representative cross-sectional survey that captures information on demographics, substance use, mental health disorders, and health service use. Sampling weights were computed to control for non-response and allow for population level estimates to be made, and 447,947 individuals 18 or older were included in the analysis. Serious psychological distress, a proxy indicator for probable severe mental illness, was defined as a score ≥13 on the Kessler SPD scale (K6). Levels of cannabis use were defined as no use, less than weekly use (1-51 days of use in the past year), and weekly-plus use (52 or more days of use in the past year). Psychiatric hospitalization was defined as any past year overnight stay in a psychiatric unit. To ascertain trends in cannabis use, we estimated multivariate logistic regression models with an interaction term between year and SPD on any cannabis use and then weekly-plus cannabis use, adjusting for race/ethnicity, sex, income, age, income, health insurance, education and heavy alcohol use. Next, multivariate logistic regression models were estimated on psychiatric hospitalization by cannabis use in individuals with SPD, conditional on the same covariates. Predictive margins methods were used to both decompose the interaction term in logistic regression as well as allow for conversion to predicted probabilities for interpretability.

Results: In unadjusted results, at all time points individuals with past year SPD (14.95% of the sample) had higher significantly rates of less than weekly cannabis use (13.56% vs 6.10%) and weekly plus cannabis use (13.86% vs 5.56%) compared to individuals without past year SPD. In adjusted models, any and weekly-plus cannabis use both increased at the same rate, but starting in 2015 rates of cannabis use rose significantly more quickly in individuals with SPD compared to the general population. ($p = 0.049$ in 2015, $p < 0.001$ in 2019).

Regarding rates of psychiatric hospitalization, in both adjusted analyses individuals with SPD who did not use any cannabis had the lowest rates of hospitalization (4.1%). Both less-than-weekly use (5.0%, $p = 0.023$) and weekly-plus use (5.4%, $p = 0.001$) were associated with significantly greater rates of psychiatric hospitalization compared to no use. There was no significant difference between less-than-weekly use and weekly-plus use ($p = 0.43$).

Conclusions: Cannabis use rates rose more quickly among individuals with probable serious mental illness from 2015-2019 compared to the general population, and in this population any cannabis use was associated with elevated risks of psychiatric hospitalization. This is the first study finding accelerating rates of cannabis use in individuals with SPD, which is concerning given its association with psychiatric hospitalization and probable worsening symptom burden in our findings. This timeline tracks with the accelerating adoption of medical and recreational cannabis legalization measures in the United States, which have previously been associated with decreased risk perceptions and increased perception of health benefits of use. While the direction of causality between cannabis use and psychiatric hospitalization in SPD cannot be known at this time, future longitudinal studies should investigate timing of cannabis use on psychiatric hospitalization as well as whether changes in cannabis marketing and product availability change these trends.

Keywords: Cannabis, Serious Mental Illness, Psychiatric Hospitalization

Disclosure: Nothing to disclose.

P651. Exploring the Associations Between Dimensions of Adverse Childhood Experiences and Alcohol Use Outcomes Among Hispanic Young Adults

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Background: In 2021, 10.3% of Hispanics met DSM-5 criteria for an Alcohol Use Disorder, but only 1% received treatment. In view of the limited access to treatment, preventing escalation to problematic alcohol use is a priority. A significant body of research links the history of Adverse Childhood Experiences (ACEs) with alcohol use, however, most evidence has focused on the number of experiences. The current study aims to explore the relationship between multiple dimensions of ACEs and alcohol use outcomes among a sample of Hispanic young adults.

Methods: We analyzed data from 195 Hispanic young adults (Mage = 21.8 [SD = 0.8]; 52% female) recruited in South Florida. The outcomes of interest included: age of alcohol use onset, frequency (days) and quantity (drinks) of alcohol used in the past 6 months, and DSM-5 alcohol use disorder in the past 12 months. Explored dimensions of ACE's included the number of ACE's (range = 0 to 14), score of self-reported ACE's severity (Likert scale with values ranging from 0 - "not at all affected" to 6 - "extremely affected" for each ACEs), chronicity (age periods at which the ACE's occurred), and timing (age at which the first ACE's occurred) Associations were explored using multiple logistic regressions and negative binomial models.

Results: Nearly two-thirds of the sample (69%) reported at least exposure to one ACE; among them, 73% occurred during childhood. The regression models indicate that the number of adverse events experienced (β = 1.15, 95% CI: 1.04, 1.27) and ACE's chronicity (β = 1.06, 95% CI: 1.01, 1.12) were positively associated with the quantity of alcohol consumption in the past six months. The number of ACEs was associated with meeting criteria for an Alcohol Use Disorder (OR = 1.20, 95% CI: 1.04, 1.4).

Conclusions: Our results concur and extend prior findings on the association between ACEs and alcohol use by documenting a relationship between the number of ACEs experienced and chronicity of ACEs, and the quantity of alcohol consumed or developing an alcohol use disorder.

Keywords: Childhood Adversity, Alcohol Consumption, Alcohol Use Disorder, Hispanic/Latinos

Disclosure: Nothing to disclose.

P652. Cell Adhesion Molecule 2: A Preclinical Validation of a Cannabis Use Candidate Gene

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Background: Genetic variation in Cell Adhesion Molecule 2 (CADM2) is among the strongest and most reliable genetic predictors of lifetime cannabis use. CADM2 is thought to affect reward circuitry and neurotransmitter systems acted on by the primary psychoactive constituent of cannabis, Δ9-tetrahydrocannabinol (THC). However, CADM2's relationship with cannabis use may be proximal to its other genetic associations that are also predictive of substance experimentation, namely impulsivity and risk-taking behavior, which we previously corroborated in humans and animals. Whether CADM2 variation predicts cannabis use vulnerability by affecting the pharmacological actions of cannabis and THC has yet to be established. Given that CADM2 associations with cannabis use are more robust than other commonly used substances, we hypothesized that deletion of the mouse Cadm2 ortholog would reduce cannabis and THC intake, as well as alter pharmacological and neural responses to THC.

Methods: Using male and female Cadm2 mutant knockout (KO), heterozygous (HT), and wildtype (WT) mice, we explored Cadm2 associations with cannabis and/or THC drug preference, cognition, pharmacology, and prelimbic neuron activity. Voluntary intake of THC and commercial cannabis oil (n = 11-18 mice/genotype/experiment) was assessed with a two-edible choice preference test, in which drug dough intake was recorded across consecutive days and escalating doses (0.2-2.0mg THC/g of dough). Drug- and genotype-dependent differences in cognition were evaluated with the 5-choice serial reaction time task (5CSRTT; executive functioning) and prepulse inhibition test (PPI; sensorimotor gating) 30 minutes after administering 2mg/kg, i.p. of THC (n = 12-16 mice/genotype). Blood serum levels of THC and its bioactive metabolite 11-OH-THC were measured 30 or 120 minutes after delivering 10mg/kg, i.p. of THC (n = 12-13 mice/genotype/time) using liquid chromatography-tandem mass spectrometry. Pharmacodynamic response to 3 or 10mg/kg, i.p. of THC was assessed with the cannabinoid tetrad assay following acute and chronic THC exposure (n = 12-16 mice/genotype/dose). Finally, whole-cell patch clamping was used to determine the electrophysiological properties of layer 5 pyramidal WT and KO neurons in prelimbic cortical slices at baseline (n = 49-56 neurons/genotype). We also investigated changes to spontaneous excitatory or inhibitory postsynaptic currents (sEPSC/sIPSC; n = 18-27 neurons/genotype/recording) following bath with 1μM THC. All parametric data were analyzed using one-way or repeated measures ANOVA; nonparametric data were analyzed using Kruskal-Wallis H test or aligned rank transformed ANOVA where appropriate.

Results: Genotype significantly affected preference for THC ($F(2,32) = 4.85$, $p < 0.05$) and cannabis oil-containing edibles ($F(2,34) = 4.65$, $p < 0.05$), with KO mice showing lower preference for both drugs in comparison to WT and HT littermates. THC challenge before the 5CSRTT did not modify impulsivity or attentional performance across any genotype, and genotype did not differentially affect serum levels of THC or 11-OH-THC at either time. Preliminary PPI data suggests that sensorimotor gating is impaired in KO females under drug-free conditions. Baseline locomotion ($F(2,76) = 37.90$, $p < 0.001$) and thermal pain tolerance ($F(2,77) = 4.64$, $p < 0.05$) varied by genotype such that KO mice were hyperlocomotive and had reduced thermal nociception compared to WT littermates. In the cannabinoid tetrad assay, KO mice showed sensitization to the antinociceptive properties of THC (3mg/kg: $F(2,32) = 6.23$, $p < 0.01$), had no hypothermic response to THC (10mg/kg: $F(2,36) = 13.52$, $p < 0.001$), and showed exacerbated hyperlocomotive response to THC (3mg/kg: $F(2,34) = 50.92$, $p < 0.001$; 10mg/kg: $F(2,35) = 63.69$, $p < 0.001$). Finally, whole-cell patch clamp electrophysiology revealed greater membrane resistance ($F(1,99) = 6.71$, $p = 0.01$) and lower resting membrane potential ($F(1,101) = 3.99$, $p < 0.05$) in KO mice compared to WT. In neurons from male KO mice, afterhyperpolarization amplitudes ($F(2.12,209.55) = 7.23$, $p = 7.24E-04$) and firing frequency ($F(1.76,177.60) = 3.61$, $p = 0.03$) at lower stimulation intensities were attenuated compared to WT neurons. There were no genotype differences in sEPSC/sIPSC changes after THC bath application.

Conclusions: These data implicate Cadm2 in cannabinoid preference and consumption, pharmacodynamic response to THC, and neuronal activity in a brain area relevant to executive functioning and drug intake behavior. The reported Cadm2-dependent differences in THC pharmacodynamics could suggest these mice are uniquely susceptible the effects of THC and cannabis, thus explaining reductions in voluntary drug intake. Furthermore, we find that Cadm2 may have sex-specific functions in cognition and neuronal activity, highlighting sex as an important variable to consider in future clinical and preclinical investigations. This preclinical evidence supports the validity of human genetic associations between CADM2 polymorphisms and cannabis use risk, and points to a dual role for this gene in mediating both cognitive features predictive of substance use and subjective cannabis response.

Keywords: Cell Adhesion Molecules, Pharmacokinetic and Pharmacodynamic, Executive Function, Slice Electrophysiology, Delta9-Tetrahydrocannabinol

Disclosure: Nothing to disclose.

P653. RATTACA: A Novel Genetic Paradigm for Producing Outbred Rats With Genetically Predicted Phenotypes

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Background: Genetic correlations between traits are frequently studied in humans and model systems as a first step towards identifying causal pathways and mechanisms. In model systems, genetic correlations have been studied using several approaches. One of the simplest but also most prone to misinterpretation are correlations observed in pairs of inbred strains. Using this approach, a pair of strains that is divergent for one trait might be examined to determine if a second putatively causal factor also differs between the two strains. Such an approach can be misleading because numerous traits may differ between a pair of strains without having any causal relationship. Another approach would be to examine two factors in an outbred population; however, any observed correlations could be due to genetic or environmental causes. Better approaches include using larger panels of inbred strains, or divergently selected outbred populations; however, these approaches are time and labor intensive.

Methods: Here we introduce a novel experimental paradigm that we are calling RATTACA, in which phenotypes are predicted in naïve rats using extant rat GWAS data. Prediction is based on standard polygenic methods (e.g., BLUP) that are already widely used in agricultural and human genetics. Performance improves with the heritability of the trait and with sample size of the GWAS training data. RATTACA allows us to produce cohorts of rats that are predicted to be divergent for a trait. These divergent cohorts can be examined for a second putatively correlated trait to see if the second trait is genetically correlated with the first trait that used for prediction.

Results: We will present two examples of the application of RATTACA. In the first, electrophysiological differences were observed in amygdala slices taken from rats that were predicted to take very large or very small amounts of cocaine. Because none of the rats had ever been exposed to cocaine, electrophysiological differences are not secondary to differential cocaine exposure. In the second, rats predicted to take large or small amounts of cocaine were found to also self-administer a novel compound that appears to have significant abuse potential.

Conclusions: One critical advantage of this approach is that by using prediction rather than directly measuring the first trait, the second trait can be measured in naïve rats. This paradigm is especially attractive for lower throughput traits since they cannot be easily measured in large cohorts. We can also produce rats that are predicted to be divergent for multiple traits. Another possible application is to examine rats that are predicted to be divergent for the expression of one or more genes. Finally, for genes with LoF mutations, cohorts could be produced that resemble wild type, heterozygous and knock out populations, but with the benefit of an outbred genetic background, thus improving robustness. We are currently providing cohorts for free or at deeply subsidized prices to qualified investigators.

Keywords: Genetic Association Study, Polygenetic Risk Score, Substance Abuse Disorders, Behavioral Phenotyping

Disclosure: Vivid Genomics: Advisory Board (Self).

P654. Epitranscriptomic Landscape in the Amygdala After Acute Ethanol Exposure

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Background: Acute alcohol exposure leads to anxiolysis, which contributes to the initiation and maintenance of alcohol use disorder (AUD). We recently reported that acute ethanol rapidly produces transcriptomic and epigenomic changes in the amygdala, some of which are responsible for anxiolytic effects in rats. N6-methyladenosine (m6A) is the most abundant mRNA modification and regulates gene transcription. Its presence and function are regulated by the methyltransferase complex (METTL3, METTL14, and WTAP), demethylases (FTO and ALKBH5), and reader proteins (YTHDF1, YTHDF2). The role of epitranscriptomic changes such as m6A methylation in AUD is unknown. We investigated whether changes in the abundance of RNA methylation modifiers alter transcript-specific m6A levels after acute alcohol exposure that may contribute to development of alcohol dependence.

Methods: Adult male rats received an intraperitoneal injection of saline or ethanol (1 gram per kilogram of body weight). After one hour, amygdala brain tissue was collected and immediately frozen. Following RNA isolation, qPCR was performed to measure the expression of methylases, demethylases, and readers. Methylated RNA immunoprecipitation sequencing (MeRIP-seq) using an m6a-specific antibody was performed on RNA samples to detect transcript-specific changes in m6A levels.

Results: Levels of the demethylase FTO and readers YTHDF1 and YTHDF2 were significantly increased in the amygdala of acute ethanol-treated rats compared to controls. MeRIP-seq analysis revealed several m6A peaks that were differentially expressed across groups. Ingenuity Pathway Analysis (IPA) of genes associated with these peaks yielded two primary networks: m6A methylation of CDS2, LIF receptor, GPR161, Celsr2 transcripts was significantly increased, while m6A methylation of IGF2, PPP2r2b, FKBP9, SLC7a14, NUP50, Dync1i1, and MYH11 was significantly decreased in ethanol-treated rats compared to controls. We further validated m6A modification of PPP2r2b and Dync1i1 transcripts by MeRIP-qPCR, which correlated with their mRNA expression as analyzed by qRT-PCR.

Conclusions: Our data suggest that a single ethanol exposure induces alterations in RNA methylation modifiers and gene-specific m6A levels, which may reflect transcriptomic changes that could prime the amygdala for the development of AUD (Supported by NIH-NIAAA P50AA022538, UO1AA-019971, U24AA024605 [NADIA], and by the VA Senior Research Career Scientist award to SCP).

Keywords: Amygdala, RNA Methylation, Alcohol, Epitranscriptome, Anxiety

Disclosure: Nothing to disclose.

P655. Changes in Alcohol-Related Outcomes During the COVID-19 Pandemic as a Function of Smoking Status and the Functional Nicotine Receptor Gene (CHRNA5) Variant in Individuals With and Without a History of Alcohol Use Disorder

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Background: The co-use of alcohol and nicotine are well documented, and both substances are known to interact with the nicotinic acetylcholine receptor (nAChR) system and to alter reward-related responses. The CHRNA5 gene encodes the α5 nAChR subunit and has been studied in animal models and humans for its association with nicotine and alcohol use. A missense single-nucleotide polymorphism (SNP) rs16969968 (G>A) in the CHRNA5 gene has shown a strong association with nicotine use disorder and smoking behavior, but less is known how this variant is associated with alcohol-related phenotypes. The negative impact of the pandemic on mental and physical health has been well documented, while reported changes in alcohol consumption during the pandemic have shown considerable heterogeneity. Given the role of CHRNA5 in both nicotine and alcohol use disorder (AUD), our aim was to examine changes in alcohol consumption, as well as alcohol-related measures collected during the pandemic, and the impact of smoking status and the missense polymorphism rs16969968 on these measures across individuals with and without a history of an AUD.

Methods: Participants (n = 288; mean age = 46.2 years; 51.0% male; 38% with history of AUD) previously enrolled in the NIAAA Natural History Protocol were recruited for a follow-up longitudinal survey study. Individuals across the spectrum of alcohol use and alcohol use disorder, who had previously participated in the NIAAA natural history study between 2015 and 2020, were contacted via phone for study participation. After consent, participants completed an initial survey to obtain a pre-pandemic baseline and to capture the initial impact of the pandemic. Assessments on alcohol use and associated behaviors included the Alcohol Use Disorder Identification Test (AUDIT) to measure alcohol consumption (AUDIT-C), Penn Alcohol Craving Scale (PACS), Drinking Motives Questionnaire (DMQ) and Impaired Control Scale (ICS). Additionally, mental health measures of depression and anxiety were measured using the Patient Health Questionnaire-9 and Generalized Anxiety Disorder Questionnaire-7, respectively, Stress was measured using the Perceived Stress Scale (PSS), and we also examined measures of psychological, physical health, social relationships, and environmental Quality of Life (QoL). Among the total participants, 58.5% were GG homozygotes, 37.5% were GA heterozygotes, and 4% were AA homozygotes. For analyses, GA and AA were combined into 1 group (41.5%). Data were analyzed using general linear models covarying for history of AUD, sex, age, and AIMs score for Africa, Europe, and Asia ancestries.

Results: Among study participants with GG genotype, 23% were smokers, while among the participants with the AA/AG genotype, 27.6% were smokers. There was a main effect of smoking on change in alcohol consumption (from pre-pandemic to pandemic) in which smokers showed an increase in alcohol consumption ($p < 0.001$). There was a significant interactive effect between smoking status and rs16969968 genotype wherein smokers who were in the GG group showed a decrease in alcohol consumption during the pandemic, while smokers who were in the AA/AG genotype showed an increase in alcohol consumption ($p < 0.001$). There was also a significant main effect of smoking observed across genotype groups. Specifically, smokers reported greater anxiety and depression during the pandemic, reported greater craving for alcohol, drinking to cope, and greater failed attempts to control their drinking compared to non-smokers (all p's < 0.001). Smokers also had poorer quality of life across all measures (all p's < 0.001). History of AUD was significant in all models (all p's < 0.001).

Conclusions: In summary, our findings confirm the strong effect of smoking status on alcohol consumption and other alcohol related behaviors, mental health, quality of life, and in individuals with an AUD. We also showed a preliminary indication of an rs16969968 'AA/AG' genotype effect on increased alcohol consumption from pre-pandemic to during the pandemic. This highlights the comorbid risk of the co-use of nicotine and alcohol in vulnerable populations, and the need for further examination of this functional CHRNA5 variation on changes in alcohol consumption in relation to smoking status.

Keywords: Alcohol, CHRNA5, Nicotine

Disclosure: Nothing to disclose.

P656. Genome-Wide DNA Methylation Patterns Associated With Alcohol Response and Alcohol Use Disorder

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Background: Patterns of excessive alcohol use and misuse have been associated with a range of acute and chronic consequences. There are a range of genetic and environmental factors that can contribute to the heterogeneity in alcohol use and alcohol use disorder (AUD), and understanding the underpinnings of these sources of variability can greatly help improving the diagnosis and prognosis of AUD. One of these determinants is the level of response to alcohol (LR), which has been shown to be genetically influenced, and associated with an increased risk for heavy alcohol use and AUD. Recent studies have also identified a critical role of epigenetic changes that can modulate the effect of genetic and environmental variables on gene expression in a number of psychopathological conditions, including AUD. Thus, the objective of this study was to identify epigenetic changes, specifically DNA methylation patterns, that are associated with AUD and measures of alcohol response in a sample of individuals with AUD and non-AUD controls.

Methods: Participants were enrolled in the NIAAA Natural History Protocol, and included individuals with AUD (n = 179) undergoing inpatient treatment as well as individuals without AUD (n = 95) that were participants in research studies conducted at the NIH Clinical Center. Participants provided a blood sample for genetic analysis. Genome-wide DNA methylation data were assessed from whole blood samples using the Illumina Methylation EPIC Bead Chip (850K). Differentially methylated positions (DMPs) were analyzed to identify hypo- and hypermethylated regions between AUD and non-AUD groups after controlling for age, sex, race, principal components of control probes, and cell compositions. Given the impact of childhood trauma on long-term mental and physical health and associated epigenetic changes, the analysis also controlled for Childhood Trauma Questionnaire scores. The LR to alcohol was measured with the Self-Report of the Effects of alcohol (SRE) questionnaire evaluating the number of standard drinks required to feel four different effects of alcohol. To identify DMPs associated with alcohol response, the SRE recent (past 3 months) and total (lifetime) scores were included in the general linear model.

Results: Results of the comparison in DNA methylation between AUD and non-AUD groups identified 4,430 differentially methylated CpG sites, and included 2,719 hypermethylated and 1,711 hypomethylated sites across exon, 3'UTR, 5'UTR, and gene body regions. After including alcohol response measures (SRE recent and total scores), the AUD group showed 909 differentially methylated CpG sites compared to the non-AUD group, and

included 631 hypermethylated and 278 hypomethylated sites across the 3'UTR, 5'UTR, and gene body regions. Among the significantly altered CpG-associated genes in both models was the dopamine transporter (DAT) gene SLC6A3, which has been previously associated with heavy alcohol consumption and AUD. The 5'UTR CpG in the SLC6A3 gene was significantly hypomethylated in the non-AUD group compared to the AUD group.

Conclusions: In conclusion, this study has identified several novel CpG sites/genes associated with AUD and measures of level of response to alcohol. These findings providing supportive evidence for the previously identified association between the SLC6A3 dopamine transporter gene and AUD and alcohol response. Future work will include examining gene pathways related to AUD and alcohol response, including replication of the study findings using human laboratory measures of alcohol response and AUD risk, as well as evaluating the relationship between alcohol response measures and epigenetic age acceleration.

Keywords: Epigenetics, Alcohol Response, DNA Methylation, Alcohol Use Disorder

Disclosure: Nothing to disclose.

P657. A Multi-Omic Mendelian Randomization Study of Epigenetic Aging and Human Longevity Identifies Novel Genes, Biological Pathways, and Potential Drug Targets for Healthy Aging

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Background: Biological aging is accompanied by increasing morbidity, mortality and healthcare costs; however, its molecular mechanisms are poorly understood. DNA methylation-based aging biomarkers and longevity are influenced by common genetic variants and the use of novel multi-omic computational techniques offers the opportunity to identify their transcriptomic, metabolomic, and cellular signatures, thereby elucidating the biological mechanisms linking epigenetic age acceleration (EAA) and longevity and also informing the development of therapeutics aimed at improving aging.

Methods: Using summary-level genome-wide association study data of four EAA models (HannumAge, IEAA, GrimAge, and PhenoAge) and human longevity, we performed transcriptome-wide association studies (TWAS) and post-TWAS analyses prioritizing genes and biological pathways, diseases, and genetic loci underlying EAA and longevity. We also leverage Mendelian randomization (MR) to investigate the impact of genetic drug targets and circulating metabolites on EAA and longevity to guide therapeutic development. Finally, we performed single-cell enrichment analyses to prioritize cell types associated with aging-related phenotypes.

Results: Our TWASs and sensitivity analyses yielded 23 high confidence associations with EAA and 7 high confidence associations with human longevity. FLOT1, KPNA4, and TMX2 are novel EAA-associated genes. Gene set enrichment implicated the immune-hub chr6p21 and immune system-related disorders in the genomic architecture of our age-related phenotypes. Drug-target MR further identified two immune-related genes, TPMT and C4B, that significantly impacted multiple aging phenotypes. Metabolome-wide MR identified an adverse impact of increased non-HDL cholesterol and associated lipoproteins on longevity but failed to find evidence of metabolomic effects on EAA. Finally, single cell enrichment implicated immune cells and their precursors in the biology of EAA and longevity and follow-up MR of 731 immune cell traits on EAA and longevity confirmed a role of lymphocyte subpopulations and lymphocytic cell surface molecule composition for both EAA and longevity.

Conclusions: Our findings highlight the power of integrating genetics, transcriptomics, and metabolomics to elucidate biological underpinnings of EAA and longevity. We identify genes that link EAA and longevity and find shared immunological pathways that together may guide drug development for anti-aging therapeutics and generate hypotheses for future investigating EAA and longevity.

Keywords: Aging, TWAS, Human Genetics, Methylation, Epigenetics

Disclosure: Nothing to disclose.

P658. Parental History and Polygenic Score of Problematic Alcohol Use Confer Risk to Alcohol Use Disorder Through Incentive Salience Factors of the Addictions Neuroclinical Assessment

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Background: Alcohol use disorder (AUD) is a heterogeneous disorder driven by both genetic and environmental factors. Genetic factors contribute approximately 50% of the risk of AUD, and genome-wide association studies have identified specific genes associated with problematic alcohol use (PAU). However, associations between individual genes and AUD are weak due to AUD's complex and heterogenous phenotypic representation. Recent approaches have utilized polygenic risk scores (PRS) to quantify the overall genetic risk underlying a given phenotype. In addition to genetic factors, family history of PAU (FH) is a strong predictor of AUD and captures both genetic and early life drivers of AUD. However, disentangling the genetic and environmental effects of FH on AUD remains difficult. To elucidate the role of genes and early life environment in AUD, we utilized the Addictions Neuroclinical Assessment (ANA), a clinical framework developed to understand the heterogeneity and etiology of AUD through three neurofunctional domains: Incentive Salience, Negative Emotionality, and Executive Function. We used polygenic risk scores of problematic alcohol use (PRS-PAU) as a proxy for the overall effect of genetic factors of AUD. Here, we investigated how parental FH is associated with PRS-PAU, and how these two factors confer risk for AUD through the incentive salience factors of ANA.

Methods: The primary dataset consisted of $N = 300$ individuals recruited from the National Institutes of Alcohol Abuse and Alcoholism Natural History Protocol (41.0% female, 50.5% Caucasian White, 70.0% with current AUD). Participants completed the ANA battery, consisting of self-report questionnaires and neurocognitive tasks that assessed the ANA domains. Measures of incentive salience include approach-avoidance bias, implicit alcohol associations, alcohol demand, Self-rating of the Effects of Alcohol questionnaire, Obsessive-Compulsive Drinking Scale, Alcohol Dependence Scale, and the Penn Alcohol Craving Scale. Factor analyses were used to identify the latent factors underlying the incentive salience domain. Parental FH (FH-Mother and FH-Father) was captured using the family tree questionnaire. Current AUD was determined using the Structured Clinical Interview for the DSM-5. Effect sizes and p-values of genes associated with PAU were obtained from a genome-wide meta-analysis of PAU

(n = 865,041) and were used as our discovery dataset to compute PRS-PAU in our primary dataset. Associations between parental FH, PRS-PAU, incentive salience factors, and current AUD were modeled using structural equation models with age, sex, and race as covariates.

Results: Two factors underlie the incentive salience domain: Alcohol Motivation and Alcohol Sensitivity (CFI = 0.99, TLI = 0.98, RMSEA = 0.04). Individuals with AUD exhibited greater PRS-PAU, greater Alcohol Motivation, and lower Alcohol Sensitivity (p's0.05). Alcohol Motivation was associated with both PGS-PAU ($p = 0.005$) and parental FH (p's0.05). Both Alcohol Sensitivity and Alcohol Motivation were associated with current AUD, after controlling for both parental FH and PRS-PAU. The relationship between PRS-PAU and AUD was mediated by Alcohol Motivation ($p = 0.008$), but not Alcohol Sensitivity (p>0.05).

Conclusions: The incentive salience domain of ANA consisted of two factors: Alcohol Motivation and Alcohol Sensitivity. Both factors were associated with the risk of current AUD, even after controlling for parental FH and genetic factors underlying PAU. Individuals with AUD were more likely to have at least one parent with a history of PAU. However, paternal history of PAU (FH-Father) played a more substantive role than maternal history of PAU (FH-Mother) in conferring the risk of AUD to the offspring through both genetic and non-genetic pathways. Finally, Alcohol Motivation, but not Alcohol Sensitivity, mediated the relationship between genetic propensity for AUD and current AUD. These results elucidated the complex interplay between genes and environment on the etiology of AUD. Future work will focus on the negative emotionality and executive function domains of ANA, and exploring psychiatric comorbidities frequently observed with AUD.

Keywords: Polygenic Risk Score, Incentive Salience, Family History of Alcohol Use Disorder, Addictions Neuroclinical Assessment

Disclosure: Nothing to disclose.

P659. Sex Differences in Brain Circuitry Engaged During Binge-Like Drinking

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Background: Male and female mice exhibit distinct transcriptional responses in the nucleus accumbens core (NAcc; a brain region important for alcohol use disorder) following limited access binge-like drinking. Moreover, chemogenetic manipulation of the NAcc results in opposite effects on binge drinking in male and female mice (where inhibition decreased drinking in males and stimulation decreased drinking in females). To determine whether brain regions and NAcc brain circuitry is differentially engaged in males and females during binge-like ethanol drinking, we employed whole brain triple fluorescent imaging allowing for quantification of c-Fos (immediate early gene), NeuN (neuronal marker), and GFP (where a viral retrograde tracer AAVrg hSyn GFP was used to label projections to the NAcc).

Methods: Male and female C57BL/6J mice (n = 17-19/sex/fluid) underwent stereotaxic surgery (0.5uL AAVrg-hSyn-eGFP bilaterally into NAcc). After three weeks of recovery, we carried out a 4-day Drinking in the Dark assay (DID; 2hr drinking on days 1-3, 4hr on day 4) with 20% ethanol (experimental group) or water (control group). Following DID, blood was collected for determination of blood ethanol levels, and mice were deeply anesthetized prior to intracardial perfusion. Brains were collected, processed for whole-brain clearing, immunolabeled (for NeuN, c-Fos, and GFP), and imaged via light-sheet microscopy (1.8 x 1.8 x 2um resolution). Image atlas registration and cell detection were conducted using SmartAnalytics software. The data distribution for c-Fos was skewed, therefore data were square root transformed prior to analysis. Principal components analysis, ANOVA, hierarchical clustering, Pearson's correlations, weighted covariance network analysis, and Student's t-test were performed to identify regions and networks engaged by binge-like drinking.

Results: There were no sex differences in drinking behavior or blood ethanol levels. >1100 structures were detected, allowing detailed, hemisphere specific data for quantification. Principal component analysis of c-Fos levels revealed that fluid group and sex are associated with significant amounts of the total explained variance (p's < 0.05, one-way ANOVA). The Edinger-Westphal nucleus, as well as several other regions (e.g. subfornical organ, superior olivary complex, dorsal tegmental nucleus) were engaged in binge-like ethanol drinking (p's < 0.05, with > 0.5 log fold change; as compared to water drinking mice).

Statistics

a. ANOVAs for male and female ethanol or water drinking data, and Student's t-test for BEC data (all n/s).
b. Top Principal Components associated with factors of interest (by % and ANOVA value): Fluid group is significantly associated with PCs 5 and 6, and corresponds to 5.77% of the total explained variance (PC5: 3.19%, $p = 0.0014$, PC6 2.58%: $p = 0.005$). Sex is associated with PC1 (41.8%: $p = 0.072$) and significantly with PC7 (2.56%: $p = 0.029$) and corresponds to 2.557% of the total explained variance. Sex and fluid group interactions are associated with PCs 5,9, and correspond to 5.128% of the total variance (PC9: 1.94%, $p = 0.04$, PC5 3.19%: $p = 0.012$).
c. Student's t-test for c-Fos (density; counts/mm^3) comparisons, only noting regions where p < 0.05.

Conclusions: Binge-like drinking impacts brain activity in females more than in males. Hemisphere differences in c-Fos levels, independent of sex or fluid group, indicate laterality of cellular function, necessitating consideration as a factor when rodent brain tissue is collected and analyzed.

Keywords: Binge Drinking, Whole-Brain Rodent Imaging, Circuitry-Based Approach, Retrograde Tracing, c-Fos

Disclosure: Nothing to disclose.

P660. Synaptic Density in Youth With a Substance Use Disorder: Positron Emission Tomography Studies of [18F] SynVesT-1 In The Toronto Adolescent and Youth (TAY) Cohort Study

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Background: Adolescents and young adults with a mental health challenge have a high-rate of pathological drug use which increases their risk of developing substance use disorders (SUD) and other psychiatric illnesses. While the biological basis for this vulnerability is unknown, it has been proposed, based on converging preclinical and indirect neuroimaging data, that exposure to drugs of abuse during neurodevelopment may lead

to impairments in synaptic density. Here we used positron emission tomography (PET) imaging of the synaptic vesicle glycoprotein 2A (SV2A) radiopharmaceutical [18F]SynVesT-1 to investigate whether synaptic density is lower in the brain of mental health service-seeking youth with a SUD relative to those without.

Methods: Participants enrolled in the TAY cohort study (https://www.taycohort.ca/) underwent clinical assessments and were invited to complete a PET scan with [18F]SynvesT-1 and arterial blood draws. All participants also completed a T1-weighted MRI scan for the purpose of region of interest (ROI) delineation. [18F]SynVesT-1 volume of distribution (VT), a measure of SV2A binding, was computed for five brain ROIs using a 1-tissue compartment model. ROIs included the prefrontal, temporal and cingulate cortices, the hippocampus, amygdala and full striatum as these brain regions have been implicated in SUD. Image preprocessing and kinetic modeling were done using PMOD (4.2). An analysis of variance was conducted to test our hypothesis.

Results: Participant scans (n = 22) were analyzed with arterial input function (10 M, 12 F; 20.5 years old). 11 (50%) participants had no history of SUD (6M, 5 F; 18.9 years old) and 11 met DSM-5 diagnostic criteria for a SUD (4 M, 7 F; 22.2 years old); of those, 7 met criteria for cannabis use disorder. Age was significantly different between the SUD and non-SUD groups ($p = 0.009$). The SUD group had a higher rate of trauma and stress related disorder (8/11 vs 2/11; $p = 0.01$) and a marginally lower rate of anxiety disorder (8/11 vs 11/11; $p = 0.06$). The ANCOVA with age found no significant differences in [18F]SynVesT-1 VT in SUD vs non-SUD across the 5 ROIs (-4%; $F(1, 19) = 0.28$; $p = 0.6$). Differences in VT values were the most marked in the prefrontal cortex: −9% lower (non- significantly) in SUD vs non-SUD. The co-efficient of variation was 15% across all ROIs.

Conclusions: This study was the first to explore the relationship between SUD and synaptic density in youth with a mental health challenge. Further work is ongoing to increase the sample size.

Keywords: PET Imaging, Synaptic Density, Substance Abuse, Adolescence

Disclosure: Nothing to disclose.

P661. Dose-Dependency of Dopamine Receptor Internalization After Stimulants: Evidence From PET/fMRI in Nonhuman Primates

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Background: Psychostimulant drugs cause short and long-term changes to the dopamine (DA) system. Amphetamine is known to powerfully increase extracellular DA and rapidly induce receptor internalization. Psychostimulant drugs, such as amphetamine (AMP) or methylphenidate (MPH), acutely increase brain DA levels. Acute stimulant-induced increases in synaptic DA can also trigger receptor internalization, as one of the initial responses of the reward circuitry to adapt to overwhelmingly high stimulation by DA. However, microdialysis and functional imaging studies have reported discrepancies between the expected timeline of DA release and paradoxically prolonged reductions in PET signal. In vitro data have suggested that the rate of receptor recycling can vary between the D1 and the D2R subtypes, yet, little is known about the functional effects and dose-dependency of this mechanism in vivo. In this study, we investigated differential D1 and D2R trafficking mechanisms following two doses of stimulant exposure by imaging with combined PET/fMRI during repeated injections in non-human primates.

Methods: Simultaneous PET/fMRI was acquired in three anesthetized rhesus macaques in a total of 28 imaging sessions: The D2/D3R PET radiotracer [11C]raclopride was administered as a bolus+infusion, while gradient-echo EPI was acquired continuously with a total scan duration of 2h. In 14 sessions, a first (0h) AMP dose (0.6 mg/kg) was given intravenously at 40 min after radiotracer administration as a within-scan challenge, followed by a second AMP injection after 3h or 24h using the same PET/fMRI acquisitions. To compare a moderate dose with a lower stimulant dose, this data was compared to a 0.2 mg/kg AMP and 0.6 mg/kg MPH dose at 0h and 3h. In an analogous set of 12 imaging sessions, SCH23390 was administered as a bolus+infusion (0.1mg/kg + 0.09mg/kg/h) to block D1R prior to radiotracer injection. FMRI data were analyzed with a general linear model and converted to relative changes in cerebral blood volume (CBV). Binding potential (BPND) and receptor occupancy were quantified using a modified simplified reference tissue model with a time-dependent binding term.

Results: After each amphetamine injection, a reduction in striatal [11C]raclopride-PET binding was observed, driven by amphetamine-induced DA release. Amphetamine-induced CBV changes demonstrated both a positive and negative CBV component that were modulated and interestingly shifted in sign with repeated injections. For the first 0h AMP injection, we observed a reduction in [11C]raclopride BPND, equivalent to a mean peak occupancy of 27.3% [19.1; 35.1] (mean, [95% CI]). The repeated AMP administration after 3h caused an additional increase with a total peak D2/D3R occupancy of 46.6% [37.2; 94.6]. In contrast to the first 0h 0.6 mg/kg AMP injection, which showed a short-lived decrease in CBV with a negative peak of −11.2% [-13.4; −9.0] in the putamen, the CBV response at 3h showed a long-lasting increase in CBV with a peak change of 14.2%. The lower 0.2 mg/kg AMP dose and 0.6 mg/kg MPH produced a robust negative CBV peak of −8.2% at 0h, whereas a repeated injection at 3h produced a smaller short-lived negative CBV peak of −4.2%. At 24h, D2/D3R occupancy returned to baseline but showed reduced acute amphetamine-induced occupancy of 13.9% [2.2; 25.6]. The repeated amphetamine administration at 24h caused a biphasic CBV response, consisting of a negative component, similar to the 0h response, but exhibiting a longer-lasting positive component, as seen with the 0.6 mg/kg AMP injection at 3h. Administration of SCH23390 neither altered the overall shape of the amphetamine-induced changes in CBV at 0h nor affected BPND. Interestingly, the long-lasting positive CBV response at 3h was reduced to a short-lived positive response, demonstrating the D1-specificity of the functional response.

Conclusions: The results from this study demonstrate (i) persistent D2/D3 receptor internalization after amphetamine exposure that lasts for up to 24h, (ii) continued DA release with repeated injections, (iii) differences in functional activation between D1 and D2/D3R within 3h, and (iv) a potential dependency of receptor internalization on the concentrations of DA release. Our data uniquely show the adaptation of receptors over time in vivo, thereby providing insight into adaptive changes beyond the D2/D3Rs, and the important role of not only D2 but also D1R dynamics for understanding the molecular mechanisms of stimulant drugs.

Keywords: Positron Emission Tomography Imaging, Functional MRI (fMRI), D2 Dopamine Receptor, Psychostimulant, Dopamine

Disclosure: Nothing to disclose.

P662. Nicotine Use Engenders Sex-Specific Suppression of Neuroimmune Signaling in the Brain Reward Pathway That is Differentially Regulated by Steroid Hormones

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Background: Craving and relapse to smoking appear to vary as a function of biological sex. Women face unique challenges with use of nicotine-containing products which appear to be critically impacted by ovary-derived steroid hormones. Progesterone (P4), a steroid hormone that is anti-inflammatory, has shown clinical efficacy in promoting smoking cessation in women although the mechanisms of this are unknown. There are clinical signals implicating immune system dysregulation in women who smoke, such as changes in circulating cytokines. Preclinically, it has been shown that neuroimmune signaling plays a vital role in modulating responses through the regulation of neural activity and plasticity in key brain regions within the reward pathway.

Methods: During the steroid hormone phase of the experiment, 72 male and female Long Evans rats underwent nicotine or saline self-administration (SA) for 10 sessions (0.06 mg/kg/infusion), followed by 15 sessions in which rats received either P4 (1.75 mg/kg in 0.1 mL sesame oil, SC) or vehicle (sesame oil, SC) 15 min prior to sessions. Females were vaginally swabbed for cytology to track estrous cycle, and all rats were perfused immediately following the 25th session. Nucleus accumbens core (NAcore) tissue was then dissected for immunohistochemistry, confocal microscopy, and microglial morphometrics using the automated analysis program 3DMorph. To establish female cytokine profiles, 30 ovary-intact female Long Evans rats underwent nicotine or saline self-administration and vaginally swabbed for cytology to track estrous cycle for at minimum 10 sessions (0.06 mg/kg/infusion), followed by live decapitation immediately following post 10th session estrus phase of cycle. Ventral striatum and serum from trunk blood were then collected for cytokine analysis.

Results: We found that females display unique structural changes in NAcore microglia (ANOVAs, p's < 0.05) indicating that these cells are stuck in a homeostatic state, unable to respond to injurious stimuli following nicotine SA. We measured significant reductions in ramification index (RI) and number of endpoints (LME, p's < 0.05). Reductions in RI are associated with decreases in normal immune surveillance by microglia. In addition, P4 treatment also increased the number of microglia present following chronic nicotine SA only in females (LME; p < 0.05), thus reversing the population effects observed with nicotine SA. Supporting this female-specific structural consequence, we found that nicotine SA altered cytokine profiles within the ventral striatum as compared to saline SA controls whereby TNFα, IL-6, G-CSF, IL-1α, VEGF, leptin, among others were suppressed (t tests, p's < 0.05). However, fractalkine, which is released from neurons as a "find me" signal to recruit microglial processes for clearance of apoptotic cells and to regulate glutamate neurotransmission, was significantly increased by nicotine SA. Conversely, specific peripheral circulating cytokines in serum were increased, including TNFα, IL-6, IL-1α and IL-18 (t tests, p's < 0.05).

Conclusions: We found that neuroimmune signaling as measured via microglia morphology and cytokine profiles within the NAcore are driven by nicotine use during self-administration (SA) in a sex-specific fashion, whereby female rats were more susceptible to nicotine-induced neuroimmune consequences as compared to males which was reversed by P4 only in females. Together, these results indicate critical sex differences in nicotine-induced dysregulation of the neuroimmune and peripheral immune landscape, which may have translational implications for novel sex-specific therapeutics for smoking cessation.

Keywords: Sex-Specific Effects, Nicotine/Substance Use Disorder, Neuroimmune Mechanisms, Microglia, Progesterone

Disclosure: Nothing to disclose.

P663. Resource Scarcity Early in Life Alters the Basolateral Amygdala Transcriptome and Addiction Related Behaviors

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Background: Adversity is a risk factor for psychiatric disorders, however, stress that is not overwhelming can promote resilience. We use limited bedding and nesting (LBN) to model mild adversity. Our prior research shows LBN reduces morphine self-administration in adult males and decreases impulsive choice and risk-taking behavior. These findings suggest that LBN induces neurobiological alterations that reduce some addiction-related behaviors. These behaviors rely on cues as a driver of behavior and performance. Exposure to cues previously paired with drug taking can induce craving and drug-seeking behavior following periods of abstinence. Thus, we are investigating whether LBN alters incubation of morphine craving, a cue-driven behavior. We extend this work to another drug, cocaine, to determine whether LBN causes similar changes to cocaine self-administration. Finally, we are investigating the molecular changes induced by LBN in the basolateral amygdala (BLA), a region important for responses to stress and for the integration of cues.

Methods: Long Evans rats were reared in LBN or control housing conditions from postnatal day (PND) 2 through 9. The LBN condition consisted of dams and pups placed in a limited resource environment where a metal grate prevented access to bedding and dams were given a single paper towel to use as nesting material. Control animals were reared in standard laboratory housing conditions with ample bedding, two cotton nestles, and one enrichment tube. On PND10 LBN rats were moved back to standard laboratory housing conditions.

E1: Rats (n = 10-11/group) were placed in operant chambers and permitted to lever press on a fixed ratio 1(FR1) schedule for morphine infusions (0.75 mg/kg/infusion). Presses on the "active lever" resulted in one infusion accompanied by a 5-s light cue and a subsequent 20-s timeout period during which the house light was off and lever presses were recorded but had no corresponding drug infusion. Sessions began at the start of the animals' dark cycle (8PM) and ended 12 hours later (8AM) resulting in 12 hours of access to the drug. This was performed on 10 consecutive days. Following the 10 days on FR1 morphine self-administering rats were tested for behavioral signs of drug seeking during early (day 1) and late (day 30) abstinence utilizing a within-subjects design. during drug-seeking tests, lever presses were reinforced by previously drug-paired cue presentations, but morphine was not available.

E2: Rats had daily 6-hour access to cocaine self-administration (0.5 mg/kg/infusion) on an FR1 reinforcement schedule for 10 days. Presses on the "active lever" resulted in one infusion accompanied by a 5-s light cue and 20-s timeout period. Sessions began at the start of the animals' dark cycle and ended 6 hours later resulting in 6 hours of drug access. This was performed for 10 consecutive days.

E3: RNA sequencing was conducted to delineate the effect LBN had on the transcriptional profile of the BLA in adult rats (n = 4-5 rats/group). BLA tissue from adult, behavioral naive rats were sequenced on an Illumina HiSeq 4000. Fastqc version 0.11.8 was used to evaluate the quality of reads with adaptors and non paired reads removed using Trimmomatic version 0.39. The Rank-Rank Hypergeometric Overlap (RRHO) version 2 test evaluated the

degree of overlap in gene signatures between sexes. Differentially expressed genes (DEGs) were identified using an adjusted P value of <0.1 and a 50% change in the expression as cutoffs to determine significance.

Results: We investigated whether rats exposed to LBN had alterations in incubation of craving of morphine during early and late abstinence. At this dosage (0.75 mg/kg) there is no difference in morphine taking between LBN and control rats. Both LBN and control rats showed the incubation effect, pressing more after 30 days of abstinence than 1 day ($F(1,18) = 25.94$, $p < .0001$). However, there was no difference in lever pressing between LBN and control rats in males ($F(1,18) = .325$, $p = .5757$) or females ($F(1,20) = .217$, $p = .646$). Therefore, LBN does not reduce craving behavior elicited after prolonged abstinence suggesting that LBN does not elicit protective effects on the relapse model of craving associated with morphinetaking behavior.

Our preliminary behavioral analysis demonstrates that LBN does not alter cocaine self-administration in male or female rats.

LBN-induced sex-specific changes in transcription. RRHO analysis revealed distinct genes upregulated and downregulated in males and females due to LBN. There was minimal overlap between genes either upregulated or downregulated in males and females. A large proportion of genes were upregulated by LBN in males and downregulated in females. We narrowed our analysis to genes showing a significant difference between control and LBN and found 209 DEGs in females and 149 DEGs in males. These gene expression changes were predominantly sex specific as only 11 genes were altered by LBN in males and females. Heatmaps organized by fold change of LBN DEGs displayed different patterns of upregulated and downregulated genes in males and females.

Conclusions: LBN reduces morphine self-administration in male rats but does not alter cue-driven incubation of morphine craving. LBN induces sex-specific patterns of gene transcription within the BLA. These findings contribute to the notion that some mild early life adversity can be protective and promote later resilience. Understanding neurobiological mechanisms that promote resilience can lead to better therapeutical techniques.

Keywords: Basolateral Amygdala, Transcriptome, Cocaine, Early life Stress (ELS)

Disclosure: Nothing to disclose.

P664. Mechanisms for Estrogen Control of Alcohol Drinking and Anxiety Behavior in Mice

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Background: The overconsumption of alcohol is a risk factor linked to numerous physiological and neuropsychiatric disease states, including alcohol use and anxiety disorders. Women exhibit an accelerated onset of and a higher probability for developing anxiety and are at increased risk for becoming alcohol-dependent with the same history of alcohol use as their male counterparts, but the mechanisms underlying potential sex differences in binge alcohol drinking and anxiety behavior have not been well described. The sex steroid hormone estrogen (E2) may be a key neuromodulator underlying these behaviors. E2 is synthesized in large volumes in the ovaries of females, where it fluctuates cyclically during the human menstrual/mouse estrous cycles to control many adaptive behaviors. In the brain, E2 can act as a neurotransmitter via binding to membrane bound estrogen receptors (ERs) to rapidly mediate synaptic transmission and downstream behavior. The bed nucleus of the stria terminalis (BNST), an E2 sensitive limbic brain structure, has been shown to be a site of rapid E2 transmission. We sought to investigate the role of ovarian-derived E2 BNST signaling actions in binge drinking and basal anxiety behavior in females, particularly via its actions at stress-sensitive neurons that synthesize and release the stress neuropeptide corticotropin-releasing factor (CRF).

Methods: Intact female adult C57BL/6J mice were used in all experimental procedures. Estrous status was determined daily in females via minimally invasive vaginal lavage, in which vaginal epithelial cells were collected and analyzed for cell types corresponding to the different estrous cycle stages. The standard Drinking in the Dark (DID) binge alcohol paradigm and avoidance behavior measures were used in conjunction with behavioral pharmacology, GCaMP fiber photometry, and RNA interference to assess the role of E2 signaling on binge drinking and anxiety-like behaviors. Slice electrophysiology recordings were used to assess the effects of E2 on the synaptic transmission and excitability of BNST CRF neurons, which promote binge drinking and modulate avoidance behavior.

Results: We found that in female mice, binge alcohol consumption and basal anxiety fluctuated across the estrous cycle, with higher levels of drinking in the proestrus (high plasma E2) versus metestrus (low plasma E2) stages. BNST CRF neuron calcium activity during these behaviors was significantly different between estrous cycle stages, and BSNT CRF neuron synaptic excitation and excitability was increased in high ovarian E2 vs. low ovarian E2 mice. Further, acute E2 delivery to the BNST in low ovarian E2 females recapitulated the pro-drinking behavioral effects of ovarian E2, and site-directed BNST infusion of an ERα antagonist, but not ERβ antagonist, rapidly reduced binge drinking in high ovarian E2 status females. In contrast, rapid E2 signaling in the BNST did not modulate avoidance behavior. Acute bath application of E2 increased synaptic excitation onto BNST CRF neurons in slice within minutes, recapitulating the effects of high ovarian E2 status.

Conclusions: Altogether, these results suggest that both alcohol drinking and anxiety-like behavior are regulated by ovarian hormones in females, but that the rapid effects of E2 signaling in the BNST (via ERα) bidirectionally control binge alcohol drinking but not avoidance behavior in female mice. Rapid E2 potentiation of excitatory synaptic transmission onto BNST CRF neurons may serve as a mechanism by which rapid E2 signaling has its effects.

Keywords: Estrogen Receptors, Estradiol, BNST, Limbic System, CRF

Disclosure: Nothing to disclose.

P665. Investigating the Role of Inflammatory Signaling in Opioid-Induced Sleep Disruption

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Background: Opioid use disorder (OUD) remains a public health burden with over 80,000 opioid-induced overdose deaths in 2022 alone. A ubiquitous though often understudied symptom of OUD is sleep disruption. Most patients experience persistent sleep disruption which fails to abate on maintenance therapy and can last months following opioid cessation. Importantly, this often a risk factor for relapse. Fentanyl and its analogs have supplanted prescription opioids and heroin as the main drivers of the opioid epidemic accounting for over 80% of opioid-induced overdose deaths in the same year. Coupled with the emerging research that

indicates that fentanyl pharmacology is unique compared to other opioids there is a strong imperative to study how fentanyl affects the brain, including sleep. Further understanding of molecular mechanisms that contribute to fentanyl-induced sleep disruption could lead to novel non-opioid therapeutics that reduce the risk of relapse. A promising avenue gleamed from snRNA-seq of human post-mortem brain tissue suggests that opioid use induces a pro-inflammatory state. Moreover, many pro-inflammatory cytokines are also sleep regulatory substances. It remains unclear, however, whether changes in pro-inflammatory signaling are associated with fentanyl-induced sleep disruption and which may be responsible. Utilizing a mouse model of fentanyl addiction, we investigated the changes of the pro-inflammatory cytokines and chemokines of sleep-related brain regions.

Methods: Male and female C57BL/6J mice underwent fentanyl exposure or saline as a control group (n = 6 per group/sex). Sleep-wake behavior was recorded simultaneously and changes in percent as well as bout duration of wake, non-rapid eye movement sleep (NREMS), and rapid-eye movement sleep (REMS) were quantified. A two-way repeated measures ANOVA (treatment x zeitgeber) was used to assess significance. Brain tissue was collected following fentanyl exposure. The suprachiasmatic nucleus (SCN), thalamic reticular nucleus (TRN), and dorsal medial medulla (dmM) were micropunched from frozen brain tissue sections (n = 72 total samples). These brain regions were chosen to each represent a core facet of sleep-wake behavior. SCN controls the circadian system, the TRN is important for generation of NREMS, and the dmM is important for REMS generation. Using a multiplex immunoassay we then profiled a panel of cytokines and chemokines in each brain region to understand how pro-inflammatory signaling changes in sleep-related brain regions during fentanyl exposure.

Results: We show for the first time that fentanyl exposure during the light period reverses the typical pattern of sleep-wake with mice awake mostly during their inactive phase (light phase) and asleep during their active phase (dark phase) ($p < .05$). Both NREMS and REMS were elevated during the inactive phase and remained elevated for the entire seven days of exposure. While the same direction of effect was observed in both males and females the effect was stronger in males with wake decreased by ~55% compared to a decrease of ~45% in females ($p < .05$) across the seven days. Within in each brain region we found broad changes in pro-inflammatory cytokines.

Conclusions: Our findings show sex-specific effects on sleep-wake behavior due to fentanyl exposure and begin to identify inflammatory changes in brain circuitry relevant to sleep that may be important for fentanyl-induced sleep disruption.

Keywords: Fentanyl, Sleep Disturbances, Opioid Use Disorder, Inflammation

Disclosure: Nothing to disclose.

P666. Alterations to the Blood-Brain Barrier Promote Changes in Alcohol Preference

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Background: Major depression is a serious public health concern and is commonly comorbid with alcohol use and abuse. Previous work highlights the role of blood brain barrier (BBB) tight junctions in appropriate stress-responding, but less is known about how these proteins are involved in alcohol-related behaviors. To address this gap, the following experiments investigated the role of Claudin 5 (CLDN5), the tight junction protein, in stress-induced alterations in alcohol reward.

Methods: Adult male mice were exposed to chronic social defeat stress and then given intermittent access to alcohol (20%; three 24-hour bouts/week) in a volitional drinking paradigm that models moderate binge drinking and is thus very relevant to both recreational and potentially problematic alcohol consumption. After eight weeks of alcohol exposure mice were tested in a behavioral battery to assess stress reactivity. To further investigate the role of CLDN5in alcohol reward we adopted a virally-mediated approach to down-regulate CLDN5 in the nucleus accumbens and assessed alcohol-related behaviors (escalating preference paradigm [water vs alcohol 3, 6, 10, 20%] and alcohol conditioned-place preference (CPP) [1.0 and 2.0 mg/kg]).

Results: Stress-exposed mice drank more alcohol across the duration of the drinking paradigm and did not show any attenuation of stress-related deficits. Knockdown of CLDN5 in nucleus accumbens (NAc) of male mice increased their drinking behavior at lower doses of alcohol (3%) compared to their control counterparts. In a separate group of mice we assessed the effects of CLDN5 knockdown in NAc on alcohol CPP and found that Claudin 5 knockdown promoted preference for lower doses of alcohol. Interestingly, exposure to a subthreshold stressor shifted alcohol preference regardless of CLDN5 knockdown.

Conclusions: These data suggest that alcohol-induced stress-susceptibility can be recapitulated by artificially opening the BBB. Furthermore, in the absence of stress or alcohol, artificial downregulation of CLDN5 shifted the rewarding properties of alcohol by enhancing preference for lower concentrations of alcohol. These data highlight that BBB integrity plays a role in the rewarding properties, and thereby abuse liability, of alcohol.

Keywords: Blood-Brain-Barrier, Alcohol, Acute Stress

Disclosure: Nothing to disclose.

P667. Altered Gut Microbiome Diversity and Function in People With Alcohol Use Disorder: A Preliminary Case-Control Study

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Background: In animals and humans, chronic, heavy alcohol use is associated with changes in the amount, type, and function of gut bacteria. This alcohol-induced gut dysbiosis may contribute to increased gut membrane permeability, systemic inflammation, and organ damage. Most human studies in this area have examined abstinent individuals with severe alcohol use disorder (AUD), often with alcohol-associated liver disease (ALD), or young, heavy drinkers without AUD. In order to advance our knowledge on the potential role of the gut microbiota in AUD, studies in actively drinking individuals with AUD, prior to developing ALD, are needed to better characterize the effects of alcohol on the gut microbiome.

Methods: This study included actively drinking, non-treatment seeking individuals aged 25-65 with DSM-5 AUD (n = 17; ≥ 14 or 7 drinks per week for men and women, respectively, and ≥ 5 heavy drinking days [HDD] per month [HDD = ≥ 5 drinks in a day for men, ≥ 4 drinks for women]) and healthy controls (n = 15; < 14 or 7 drinks per week for men and women, respectively and ≤ 2 heavy drinking days over the last 6 months) who were recruited from the general community and eligible to participate in a randomized

controlled medication trial (NCT04210713). Participants in both groups had to be generally healthy and medically cleared to participate in a medication trial; no participant with AUD had a clinically significant ALD. Participants completed an in-person screening session to assess eligibility for the parent trial, which included a physical exam, diagnostic interviews to assess psychiatric disorders (e.g., the Structured Clinical Interview for the DSM-5), and various other assessments. At this session, participants were provided a take home fecal sample collection kit consisting of a test tube with chaotropic solution (DNA/RNA Shield, Zymo Research) as well as collection and storage materials and instructions. Participants were instructed to collect a stool sample as close as possible to their scheduled study commencement date and immediately store their sample in their freezer until that time. Upon arriving to the study baseline with their frozen sample, fecal samples were then stored in a −80ºC freezer until sequenced. Hypervariable regions V3 and V4 of the bacterial 16S rRNA gene were amplified with primers 319F and 806R. High-quality amplicon sequences were obtained on an Illumina HiSeq 2500 modified to generate 300 bp paired-end reads. Amplicon sequence variants were generated by DADA2 and taxonomically classified using the RDP Naïve Bayesian Classifier trained with the SILVA v128 16S rRNA gene database. Microbial community data were analyzed with the phyloseq R package; all analyses included group (AUD vs control) as independent variables and controlled for BMI. Comparisons of alpha diversity and taxa differences were performed using multivariable linear regressions. Principal Coordinate Analysis using Bray-Curtis dissimilarity was performed to assess beta diversity. Permutational multivariate analysis of variance (PERMANOVA) was conducted to test whether the bacterial communities sequenced have different centroids based on AUD diagnosis. To determine whether assumptions are met for PERMANOVA, a test of heterogeneity (ensure homogenous dispersion) was performed. In addition, multivariate association with linear models (MaAsLin2) was used to efficiently determine multivariable association between group and 16S rRNA gene sequence data. MaAsLin2 parameters for taxa analysis were set as follows: α level was set at 0.05, the minimum abundance for each taxon was set to 1%, and the minimum percent of samples for which a taxon is detected at 1% was set to 10%. Lastly, to assess functional differences in taxa, KEGG Orthology profiles were generated using the tool Tax4fun.

Results: The AUD group, vs. controls, exhibited significantly higher microbial diversity (i.e., richness and evenness), as measured by Shannon ($B = -0.27$, $t = -2.10$, $p < 0.05$) and Simpson indexes ($B = -0.02$, $t = -2.83$, $p < 0.01$). There was no significant difference between the AUD and control groups in beta diversity ($p = 0.15$). Several group differences were identified at the taxa level, including participants in the AUD group, vs. healthy controls, having significantly lower abundance of Akkermansia ($B = 0.03$, $t = 2.08$, $p < 0.05$) and Lachnospiraceae UCG 001 ($B = 0.00$, $t = 2.17$, $p < 0.05$) and higher abundance of Phascolarctobacterium in the AUD group ($B = -0.03$, $t = -2.32$, $p < 0.05$). Multiple significant functional differences were identified between groups, including reduced representation of the tetrahydrofolate biosynthesis pathway in the AUD group ($p < 0.05$).

Conclusions: Actively drinking individuals with AUD showed greater gut microbial diversity than healthy controls, and this effect may have been driven by changes in abundance of several taxa. Healthy controls showed greater abundance of genera associated with anti-inflammatory processes and short chain fatty acid production (Akkermansia) and healthy diet (Lachnospiraceae UCG 001). Conversely, individuals with AUD had higher abundance in the Phascolarctobacterium genus, previously shown to be associated with alcohol consumption and obesity, and a functional reduction in a pathway responsible for folate synthesis. These findings suggest individuals with AUD, prior to the onset of clinically significant ALD, show gut dysbiosis. Longitudinal studies in larger samples are needed to replicate the present findings.

Keywords: Alcohol Use Disorder, Gut Microbiome, Healthy Controls

Disclosure: Nothing to disclose.

P668. Effect of Acute Vapourized Cannabis Exposure on Microglia

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Background: Microglia, the brain's resident immune cells, are increasingly becoming recognized for their physiological, as well as immunological, roles. Microglia possess cannabinoid receptors and respond to cannabinoids, such as phytocannabinoids. Administration of the primary phytocannabinoids, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), typically leads to anti-inflammatory responses, but this is dependent on the route of administration (i.e., generally injection or ex vivo), the duration and compounds delivered. Our goal is to understand how another relevant route of administration, inhalation, affects microglial physiological functions.

Methods: Whole cannabis plant was administered to adult, male, C57BL/6J mice for 15 min (one 15 second puff every 5 min; 3 puffs total; 0.15 g flower/puff). Four groups were utilized, mice that received control air vapor, and mice that were exposed to either: high CBD/low THC [CBD], high THC/low CBD [THC], or balanced THC/CBD [Balanced] cannabis strains. Brains were isolated 30 min post-cannabis administration onset, when THC levels peak in the brain. We stained the tissue with antibodies against IBA1 (microglia and macrophages) and TMEM119 (more specific for microglia). We looked at IBA1+ cell density, nearest neighbor distance and spacing index (changes in number and distribution), in regions important for cognition, memory, and emotional regulation, specifically focusing on the prefrontal cortex. We also investigated IBA1 and TMEM119 colocalization, as well as IBA1+ cell morphology.

Results: Our preliminary data indicates that the distribution and spacing of microglia, as ascertained by nearest neighbor distance, was altered in the prefrontal cortex of male mice, and differently in the infra- and prelimbic cortices. Specifically, density and distribution of IBA1+ cells in the infralimbic area, but not the prelimbic cortex, were potentially altered in CBD high strain versus others. In the prelimbic cortex, all IBA1+ cells analyzed were colocalized with TMEM119, indicating these cells were likely all resident/homeostatic microglia. Furthermore, in the prelimbic area, although there were no changes in density or distribution, microglial morphology was altered. In the balanced strain exposed group compared to control air exposed group, microglial fractal dimension was reduced ($p < 0.05$), indicating a less complex morphology; and microglial lacunarity ($p < 0.01$) was increased, indicating altered shape. Additionally, exposure to the CBD strain altered microglial arbor (process morphology) in the infralimbic cortex ($p < 0.05$), in a way that indicates a potential reduction in surveillance. Furthermore, the relative distribution of the microglia morphology in all cannabis strains was significantly different than microglia from the control group ($p < 0.01$—$p < 0.0001$), even if there was not an overall significant differences in the animal/group averages—potentially indicating the emergence of different states of microglia in response to acute cannabis exposure.

Conclusions: Our preliminary data indicates that acute cannabis exposure modifies microglial morphology in the

prelimbic cortex. We will next use electron microscopy to investigate potential changes in microglial organelles and interactions with parenchymal elements. This work will lay the foundation for understanding how vaporized cannabis exposure alters microglial form and function, and determining how these parameters change with chronic exposure and in response to stress, infection or disease. We are also investigating potential sex differences in the response of microglia to acute cannabis exposure.

Keywords: Microglia, Cannabis, Mouse Models, Cortex

Disclosure: Nothing to disclose.

P669. Preliminary Results of Deep Transcranial Magnetic Stimulation to the Dorsal Anterior Cingulate Cortex Reveal Increased Neural Target Function and Abstinence in Veterans with Alcohol Use Disorder

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Background: Transcranial Magnetic Stimulation (TMS) offers a promising treatment avenue to modulate brain function directly in alcohol use disorder (AUD). New technologies have emerged to stimulate deeper cortical targets that have previously been associated with relapse. For example, the dorsal anterior cingulate cortex (dACC) plays a critical role in our understanding of addiction. Specifically, individuals who go on to relapse within three months of treatment demonstrate blunted activation to a negative, salient emotion processing, suggesting that increasing activation in this region may improve treatment outcomes. To this end, we sought to determine whether an FDA approved TMS protocol for obsessive compulsive disorder, which targets the dACC, would increase dACC activation in response to salient faces and reductions in relapse rates relative to treatment as usual.

Methods: This preliminary pilot study enrolled a total of $N = 6$ Veterans (2 women) currently in residential treatment for AUD. Two individuals were discharged irregularly during the course of the study but their pre-assessment data were used in the current analyses. Participants completed pre and post treatment assessments to evaluate symptoms, cognition and brain function. Consistent with existing protocols, participants completed 30 sessions of 20Hz dTMS to the dACC, including a personalized provocation protocol to prime individuals prior to each dTMS session. In order to finish treatment within the 28 day residential treatment program, participants completed 3 dTMS sessions per day for 10 consecutive business days. Brain function was measured during a facial affective processing task while scanned with 3T functional MRI. Given the very small sample size, we report Cohen's d effect sizes in order to design future, well powered clinical trials.

Results: Relative to the participants receiving treatment as usual who have an abstinence rate of 55% by 3 months post treatment and who did not receive dTMS, the individuals who received at least 15 sessions of dTMS currently have 80% complete abstinence. In addition, relative to pre-treatment assessment, individuals demonstrated an increase in dACC activation during the facial affective processing task (threat versus neutral contrast) that was a medium to large ($d = 0.63$) effect. In addition, we explored changes in functional connectivity between pre- and post-TMS and observed similar medium to large effects in increasing functional connectivity between the dACC and dorsomedial prefrontal cortex (dmPFC; $d = 0.68$) as well as decreasing functional connectivity between the dACC and dorsal striatum (DS; $d = -0.85$). Given the limited sample size of this preliminary pilot study, the impact of gender was not explored.

Conclusions: Findings highlight the potential of this approach to increase abstinence rates and neural target engagement associated with treatment outcomes in Veterans with AUD. Further, this pilot trial demonstrates the feasibility of this treatment and tolerability of treatment in this clinical population. Future well-powered, double blind, sham controlled clinical trials to examine the efficacy of this novel intervention are warranted.

Keywords: Alcohol Use Disorder - Treatment, Repetitive Transcranial Magnetic Stimulation (rTMS), Dorsal Anterior Cingulate Cortex, Salience Network, Abstinence

Disclosure: Nothing to disclose.

P670. Pharma-Psychosocial Treatment for Individuals With Substance Use Disorders in a Canadian Forensic Program

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Background: Substance misuse is disproportionately prevalent among individuals in the criminal justice system, often complicating their assessment, diagnosis, treatment, and rehabilitation. While pharmaco-psychosocial therapy is beneficial to manage concurrent disorders in this population, the efficacy of psychosocial therapy for substance misuse may be limited if not well-designed to target patients' risk factors and resources. This study describes lessons learned from the implementation of a semi-structured manualized treatment program (SSMTP) for substance misuse in a forensic program to model treatment for substance misuse among this population.

Methods: Pre- and post-treatment data of forensic adult patients ($n = 96$) referred for SSMTP was reviewed. The SSMTP offered a multi-levelled comprehensive substance use treatment program for individuals with concurrent disorders using motivational enhancement and cognitive behavioural models to assist with positive social and coping skill development to avoid relapse. Readiness was assessed pre- and post-SSMTP using the Stage of Change Readiness and Treatment Eagerness Scale (SOCRATES). We conducted paired t-tests to compare participants' performance on measures of substance use behaviours (SOCRATES) pre- and post-treatment with the SSMTP. A two-tailed p-value ≤ .05 was considered statistically significant.

Results: The participants had a mean age of 40.25 ($SD = 10.04$) years and were predominantly males (90.6%). Regarding alcohol use problems, ambivalence increased significantly between pre-treatment ($M = 10.0 \pm 5.1$) and post-treatment ($M = 11.4 \pm 5.4$) analysis, $t(34) = 2.3$, $p = .025$. For participants using cannabis, cocaine, and tobacco, there were significant increases in recognition and taking steps between pre- and post-treatment groups. Cannabis recognition scores increased from $M = 21.2$ (± 8.2) to $M = 23.6$ (±8.7), $t(38) = 2.4$, $p = .024$, and taking steps scores increased from $M = 33.1$(± 7.3) to $M = 35.5$ (± 6.1), $t(41) = 2.79$, $p = .008$. Cocaine recognition scores increased from $M = 24.4$(± 6.9) to $M = 26.7$(±6.3), $t(15) = 2.1$, $p = .05$, and taking steps scores increased from $M = 34.5$(±4.7) to $M = 37.4$(± 3.4), $t(16) = 2.9$, $p = .011$. Tobacco recognition scores increased from $M = 19.0$(±8.0) to $M = 21.3$(±9.1), $t(42) = 2.4$, $p = .022$, and taking steps scores increased from $M = 32.6$(±7.4) to $M = 34.7$(±6.1), $t(44) = 2.4$, $p = .021$.

Conclusions: The study findings suggest SSMTP may increase the level of recognition and degree of steps taken towards cessation and abstinence from cannabis, cocaine, and tobacco use among forensic populations with concurrent disorders. Optimally,

implementing similar intervention programs in forensic populations can improve rehabilitation and mitigate risks to public safety associated with substance use in this population. Future well-powered clinical trial studies are needed to replicate and build upon findings in the present study to inform evidence-based recommendations.

Keywords: Addiction, Concurrent Disorders, Correctional Psychiatry, Forensic Psychiatry, Psychosocial Intervention

Disclosure: Nothing to disclose.

P671. Left Dorsolateral Prefrontal Cortex Intermittent Theta Burst Stimulation Improves Treatment Outcomes in Veterans With Alcohol Use Disorder: A Randomized, Sham-Controlled Clinical Pilot Trial

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Background: Transcranial Magnetic Stimulation (TMS) offers a promising treatment avenue to modulate brain function in Alcohol Use Disorder (AUD). To the best of our knowledge, this pilot study is the first randomized, double-blind, sham-controlled trial to deliver intermittent theta burst stimulation (iTBS) to the left DLPFC among U.S. Veterans with AUD. We hypothesize that 20 sessions of real TMS will be tolerable and feasible. As a secondary line of inquiry, we hypothesize that, relative to sham TMS, individuals receiving real TMS will experience greater reductions in 6-month relapse rates, anhedonia, and alcohol cue-reactivity.

Methods: United States Veterans (n = 17,1 woman) were enrolled in a double-blind, sham-controlled trial (2-3 sessions/day; 7-10 days; 600 pulses/session; 20 sessions). Pre- and post-treatment assessments included self-report questionnaires and fMRI alcohol cue-reactivity. Alcohol consumption was assessed for 6 months. Linear mixed-effects models were constructed to predict post-treatment craving, mood and cue-reactivity.

Results: Individuals who received active iTBS were less likely to relapse for 3-months after treatment (OR = 12.0). Greater reductions in anhedonia were observed following active iTBS (Cohen's d = −0.59), relative to sham (d = −0.25). Alcohol cue-reactivity was reduced following active iTBS and increased following sham within the left insula (d = −0.19 vs 0.51), left thalamus (d = −0.28 vs 0.77), right insula (d = 0.18 vs 0.52), and right thalamus (d = −0.06 vs 0.62).

Conclusions: Relative to sham, we demonstrate 20 sessions of real left DLPFC iTBS reduced the likelihood of relapse for at least 3 months. The potential of this approach is underscored by decreases in anhedonia and alcohol cue-reactivity – strong predictors of relapse among Veterans. These initial data offer a valuable set of effect sizes to inform future clinical trials within this population.

Keywords: Transcranial Magnetic Stimulation, Alcohol Use Disorder, Veterans, Theta-Burst Stimulation, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P672. FARESHARE: An Open-Source Device for Measuring Drinking Microstructure in Socially Housed Rats

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Background: Social factors have been shown to play a significant role in alcohol consumption. Studying the role of social context on alcohol drinking is important to understand the factors that contribute to initiation or maintenance of casual and problematic alcohol use. A large body of preclinical research has shown that social environment plays an important role in alcohol consumption and preference, though the extent of these effects have been obfuscated by methodological differences and technical challenges. Robust individual differences in alcohol intake in socially housed rats are difficult to track when sharing a common fluid source. Commercial solutions are prohibitively expensive and are limited by proprietary software and hardware. Here we describe FARESHARE (Fluid Acquisition REcording in Socially Housed Animal REsearch), an affordable, open-source solution for tracking fluid consumption in socially housed rats.

Methods: FARESHARE uses RFID to identify rats, a lickometer to activate fluid delivery, a custom low-profile PCB that sits on top of an Arduino-based microcontroller, fluid delivery via custom peristaltic pump for accurate measurement of consumption volume, OLED display, and continuous data logging to an SD card. Here we validated our design via an alcohol two-bottle preference task. Four female Sprague Dawley rats were implanted with RFID tags and housed together. Animals were given access to two FARESHARE devices: one for water, and the other for 10% ethanol. Fluid intake was tracked for 9 days. All animal procedures were performed in accordance with the University of Guelph animal care committee, the University of Western Ontario Animal Use Subcommittee, and were consistent with guidelines established by the Canadian Council on Animal Care.

Results: Rats showed a significant preference for alcohol, as well as unique drinking behaviour depending on fluid type, with greater max and mean bout sizes and greater volume per lick for alcohol than water. Drinking showed strong circadian patterns, with most drinking occurring during the dark cycle.

Conclusions: Having a robust, affordable method for measuring drinking microstructure in socially housed animals will be of considerable use in preclinical addiction research and a step toward more translationally relevant animal models of fluid consumption. The added dimension of time allows for the analysis of circadian-linked consumption and the discrimination of continuous or binge-like drinking behaviours. Additionally, being open-source enables researchers to customize the device for more advanced applications such as sending signals to additional peripherals (e.g., optogenetic stimulation) or software on drinking initiation for time-locked or closed-loop interventions, manipulations, and measurements.

Keywords: Alcohol, Social Behavior, Social Drinking Motives, Open Neuroscience

Disclosure: Nothing to disclose.

P673. Independent Component Analysis Reveals Significant Differences in β2*-Nicotinic Acetylcholine Receptor Components in People Who Smoke Tobacco

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Background: Nicotine, the primary addictive chemical in tobacco smoke, exerts its initial reinforcing effects by binding to β2 subunit-containing (β2*) nicotinic acetylcholine receptors (nAChRs) in brain. The radiotracer [18F]Flubatine binds to β2*-nAChR and used with positron emission tomography (PET) brain imaging provides crucial insights into the spatial distribution of

the whole pool of β2*-nAChR and their functional changes due to nicotine use. Independent component analysis (ICA), when conducted on group-level neuroimaging data, can isolate shared variance sources in spatial components. Previous work used ICA to separate components of D2- and D3-receptor binding from [11C] PHNO PET images[1], and analogous application of ICA to [18F] Flubatine PET images could potentially aid in the differentiation of various nAChR stoichiometries. The objective of this study was to perform ICA on a [18F]Flubatine dataset from a cohort of people who smoke tobacco and controls [2] to observe the spatial distribution of components and compare individual loading weights across groups.

Methods: [18F]Flubatine PET scans were acquired in people who smoke ($n = 18$; 6F; age = 35.5 years(21-55); 7 ± 1 day abstinence) and matched control participants ($n = 23$; 8F; age = 28.3 years (20-45)). [18F]Flubatine was injected using a bolus plus constant infusion paradigm (KBol = 360 min, dose: 257.7 ± 44 MBq, 0.07 ± 0.06 μg) and image data were acquired with an HRRT scanner (Siemens) from 90-120 min. Images were reconstructed into 5 min frames with the MOLAR algorithm and denoised with the HYPR algorithm [2]. Parametric volume of distribution (VT) images were generated with equilibrium analysis with correction for radiotracer clearance (estimated across a Gaussian kernel of 7 mm FWHM) using the metabolite-corrected arterial blood data. Magnetic resonance imaging (MRI) scans (3T Trio, Siemens) were collected for PET coregistration and subsequent registration into Montreal Neurological Institute space. VT parametric images were loaded into the source-based morphometry toolbox of the Group ICA of functional MRI Toolbox (GIFTv4.0c). Regions above a threshold of 2 (pseudo-VTs) were deemed high intensity. Source maps were overlaid with thalamic and midbrain atlases for detailed anatomical review. Individual independent components (IC) spatial loading values were computed for all three components. Group differences were statistically determined using unpaired t-tests.

Results: [18F]Flubatine ICA analysis was restricted to three IC, as additional components accounted for less than 5% of variance. The three ICs identified showed similar spatial distributions when applied to control participant ($n = 23$), tobacco smokers ($n = 18$), and pooled data ($n = 41$). These components reveal high intensity in specific regions: IC1 in the cerebellum and optic system; IC2 in thalamic and hypothalamic regions; IC3 in the locus coeruleus, superior colliculus, and retinal ganglion cells. Loading values from the pooled data highlighted significant group differences for all ICs. Tobacco smokers demonstrated significantly higher IC1 loadings (Cohen's $d = 0.96$; $p = 0.003$), and significantly lower loadings of IC2 (Cohen's $d = 1.12$; $p < 0.001$) and IC3 (Cohen's $d = 1.46$; $p < 0.001$) sources, when compared to control participants.

Conclusions: [18F]Flubatine ICs showed interesting spatial heterogeneity. We speculate that [18F]Flubatine components may correspond to different pools of β2*-nAChR conformations. This is based on similarities in the regional distribution of β2*-nAChR expression with these components, combined with [18F] flubatine's high but distinct affinity for different nAChR stoichiometries (e.g., α3β2*, α4β2*, and α6β2*-nAChRs conformations). Future work is needed to confirm pharmacological specificity of these ICs. The distinct differences observed in the loadings—but not spatial distributions—of these ICs between people who smoke tobacco and the control group potentially offer valuable understanding into neurobiological adaptations that occur from chronic tobacco smoking and early stages of abstinence.

References:

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[2] Calakos KC et al. Cholinergic system adaptations are associated with cognitive function in people recently abstinent from smoking: a (-)-[18F] flubatine PET study. Neuropsychopharmacology. 2023.

Keywords: PET Imaging Study, Nicotine Addiction, Nicotine Withdrawal, Independent Component Analysis, a4b2 Nicotinic Acetylcholine Receptors

Disclosure: Nothing to disclose.

P674. Evaluation of the Abuse Liability of Oxycodone, Pregabalin, Duloxetine and Gabapentin Using Intravenous Self-Administration and Conditioned Place Preference in Male and Female Rats

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Background: In collaboration with the NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP), we evaluated the abuse liability properties of oxycodone, pregabalin, duloxetine and gabapentin using intravenous self-administration (SA) and conditioned place preference (CPP) assays in male and female Sprague Dawley rats. These two assays are the most commonly used to assess abuse liability with high validity.

Methods: Intravenous self-administration (SA): SA took place in sound attenuated operant chambers (Med Associates, VT) where rats pressed an active lever that delivered the test compound intravenously through a jugular vein catheter. Food restricted rats, maintained at ~85% of free feeding body weight, were first trained to press the active lever to receive food reinforcement. Rats that met the criteria of receiving 50 food pellets during the 1 hour session, using a FR3 schedule of reinforcement (three lever presses for one food reward), were transitioned acquisition training of test compound. Acquisition training lasted 20 days. Progressive Ratio was conducted one day following the last acquisition training. The following compounds and doses were tested: oxycodone (0.01, 0.03, 0.06 and 0.1 mg/kg/infusion) pregabalin (0.1, 0.3, 1 and 3 mg/kg/infusion) and gabapentin (0.3, 1, and 3 mg/kg/infusion).

Conditioned place preference (CPP): Open field chambers [60cm (L) x 40cm (W) x 24cm (H)) with two compartments were used in these studies. Perceptive cues were applied to create a distinctive texture and visual feature for the two compartments: plastic flat mat floor with white stripes on the wall vs. plastic spiny mat with black stripes on the wall. A 10-day protocol was used in this study. The studies were videotaped on Day 1 (baseline) and on Day 10 (bias test). Days 2-9 of the test were conditioning sessions where differentiation between "drug compartment" and "saline compartment" was achieved. Rats were administered vehicle on days 2, 4, 6 and 8, and with test compound on days 3, 5, 7, 9. Animals were confined in the "drug compartment" or "vehicle compartment" immediately after drug administration for 20 minutes. Time spent in the different chambers was scored by an experimenter blinded to the treatment. The following compounds and doses were tested: oxycodone (1, 3 and 5 mg/kg; intraperitoneal injection (IP)), pregabalin (3, 10, 30 and 100 mg/kg; IP) and duloxetine (10, 30 and 100 mg/kg; oral administration).

Statistical analysis, effect size, and power analysis: Data were analyzed analysis of variance and Dunnett's post hoc test when appropriate. Treatment groups were randomized and were sufficiently powered based on previous analysis. Effects $p < 0.05$ were considered to be statistically significant.

Results: In male and female rats, oxycodone (0.03, 0.06 and 0.1 mg/kg/infusion) showed higher infusion rate compared to saline.

In CPP study, oxycodone (1, 3 and 5 mg/kg; IP) induced a significant bias between the two compartments ($P < 0.001$) when compared to saline. All doses showed equal potency in inducing CPP. Pregabalin (0.1, 0.3, 1 and 3 mg/kg/infusion) showed no significant effect on lever response compared to saline in the SA test. Neither pregabalin (3, 10, 30 and 100 mg/kg) nor duloxetine (10, 30 and 100 mg/kg) induced CPP. Similarly gabapentin (0.3, 1, and 3 mg/kg/infusion) showed no potential abuse liability as measured by the lever press response in SA. Results from ongoing studies with duloxetine in SA and gabapentin in CPP will also be presented.

Conclusions: The results of these studies indicated that oxycodone possesses strong potential for abuse liability. Meanwhile pregabalin, gabapentin and duloxetine do not appear to have abuse potential. These two assays can be used to screen the potential abuse liability of novel therapies as part of the PSPP program towards discovering novel non-addictive analgesics.

Keywords: HEAL Initiative, NINDS, PSPP, Non Addictive Analgesics, Self Administration and CPP

Disclosure: Nothing to disclose.

P675. Translational Studies of AZD4041, a First In-Class Selective Orexin 1 Receptor Antagonist for the Treatment of Opioid Use Disorder

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Background: Opioid use disorder (OUD) is a chronic relapsing disorder of opioid misuse affecting over 26 million individuals worldwide. The prognosis for OUD is poor if left untreated, with substantial morbidity and mortality resulting from opioid overdose. The hypothalamic neuropeptide orexin regulates states of arousal and motivation. Chronic opioid exposure increases the activity of orexin neurons in rodents and patients with OUD. Orexin acts through orexin 1 (OX1) receptors to regulate the motivation to seek and consume opioids as well as the craving and withdrawal symptoms that emerge during periods of abstinence. AZD4041 is a first-in-class, potent and selective, brain penetrant small molecule OX1 receptor antagonist being developed for the treatment of OUD under a collaborative grant from the National Institute on Drug Abuse (NIDA), as part of the HEAL Initiative (Helping to End Addiction Long-term) from the National Institute of Health (NIH). Here, we describe translational studies conducted to progress AZD4041 into clinical development.

Methods: Pharmacological actions of AZD4041 on OX1 receptors were investigated using cell-based functional assays. Target engagement at human and rat OX1 receptors in the brain was determined using in vitro and ex-vivo autoradiography. AZD4041 (0.3, 1, 3 and 10 mg/kg) was tested in rodent behavioural procedures relevant to OUD, including intravenous oxycodone self-administration, cue-induced reinstatement of oxycodone seeking, and oxycodone-seeking during withdrawal. Single Ascending Dose (SAD) and Multiple Ascending Dose (MAD) studies were conducted in healthy human subjects to explore the safety, tolerability, pharmacokinetics (PK) and Pharmacodynamics (PD) of single and multiple oral doses of AZD4041 over a wide dose range. PK/PD modelling was performed to predict AZD4041 occupancy of human OX1 receptors using pre-clinical concentration-receptor occupancy relationships (in vitro and rat ex-vivo autoradiography) and clinical PK data.

Results: AZD4041 was a brain penetrant (human Kpuu 0.28), potent and selective OX1 receptor antagonist (Ki 9.3 nM and >1000 nM for OX1 and OX2 receptors, respectively) and demonstrated target engagement at OX1 receptors in addiction-relevant brain regions. There were no significant differences in the affinity of AZD4041 at OX1 receptors across specifies. AZD4041 dose-dependently decreased oxycodone self-administration and attenuated withdrawal-induced oxycodone-seeking in rodents, with efficacy observed at OX1 receptor occupancies (RO) >65%. AZD4041 had favourable drug-like safety and toxicology profiles in preclinical studies. SAD and MAD studies in healthy subjects established that AZD4041 had a good safety profile and was generally well tolerated when administered as a single dose and repeat doses over 14 days of treatment. In addition, pharmacokinetic data showed slightly more than dose-proportional increases in systemic exposures after repeat dosing with a terminal half-life between 19-23 h, allowing for once daily dosing. PK/PD modelling indicated maximum human brain RO of 92% and average brain RO of 84% at steady state with the highest dose tested.

Conclusions: AZD4041 demonstrated target engagement and efficacy in preclinical models of OUD at RO >65%. Studies in humans showed a good safety profile with clinical doses predicted to have receptor occupancies of greater than 84% at steady state. AZD4041 is considered a promising drug candidate with a strong underlying rationale supporting its further development as a novel and differentiated treatment for OUD. Development of AZD4041 was accelerated combining grant support from NIDA/NIH and the neuroscience drug discovery expertise of AstraZeneca. This unique public-private partnership promises to yield a much-needed new medicine for the treatment of a major public health crisis in the US.

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Keywords: Opioid Use Disorder, Orexin Receptor Antagonist, Orexin/Hypocretin, Translational Studies, Addiction

Disclosure: Astrazeneca: Share Holder, Employee (Self).

P676. Investigation of Within-Session Cocaine vs. Sucrose Choice Behavior Using a Drug-Biased Progressive Effort Paradigm in Rats

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Background: Substance use disorder (SUD) is a complex psychiatric condition that involves maladaptive changes in motivation and reward valuation. Drugs of abuse usurp motivation circuits leading to interference with syntonic decision making. Disentangling pathological motivation to pursue drug from essential motivational processes has been a limitation of current neurobehavioral research. Animal models that incorporate a choice element (i.e., choosing between drug and alternative reward) have gained increasing popularity because they offer multidimensional insights into reward seeking behavior. Previous work from the field has demonstrated that rodents will generally prefer a non-drug reward such as sucrose pellets or social

interaction over drug taking when offered at a similar effort cost. Our lab has leveraged this observation to develop a novel behavioral paradigm that applies an increasing effort cost of sucrose pellets, a non-drug reinforcer, to observe a within-session "switch point" from sucrose to cocaine. These preliminary studies lay the groundwork for future investigation into changes in drug choice after acute behavioral or pharmacological manipulations.

Methods: Female (n = 14) and male (n = 10) SD rats were trained to respond for sucrose pellets on a progressive ratio (PR) schedule accompanied by a solid light availability cue until a response criterium was met. After initial sucrose training, rats received intravenous catheters followed by operant cocaine training (0.5 or 0.8 mg/kg/inf) at a fixed ratio (FR) with a flashing light cue on a lever across from the sucrose lever. After a short acquisition period, rats received daily access to cocaine (FR3) for 7 days during which no sucrose was available. After the 7th day of cocaine training, a "probe session" was administered during which both levers were presented accompanied by the respective cues, and animals were able to select either reward over the course of 21 discrete trials. The trial format consisted of a 7-minute decision period followed by a 2-minute timeout. After a reward was selected or the decision period expired, the effort cost of sucrose progressed to the next response interval, while the effort cost of cocaine remained at FR3. After the first probe trial, the animals received 3 more days of cocaine-only training after which another probe trial was administered. This cycle repeated for another 3 days of cocaine-only training followed by a probe trial for a total of 13 cocaine-only sessions and 3 probe sessions. The day after the 3rd probe session began the choice period. The choice period consisted of 8 daily "choice sessions" which mirrored the probe sessions exactly. Following the 8th choice session, food was withheld overnight, and a final choice session was administered under food restriction conditions.

Results: Our pilot studies yielded a total of 190 daily choice sessions. Of these sessions, 52.3% contained a single switch point at which animals that started with at least one sucrose choice transitioned to exclusive cocaine choice. Additionally, 25.7% of sessions began with at least one sucrose choice, but contained two or more switch points before exclusive cocaine choice was reached. No sessions contained exclusive sucrose choice and 20.4% of sessions consisted of exclusive cocaine choice. At a moderate cocaine dose (0.5 mg/kg/inf), female rats displayed a greater drug choice index (DCI = drug rewards/total rewards per session) than male rats (n = 5-6/group, $p < 0.05$) during D1-D8 of the choice phase. There was no significant difference in DCI between male and female rats (n = 5-8/group) at a high cocaine dose (0.8 mg/kg/inf). Among sessions that included one or more switch points starting with sucrose choice and ending with cocaine choice (78% of trials), male rats switched to their first cocaine choice after more trials than females at a moderate cocaine dose (n = 5-6/group, $p < 0.05$). There was no significant difference in switch point between male and female rats at a high cocaine dose (n = 5-8/group). As an initial probe into the tractability of this approach, we withheld food following the D8 choice session until the D9 choice session to inflate the value of sucrose. We found no significant differences in DCI or switch point within any experimental groups when comparing D8 responding to D9 after 24-hour food restriction. Nevertheless, our preliminary studies indicate that rats will adjust their responding to sucrose choice when cocaine is replaced with saline, suggesting schedule control is maintained.

Conclusions: We have devised a simple, yet unique choice model allowing for the observation of within-session switch points and overall drug vs. sucrose responding. Our preliminary experiments have uncovered an apparent sex difference in cocaine vs. sucrose choice with respect to cocaine dose. Female rats maintained on a moderate cocaine dose switch from sucrose to cocaine responding in fewer trials than male rats. Interestingly, we observe no significant differences in switch point between males and females at a higher dose, which suggests that drug sensitivity may be equilibrated at higher doses. Future studies will address how a low cocaine dose affects male and female rats. Baseline studies with attention to sex as a biological variable provide essential information for interpreting later observations using this model. Moreover, we will employ this model to further understand the neurobiology of stress as it relates to drug choice behavior.

Keywords: Drug Choice Paradigm, Cocaine, Sex Differences

Disclosure: Nothing to disclose.

P677. Care Coordination of Individuals on MAT During the COVID-19 Pandemic

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Background: COVID-19 caused a significant and challenging bio-psychosocial impact on many individuals including patients with diagnoses of Opioid Use Disorder (OUD) receiving medication-assisted treatment (MAT) like buprenorphine (BUP). Since 2012, the Buprenorphine Integrated Care Delivery Project, a collaboration between Howard University Urban Health Initiative (HUUHI) and a community-based medical practice sought to deliver accessible, high-quality care to patients receiving BUP for chronic opioid addiction by linking them to comprehensive healthcare services via care coordination. The adaptation of care coordination services delivered during the pandemic sought to address barriers such as homelessness, unemployment, legal problems, mental illness, and co-occurring diseases (e.g., HIV, hepatitis C, and diabetes mellitus) as well as implementing harm reduction and COVID-19 protocols to enhance BUP treatment success. Our findings regarding the challenges to providing continuity of care during the heightened period of the COVID-19 pandemic (2021) will be presented.

Methods: Setting: An urban healthcare facility in the District of Columbia.

Population: Participants ($N = 99$) receiving BUP therapy were recruited at the Howard University Department of Community and Family Medicine and Department of Psychiatry, as well as a community-based medical practice, and a subset of 38 completed the Government and Performance Results Act (GPRA) Client Outcome Measures For Discretionary Programs.

Inclusion criteria included participants ≥ 18 years old, met DSM-5 criteria for OUD or undiagnosed, had medical insurance in DC, and had the ability to provide informed consent.

Exclusion criteria included patients that had liver dysfunction (acute hepatitis, liver failure, or hepatic dysfunction), suicidal ideation, and hypersensitivity to buprenorphine.

Assessments: The GPRA was utilized for the intake and follow-up of the original cohort and new patients were recruited. During the pandemic, the GPRA interview administration was changed from in-person to phone when face-to-face interviews are not feasible due to public health restrictions. Key components obtained were substance use, planned services, living conditions, education, employment and income, legal, mental, and physical health problems, and treatment/recovery as well as social connectedness. Care managers conducted in-person and tele-health assessments and developed individualized treatment plans to provide patients with a medical home and access to appropriate clinical and non-clinical services.

Descriptive statistics were used to quantitatively describe study outcomes.

Results: The sample was primarily African American males (age range 43-72 years) who were single and were residing in DC. Of the 38 participants that completed the initial GPRA assessment, 79% did not have an overdose incident, and 84% (n = 32) reported quality of life from "very good" to "good." Care coordination services and harm reduction strategies were provided for clients receiving the BUP modality of MAT. During the COVID-19 pandemic, patients reported severe depression, anxiety, tension, trouble understanding, concentrating, and remembering, little to no pleasure in doing things, and feeling down. Patients with less social connectedness required more frequent contact and resources. Most participants required assistance to access community resources available through pandemic relief programs.

Conclusions: Providing wraparound service support during the COVID-19 pandemic for patients with opioid dependence improved clinical outcomes, reduced chronic disease burden, and enhanced health-seeking behaviors. The study indicates the need for care coordination services to increase access to MAT and social determinants of health resources for individuals with opioid dependence to support long-term recovery.

Keywords: Care Coordination, Opioid Addiction, Buprenorphine, Quality of Life, Pandemic

Disclosure: Nothing to disclose.

P678. Reward System Alcohol Cue Reactivity Varies as a Function of Drink Preference in Alcohol Use Disorder

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Background: Alcohol use disorder (AUD) is a chronic relapsing disorder characterized by a recurring cycle of binge/intoxication, withdrawal/negative affect, and craving (anticipation/preoccupation). The craving stage of the cycle is marked by a strong desire or urge to consume alcohol. Alcohol craving is considered a robust antecedent of alcohol use and an important predictor of relapse. Visual alcohol-cue reactivity functional magnetic resonance imaging (fMRI) paradigms have been critical for elucidating the neurocircuitry underlying alcohol craving. These tasks involve systematically presenting participants images of alcohol beverages (including beer, wine, and liquor), non-alcohol beverages, and blurred visual images while recording their subjective and neural responses. Studies employing these tasks, however, have not considered that brain responses to various alcohol-containing beverage types may vary as a function of an individual's drinking patterns and preferences. The present study aimed to address this gap in the literature by testing whether the brain's reward system responds differently to visual cues associated with an individuals' most commonly consumed, or "preferred," alcohol beverage compared with visual cues associated with less commonly consumed, or "non-preferred," alcohol beverages in individuals with an AUD. We hypothesized that individuals with a current AUD would show greater subjective craving and reward system neural activation in response to their "preferred" relative to "non-preferred" alcohol beverage.

Methods: The current study is a secondary analysis of data pulled from two clinical pharmacotherapy studies conducted in the Addictions Laboratory at the UCLA (study 1: NCT03489850; study 2: NCT03594435). These studies both examined the effect of ibudilast on neural alcohol cue-reactivity in individuals with a DSM-5 current AUD using identical neuroimaging procedures. Participants ($N = 70$) completed a standard visual alcohol cue reactivity paradigm during fMRI and the timeline follow-back interview to assess recent alcohol use. We took a behavioral approach, using participant's alcohol use patterns to infer drink preference. Specifically, participants were determined to have a "preferred" alcohol beverage if: 1) any one alcohol beverage type accounted for more than 50% of total drinks consumed, and 2) this same beverage type was consumed at least 20% more than any other beverage type. Neuroimaging data preprocessing followed conventional procedures as implemented in FMRIB Software. To measure activation, parameter estimates within each reward system region of interest were extracted using FSL's Featquery tool and input to IBM SPSS Statistical Software (SPSS) for further analysis. Repeated measures ANCOVA was used to evaluate differences in subjective craving and neural cue-reactivity in response to visual cues of individual's "preferred" compared to "non-preferred" alcohol beverages. Medication condition (ibudilast vs. placebo), study (study 1 vs. study 2), and their interaction were included in the model as between-subjects categorical variables. Sex, age, cigarette smoking (smoker vs. nonsmoker) were examined as covariates.

Results: Fifty-four (77%) of participants were determined to have a "preferred" alcohol beverage. Participants with a "preferred" alcohol beverage reported greater subjective craving ($F = 5.7$, $p = 0.02$, partial eta squared = 0.12) in response to visual cues associated with their "preferred" relative to "non-preferred" alcohol beverage. Participants exhibited greater activation in the anterior cingulate cortex ($F = 18$, $p < 0.001$, partial eta squared = 0.26), frontal medial cortex ($F = 5.7$, $p = 0.02$, partial eta squared = 0.1), and left nucleus accumbens ($F = 4.5$, $p = 0.04$, partial eta squared = 0.08) in response to visual cues associated with their "preferred" relative to "non-preferred" alcohol beverage. There were no interactions with medication or study.

Conclusions: This study represents a critical first step in examining whether subjective and neural cue-induced craving responses in the laboratory vary as a function of alcohol beverage preference in individuals with a current AUD. Results provide evidence for differences in how the brain's reward system—particularly the anterior cingulate cortex, frontal medial cortex, and nucleus accumbens—respond to distinct types of alcohol beverage cues. Specifically, we found these brain regions are preferentially activated by visual cues associated with an individual's "preferred", as compared to "non-preferred" alcohol beverages. Alcohol-cue reactivity fMRI paradigms have been critical in elucidating the neural correlates of craving in AUD. Results from this study extend the literature by demonstrating that subjective and neural craving responses in the laboratory vary as a function of alcohol beverage preference in AUD. In addition, these findings suggest that the strong brain activation previously observed in response to an individual's "preferred" alcohol beverage may be "averaged out" when combined with activation in response to "non-preferred" alcohol beverages. While prior work has shown the fMRI cue-reactivity task has a strong signal as a whole, it is possible that the signal would be even stronger should "preferred" alcohol beverages be used as the active stimuli in the task. Future studies specifically designed to test this hypothesis are warranted.

Keywords: Functional MRI (fMRI), Alcohol Use Disorder, Cue-Induced Craving, Anterior Cingulate Cortex (ACC), Nucleus Accumbens

Disclosure: Nothing to disclose.

P679. The Eyes Have It: Alcohol-Induced Impairment in Saccadic and Smooth Pursuit Eye Movements and Perception of Impairment in Older Adults With and Without Alcohol Use Disorder

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Background: Alcohol induces widespread impairment to visual processes that contributes to accidents and injury as well as premature mortality. The theory of behavioral and physiological tolerance purports that experienced drinkers with alcohol use disorder (AUD) exhibit lower impairment after alcohol consumption than their novice light drinkers (LD) counterparts. To our knowledge, there are no well-controlled studies examining acute alcohol-induced impairment in eye tracking in persons with AUD, particularly those who are middle-aged and older. This has limited our understanding of the nature and extent of behavioral tolerance in persons with chronic AUD. Our prior work in young adult non-AUD drinkers, we found that heavy social drinkers (vs. light drinkers) showed less alcohol impairment to saccadic eye movement tasks (rapid ballistic movement to a target) but not smooth pursuit (horizontal eye tracking to a consistent frequency moving target). The goal of the present study was to extend that work to older drinkers and those with AUD to more fully examine eye movement tracking and perception of impairment before and after consumption of an intoxicating alcohol dose vs. placebo beverage.

Methods: A within-subject, double-blind laboratory study was conducted in $N = 89$ participants (aged 40-63 years) including drinkers with DSM-5 alcohol use disorder (AUD; $n = 45$) and light social drinker controls (LD; $n = 44$). All were tested in two random-order, oral beverage sessions with administration of alcohol (0.8 g/kg) or placebo (1% alcohol taste mask) consumed over a 15 minute period. Women received an 85% dose modification due to sex differences in body water. Eye movements were recorded using an Eyelink 2000 Eyetracker at baseline (T0) and then repeated at 60 (T1) and 180 (T2) minutes after beverage consumption. Eye movement tasks were: 1) Pro-Saccade: promptly moving eye focus from the center fixation point to a series of 14 successive targets appearing randomly within a 15° horizontal angle from the center; 2). Anti-Saccade: targets similar to the pro-saccade task, but with the goals of promptly moving eye focus to the opposite side of the target equidistant from the center; and 3). Smooth pursuit: tracking a target stimulus moving sinusoidally (at 0.1, 0.2 or 0.3 Hz for 4 cycles each, in total 73.3 seconds) along 15° visual angle to the right and left of midline horizontally.

Lab-developed software in Matlab was used to extract gain for smooth pursuit, and latency and velocity for pro-saccade or anti-saccade tasks. Data were first cleaned by excluding bad trials. The exclusion criteria included peak velocity $>= 1000$°/sec or latency > 1 sec for pro-saccade (9.8% bad trials) or anti-saccade (7.9% bad trials), or gain < 50% or latency > 1 sec for smooth pursuit (10.5% bad trials). Generalized Estimation Equation models were used to analyze the effects of group (AUD vs LD), dose (alcohol vs. placebo) and time (baseline, T1, T2). Demographic variables related to the primary outcomes were included as covariates, i.e., age and education for pro-saccade/anti-saccade, and age, sex and education for smooth pursuit. At two intervals (30 minutes and T2), subjects rated their perceived impairment, from not at all (0) to extremely (10), for three items: impaired coordination/thinking, impaired ability to operate a car, and noticeability of intoxication. An average of these ratings were used in analyses.

Results: For both AUD and LD, alcohol significantly impaired the latency and velocity of pro-saccade and anti-saccade eye movements that was more pronounced during the peak BrAC than declining limb [Dose x Time, ps < 0.05, Latency: T2 > T4 > T0 for pro-saccade, T2 > (T4 = T0) for anti-saccade; Velocity: T2 < T4 < T0 for pro-saccade, (T2 = T4) < T0 for anti-saccade]. In contrast, alcohol-induced impairment for average smooth pursuit gain was lower in AUD relative to LD (Group x Dose x Time, $p = 0.03$). Additional tests examined each frequency and showed that AUD were less impaired by alcohol than LD at the slower 0.1 Hz frequency (Group x Dose x Time, $p = .03$; AUD > LD at T2 in alcohol session but not at other time points) with a trend for less impairment at the middle 0.2 Hz frequency ($p = .06$). However, AUD were similarly impaired as LD at the faster 0.3 Hz frequency ($p = 0.18$). Finally, AUD reported lower alcohol perceived impairment than LD at both time points (Dose x Group, $p < 0.01$).

Conclusions: While tolerance is a well-known concept in addiction, it is not evident across all effects of alcohol and this study examining eye tracking adds to our body of work showing that AUD drinkers do not exhibit global behavioral tolerance to an intoxicating dose of alcohol despite the fact that the perceive being less impaired than light drinkers. Results indicated that older persons with chronic AUD show less alcohol-induced impairment on smooth pursuit eye movements, but only at the slower and not faster target frequencies and they also do not show tolerance to pro- or anti-saccadic eye movements in terms of latency and velocity. These findings have implications for the neurobiological processes and adaptations in persons with long-term AUD and in terms of the consequences of chronic excessive drinking as it relates potentially to public safety and harm.

Keywords: Alcohol Use Disorder, Tolerance, Eye Tracking

Disclosure: Nothing to disclose.

P680. Physiological, Subjective, and Cognitive Effects From a Typical Morning "Dose" of Kratom Among Adults Who Use Regularly: Findings From a Direct-Observation Pilot Study

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Background: Use of kratom has increased considerably in the United States since 2010, with consumers now numbering in the millions. Other than three small pharmacokinetic studies using kratom tea, data on kratom effects in humans consist of case reports and cross-sectional surveys. Kratom leaves contain dozens of bioactive alkaloids, of which at least 12 have opioidergic, serotonergic, adenosinergic, and/or adrenergic activity. At least four of these (mitragynine, 7-hydroxymitragynine, speciociliatine, speciogynine) have partial agonism at mu opioid receptors. As most people who use kratom do so regularly (oftentimes daily), there is direct public health relevance in assessing kratom use in a laboratory setting with daily consumers. To our knowledge, there has been no direct observation of the effects of kratom product self-administration. We assessed clinically relevant subjective, physiological, and cognitive endpoints after participants' self-administration of their usual kratom product at their typical dose.

Methods: Between July-November 2022, 10 adults who regularly used kratom completed a direct-observation pilot study. Before their session, participants were asked to refrain from using kratom until completing (pre-dosing) baseline assessments. Participants then consumed their typical morning kratom dose under observation and were assessed across 4-5 subsequent timepoints. Endpoints included the Subjective Opioid Withdrawal Scale (SOWS), Addiction Research Center Inventory (ARCI), Drug Effects Questionnaire (DEQ), systolic and diastolic blood pressure (SBP, DBP), respiratory rate (RR), heart rate (HR), pulse oximetry (SpO2), temperature, pupil diameter (mm), psychomotor performance on computerized tasks, and driving performance using a validated simulator with a steering wheel and gas/brake pedals. Samples of kratom products, urine, and blood were collected. R (v 4.2.2) was

used to fit generalized linear mixed-effect models examining changes from baseline. Assessment timepoints were binned: Baseline (pre-dose), 0-40min post-dose (mean = 21.7 ± 13.8 min), 40-80min post-dose (mean = 59.3 ± 16.6 min), 80-120min post-dose (mean = 110.2 ± 7.9 min), 120-160min post-dose (mean = 145.9 ± 10.9 min), >160min post-dose (mean = 186.7 ± 15.0 min). MassLynx 4.2 was used for acquisition and processing of assay data. Validated processes for identifying and simultaneously qualifying alkaloids in product, urine, and blood used a Waters Acquity Class-I ultra-performance liquid chromatography coupled with a Xevo TQ-S Micro triple quadrupole mass spectrometer.

Results: Participants were 6 men and 4 women, mean age 41.2 (SD 10.3) years. Nine were White; 1 was biracial. Participants had used kratom for 6.6 (SD 3.8) years on average (range: 2.0-14.1). Past-year DSM-5 criteria for substance use disorder for kratom were met by 4 (2 mild, 1 moderate, 1 severe). Doses consisted of whole-leaf powder taken orally. Mean dose was 5.16 grams (range: 1.1-10.9). Baseline SOWS scores were mild (5.5+/-3.3) and decreased following kratom at all timepoints (b = [-4.0, −2.9], p < .01), and were lowest 80-120 minutes post-dose (b = −4.0, p < .01). DEQ "feeling drug effects" VAS ratings were 40 ± 30.5 at 40 minutes post-dose and higher at 40-80 minutes (b = 32.7, p < .01) and 80-120 minutes (b = 19.0, $p = .04$). "Liking drug effects" mean ratings were 63.4 ± 13.0 at 40 minutes post-dose. Liking increased at 40-80 minutes post-dose (b = 14.4, $p = .02$). "Wanting more kratom" ratings marginally increased at 120-160 minutes (b = 13.7, $p = .06$); by > 160 minutes, they had significantly increased (b = 26.6, p < .01). Mean ratings for "feeling high" was 15.2 ± 23.6 at 40 minutes post-dose and ratings for "feeling intoxicated" was 4.8 ± 9.8 at 40 minutes post-dose; both were constant at subsequent timepoints. Six participants showed small increases on the Morphine-Benzedrine Group (euphoria) ARCI subscale.

Relative to baseline, SBP increased within 40-80 minutes post-dose (b = 5.3, $p = .07$) and decreased within 160 minutes post-dose (b = −8.0, $p = .01$). DBP decreased post-dose for all timepoints > 160 minutes. Pupil diameter decreased (right, b = −.70, p < .01; left, b = −.73, p < .01) within 40-80 minutes post-dose and remained below baseline > 160 minutes. Participants with higher doses had greater pupil constriction > 160 minutes post-dose. Changes in HR, SpO2, RR, or temperature were nonsignificant. At the group level, there were no significant pre-post differences on psychomotor tasks or driving performance. No artificially elevated levels of 7-hydroxymitragynine were found in analyzed products, and alkaloidal concentrations were similar to those of products tested in the literature.

Conclusions: Our pilot findings represent the most sensitive assessments to date of effects of commercial kratom products in humans. Among regular consumers, we found few statistically or qualitatively significant differences pre- and post-kratom self-administration at low to moderate doses, and no intoxication or adverse effects. Indicators found here require systematic study with larger samples over longer periods.

Keywords: Kratom, Opioids, Behavioral Pharmacology, Psychostimulants

Disclosure: Nothing to disclose.

P681. IL17RB Genetic Variants as Biomarkers of Acamprosate Treatment Response in Patients With Alcohol Use Disorder: A Multi-Omics Study

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Background: Acamprosate is an FDA approved medication for the treatment of alcohol use disorder (AUD), however, only about 50% of patients will respond to acamprosate. Previously, the Mayo Clinic Center for Individualized Treatment of Alcohol Dependence study recruited 442 AUD patients who were treated with acamprosate for three months. This clinical trial was not designed to determine the efficacy of acamprosate, but rather to gather multiple omics data for analysis and to identify biomarkers associated with acamprosate treatment outcomes. At present, there are no biological measures utilized to predict the response to acamprosate treatment. The present study was designed to identify potential plasma markers associated with acamprosate treatment response. Specifically, we first set out to identify potential plasma markers that were associated with acamprosate treatment outcomes. Next, we applied a "proteomics-informed genome-wide association study (GWAS)" research strategy, in which we performed GWAS for the concentrations of proteins associated with acamprosate treatment outcomes. We then set out to determine whether SNPs identified in the course of our proteomics-informed GWAS might be associated with acamprosate treatment outcomes. This series of studies could represent an important step toward the generation of functional hypotheses that could be tested to gain insight into the molecular mechanisms underlying acamprosate treatment response phenotypes.

Methods: The Mayo Clinic Center for Individualized Treatment of Alcohol Dependence study is an acamprosate clinical trial that recruited 442 patients with AUD, all of whom were treated with acamprosate for three months. The primary outcomes were 1) relapse to alcohol use and 2) relapse to heavy drinking. Relapse was defined as return to alcohol use during the three months of acamprosate treatment, and non-relapse as abstinence from alcohol (no alcohol use) during the three months of acamprosate treatment. Heavy drinking was defined as having four drinks a day for women and five drinks a day for men. Baseline plasma samples were assayed using the OLINK "Explore Inflammation" panel. Differentially expressed proteins were identified using the Limma package in R. Age, sex, and number of sober days prior to enrollment were considered covariates in the model.

Results: We found that the protein concentrations of IL17RB, ENAH, ADGRE2, and CSF3, in the baseline plasma samples were associated with both relapse to alcohol use and relapse to heavy drinking during the three months of acamprosate treatment (p ≤ 0.05). Next, we performed GWAS using baseline proteomics profiles as quantitative biological traits to identify genetic variants associated with variations in concentrations of those proteins that were associated with acamprosate treatment response. Specifically, among the proteins that were associated with acamprosate treatment outcomes, we observed 85 signals that were genome-wide significant SNPs (p ≤ 5E-08) among the GWAS for the concentrations of IL17RB, ENAH, and ADGRE2. We should emphasize once again that the levels of these proteins were associated with acamprosate treatment outcomes in patients with AUD. As a result, we hypothesized that these 85 SNPs identified in the proteomics-informed GWAS may be associated with acamprosate treatment response.

We have recently reported that genetic variations may contribute to individual differences in AUD treatment response by performing a GWAS meta-analysis based on data from three of the largest studies of acamprosate and naltrexone completed to date, including COMBINE, PREDICT and the Mayo Clinic Center for Individualized Treatment of Alcohol Dependence study. A total of 652 AUD patients were treated with acamprosate. That study set the stage for the present study designed to determine the association with acamprosate treatment response and the

genome-wide significant SNP loci from the proteomics-informed GWAS. Strikingly, we found that 25 SNPs were associated with the time until relapse to alcohol use and the time until relapse to heavy drinking during three months of acamprosate treatment. Even more striking, all 25 SNPs were from the GWAS for IL17RB concentrations. Remarkably, the variant genotype for all 25 genome-wide significant SNPs in the IL17RB GWAS was protective against alcohol relapse. Specifically, we identified a series of genome-wide significant signals in the GWAS for IL17RB, with the lowest p value of 4.8E−20 for the rs6801605 SNP on chromosome 3. The minor allele frequency (allele A) for the rs6801605 SNP was 38% in the European American population. The rs6801605 SNP maps 4 Kb upstream of IL17RB, and intron 1 of the CHDH gene, and this SNP was associated with a decreased risk of relapse to alcohol use ($p = 0.01$) and heavy drinking ($p = 0.005$).

Conclusions: This series of studies demonstrates that IL17RB genetic variants might contribute to acamprosate outcomes. Our results revealed that the application of multi-omics approaches may be a feasible strategy for identifying biomarkers that could potentially aid in predicting acamprosate treatment response.

Keywords: Biomarker, Acamprosate, Alcohol Use Disorder - Treatment, Multi-Omics, Pharmacogenetic Response

Disclosure: Nothing to disclose.

P682. Cannabis-Impaired Driving: Insights From a Randomized, Placebo-Controlled, Double-Blind Driving Simulator Study

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Background: Driving impairment is a major concern in the United States, particularly under the influence of prescribed and recreational drugs. With the legalization of medical and/or recreational cannabis in ~75% of U.S. states, it is expected that the number of drivers intoxicated from recent cannabis use will continue to increase significantly. However, quantifying cannabis-related driving risk from epidemiological accident statistics is challenging due to varying research methodologies and assumptions. Different studies have provided a wide range of risk estimates for being involved in a cannabis-associated motor vehicle crash or driving-related injury, further complicating the issue. Thus, there is a need for a more direct and informative approach to understanding the effects of cannabis on driving safety.

Methods: To address the gaps in understanding cannabis-related driving risk, we utilized a randomized, placebo-controlled, counterbalanced, double-blind, within-subject design. The study included 38 adult participants (27 Males) from the Greater Hartford CT area, psychiatrically healthy (SCID), aged 18-40 years, with at least 2 years of recent highway driving experience and a current driver's license. 34 of 38 participants were regular cannabis users, with a history of cannabis use at least once a week for the last 3 months, except for occasional abstinence. The study design involved $N = 3$ separate, full-day appointments for each participant. They were administered a single 0.5 gm acute dose of vaporized cannabis (either 5.9% or 13% THC) or placebo in a randomized, counterbalanced manner using a computer-paced inhalation protocol, to ensure standardized route and timing of intake. Throughout each 8-hour assessment day, at 4 different time points, participants underwent simulated driving tests, including lane-keeping, car following, and overtaking tasks to capture $N = 19$ different behavioral metrics related to changes in driving behavior, theoretically linked to drugged driving. A linear mixed model in SPSS was employed to assess main effects of dose, time and dose*time adjusted for sex, time since dose, usage group and age for the above driving metrics.

Significance level was set at $p < 0.05$ adjusted using the least squares difference procedure.

Results: Analysis of blood THC metabolites revealed that primary delta-9-THC levels were significantly increased until ~22 minutes after cannabis inhalation. After this time, differences in THC levels vs placebo became non-significant. Secondary delta-9-carboxy-THC levels were significantly higher until ~83 minutes post-dose.

We identified 6 specific impaired driving outcomes that were significantly affected by cannabis use across the 3 simulated driving tasks.

Lane Keeping Task: During the lane-keeping task, participants exhibited reduced steering reversal rates (SRR) after using cannabis. This impairment in vehicle control persisted for a substantial duration, up to 5.5 hours following the 13% THC dose of cannabis and 3.5 hours following the 5.9% dose.

Car Following Task: In the car following task, participants again showed significant reductions in pedal engagement and reversal rates, indicating impaired responsiveness to changes in driving conditions. Similar to the lane-keeping task, these impairments persisted for 1-3 hours after cannabis use (only at 13% THC).

Overtaking Task: During overtaking, intoxicated drivers demonstrated a shorter median gap to cars they passed, resulting in less time estimated to a potential collision. They also spent more time in the oncoming traffic lane while passing, an effect that persisted longer in the 13% dose condition. All of these measurements improved slowly and to varying degrees as time progressed.

Interestingly, self-reported assessments indicated that approximately 66% of participants were willing to drive despite being subjectively aware that they were impaired. Objective measurements revealed significant impairments in driving performance during these periods, highlighting the potential risks associated with individuals underestimating their impairment.

Blood THC and metabolite levels were uncorrelated with objective impairment measures.

Conclusions: This study provides valuable insights into the effects of cannabis on driving behavior. The findings highlight the complex nature of cannabis-related impairments, affecting both automatic and goal-directed driving behaviors. Future research should explore the interaction between drug effects and drivers' risk awareness, to inform public health policies and legal standards for cannabis-related driving safety. Understanding how cannabis affects specific driving behaviors will be crucial in mitigating potential risks and ensuring road safety in the context of increasing cannabis use.

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Keywords: THC, Simulated Driving, Intoxication, Subjective Cognitive Impairment

Disclosure: Nothing to disclose.

P683. Sex Differences in the Dose-Dependent Analgesic and Reinforcing Effects of Smoked Cannabis: A Double-Blind, Placebo-Controlled Study

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Background: Cannabis use is increasing among women for both non-medical and medical purposes with pain cited as one of the primary reasons for medical use. Preclinical studies point to heightened sensitivity in females compared to males in both the antinociceptive and reinforcing effects of delta-9-tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis. Sex hormones—particularly estradiol—are proposed as a potential driver of these differences. The current double-blind, placebo-controlled study in healthy male and female volunteers extends preclinical findings to investigate sex differences in cannabis's dose-dependent reinforcing and associated subjective drug effects, and analgesia while controlling for female hormone levels.

Methods: Healthy participants (21-55 years of age) who use cannabis 1-7 days per week were recruited for this placebo-controlled, within-subject study. Over three outpatient sessions, volunteers smoked 560 mg of cannabis with 0% THC (0 mg THC), 4% THC (~20 mg THC), and 10% THC (~60 mg THC); one cannabis dose was administered per session in a randomized order. Female participants were regularly cycling, not on hormonal contraceptives, and completed study sessions during their mid-follicular phase (corresponding with rising estradiol and low progesterone). Subjective mood- and drug-related effects were assessed with the Mood and Physical Symptoms Visual Analog Scale (MPS-VAS) and the Smoked Cannabis Rating Form (SC-RF), respectively. Analgesia was assessed by measuring pain threshold and tolerance with the Cold Pressor Test (CPT), an experimental pain test with predictive validity for therapeutics used to treat chronic pain. Study measures were collected before and several time points after drug administration. Cannabis's reinforcing effects were measured using a self-administration task. For this task, participants chose to self-administer 0-3 puffs (cost: $1/puff) of the cannabis strength that was smoked earlier in the session. Generalized linear mixed effects models were used to test dose-, sex-, and time-dependent effects.

Results: Healthy male ($n = 32$) and female ($n = 17$) volunteers who used cannabis (males: 4.2 days/week; females: 4.7 days/week) have completed the study. Preliminary analyses revealed that active cannabis significantly increased subjective ratings of intoxication and drug effects associated with abuse liability, such as drug liking, relative to placebo ($p < 0.001$); no differences were observed between the two active doses of cannabis or between males and females. Males dose-dependently self-administered cannabis: 15% self-administered 0% THC, 28% self-administered 4% THC, and 41% self-administered 10% THC. A similar dose-dependent effect was not observed among females; only the 10% THC was administered more frequently (35%) than 0% or 4% THC (both 24%). However, the main effect of sex was not significant, nor was a sex-by-dose interaction. With respect to analgesia, both active cannabis doses increased pain threshold and tolerance in the CPT ($p < 0.05$). Although males appeared to exhibit greater pain threshold under active doses relative to females, there was no dose-by-sex interaction. For pain tolerance, a significant dose-by-sex interaction ($p < 0.001$) revealed that only females exhibited significant increases in pain tolerance under active doses ($p < 0.001$).

Conclusions: This ongoing study builds on preclinical studies pointing to heightened sensitivity to the misuse-related and analgesic effects of THC among females. Early findings suggest that when controlling for circulating levels of estradiol in female participants, they are equally sensitive to cannabis's intoxicating and misuse-related subjective drug effects and may be less sensitive to the reinforcing effects of cannabis relative to male participants. However, females appear to be more sensitive to some analgesic effects of cannabis relative to males. These data represent the first 40% of planned study observations and provide an early signal into possible sex differences in the acute therapeutic and adverse effects of cannabis, setting the stage for future analyses incorporating pharmacokinetics, hormonal, and tolerance effects.

Keywords: Cannabinoids, Cannabis, Cannabis Use Disorder, Analgesia, Sex Differences

Disclosure: Canopy Growth Corporation, True Terpenes: Other Financial or Material Support (Self).

P684. Alcohol Use Prior to Cannabis Use Elevates Peak Heart Rate: Preliminary Data From a Laboratory Study of Co-Administered Alcohol and Legal-Market Cannabis

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Background: Individuals who drink alcohol commonly report co-using cannabis, and the increasing availability of new formulations of highly potent cannabis products on the legal market means that alcohol is likely being combined with cannabis products that contain high levels of delta-9-tetrahydrocannabinol (THC). There is limited existing research on the acute effects of cannabis products containing high amounts of THC, such as cannabis concentrates, which often contain up to 90% THC. Existing laboratory data suggests that consuming alcohol and cannabis together (compared to alcohol alone) is associated with reduced BAC, delayed time to peak BAC, longer duration of intoxication, and increased subjective intoxication, but it is unknown how cannabis concentrates may interact with alcohol to influence these outcomes. There is also a lack of research regarding whether timing or order of use matters, though recent survey data from our group and others suggests that using alcohol before cannabis may confer additional risk compared to using cannabis prior to alcohol. Biological/assigned sex is another understudied variable that may influence the effects of co-administered alcohol and cannabis. The present study leveraged a federally-compliant mobile laboratory design to explore the acute effects of self-administered cannabis concentrates alongside a standardized dose of alcohol. Here we present preliminary data from $N = 29$ (55.2% female) individuals who completed this protocol. Approximately half ($n = 13$; 46.2% female) of these participants consumed alcohol before self-administering their preferred cannabis concentrate, and the other half ($n = 16$; 62.5% female) used their cannabis concentrate product prior to ingesting the standard dose of alcohol. We hypothesized that those who used alcohol first would reach a higher peak BAC and heart rate (HR), report higher levels of intoxication, and experience more craving compared to those who used cannabis first.

Methods: Twenty-nine (55.2% female) heavy drinking community members (MAge = 31.24 years; SD = 9.21; range = 21-56 years) who also regularly use cannabis products were recruited to participate in this study. The sample reported scores on the Alcohol Use Disorder Identification Test (AUDIT) suggestive of harmful use (MAUDIT = 13.43; SD = 7.01) as well as low-moderate cannabis dependence (MMDS = 3.48; SD = 2.75). The mobile laboratory visited participants' residences on their scheduled appointment day. Participants were randomly assigned to either consume a standardized dose of alcohol designed to bring their blood alcohol concentration (BAC) to approximately .08 g/dL (using the Widmark formula and inputting participant weight and sex) and then to enter their residence and use a cannabis concentrate product of their choice, or to first use their chosen cannabis concentrate product inside their residence and then consume the standardized alcohol dose in our mobile laboratory. Following alcohol and cannabis administration, participants remained in our laboratory for 4-hours. They completed a

breathalyzer and several measures of alcohol and cannabis intoxication every 30 minutes for the duration of the session. Differences in peak levels of BAC, HR, alcohol craving, as well as the stimulating and sedative subjective effects of alcohol across the two order conditions (alcohol before cannabis [AC] or cannabis before alcohol [CA]) and differences between males and females were explored using a two-way ANCOVA with participant age and percent THC in the cannabis product that the participant used during the session as covariates.

Results: In this preliminary analysis, a significantly greater peak HR was reached by individuals in the AC condition ($M = 110.92$; $SD = 18.13$) compared to individuals in the CA condition ($M = 97.56$; $SD = 15.00$), $F1,11 = 7.474$, $p = .0194$. A significant interaction was also observed between age and percent THC of the product used predicting peak alcohol stimulatory effects, $F1,11 = 6.333$, $p = .0258$. Probing this interaction revealed that among older individuals, there was a positive relationship between percent THC and peak alcohol stimulatory effects, while for younger individuals, a negative relationship was observed between percent THC and peak alcohol stimulatory effects. No other significant main effects or interactions emerged in these models.

Conclusions: Results from this preliminary study indicate that using alcohol before cannabis is associated with higher peak heart rate compared to using cannabis before alcohol. Notably, on average, the AC group exceeded 100bpm (a commonly accepted cutoff for tachycardia), while the mean HR for those in the CA group did not exceed this cutoff. This finding, if replicated, could offer a practical and straightforward harm reduction option for individuals who co-use alcohol and cannabis (i.e., avoid using alcohol before cannabis during co-use sessions to reduce the impact of co-use on HR elevation). We also observed an interaction between age and THC percentage predicting alcohol-related stimulation, such that THC appears to increase alcohol-related stimulation in older individuals and decrease it in younger individuals. This result underscores the importance of considering developmental differences in studies of alcohol and cannabis co-use. Data collection is ongoing, and analyses will be repeated with the full sample upon completion of data collection to confirm and extend the present findings.

Keywords: Alcohol, Cannabis Concentrates, Cannabis

Disclosure: Nothing to disclose.

P685. The Influence of Sex and Childhood Trauma on the Effects of Delta-9-Tetrahydrocannabinol in Persons With Opioid Use Disorder: Insights From a Randomized, Placebo-Controlled, Crossover Human Laboratory Study

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Background: Amid the opioid crisis, numerous U.S. states have authorized the medicinal use of cannabinoids, with some states allowing the substitution of opioids with cannabinoids to treat pain and opioid use disorder (OUD). Nevertheless, existing policies contrast with the dearth of clinical trials investigating the effects of cannabinoids among persons with OUD. Prior animal studies indicate that cannabinoid-induced antinociception could be influenced by factors such as sex and childhood trauma. It remains unknown, however, whether these findings extend to humans with OUD. We sought to investigate the influence of sex and childhood trauma on the acute effects of delta-9-tetrahydrocannabinol (THC), the main analgesic and psychoactive constituent of cannabis, among persons receiving methadone therapy for OUD.

Methods: We conducted a secondary analysis of a within-subject, crossover-design human laboratory study. Twenty-five persons with OUD, who were receiving methadone therapy were randomly assigned to receive single doses of oral THC (10 mg or 20 mg), administered as dronabinol; or placebo, across three five-hour test sessions. At baseline, participants completed the Childhood Trauma Questionnaire (CTQ), which categorizes childhood trauma into several types pertinent to our study: total trauma, emotional neglect, physical neglect, emotional abuse, and physical abuse. Before each test session, a urine laboratory immunoassay confirmed abstinence from non-medical substance use. Pain sensitivity in response to THC administration was measured by the Cold Pressor Test (CPT) at 4 °C, and by the McGill Pain Questionnaire (MPQ). The abuse potential of THC was measured by the Drug Effects Questionnaire (DEQ). Cognitive performance was indexed by the Hopkins Verbal Learning Test (HVLT). We used mixed-effects models to examine the main effects of sex, childhood trauma, and THC dose, in addition to interactions between sex, childhood trauma, and THC dose (10 mg, 20 mg). At a significance level of 0.05, this exploratory study had 80% power to detect medium effect sizes ($d \geq 0.5$).

Results: Approximately 24% of participants were women and 76% were men. The average age and methadone dose were 47.3 ± 12.2 years old and 96.6 ± 35.1 mg/day, respectively. Preliminary evidence of a sex-by-THC dose interaction was observed for pain sensitivity, indexed by the MPQ ($F(2,38) = 2.76$, $p = 0.07$); women experienced higher pain relief at lower THC doses (10 mg) ($t(5,38) = -2.43$, $p = .001$), while men required a higher dose (20 mg) ($t(3,38) = -2.42$, $p = .002$). The impact of THC on cognitive performance was also sex-specific, as verbal learning deficits occurred in women regardless of THC dose ($F(1,21) = 6.06$, $p = 0.02$). Notably, there was no evidence of sex differences for THC's abuse potential. Additionally, individuals with higher childhood trauma severity exhibited a blunted response to THC's acute aversive effects ($F(2, 36) = 5.91$, $p = 0.006$). However, childhood trauma did not influence pain or cognitive performance outcomes.

Conclusions: The findings shed light on the potential differential effects of THC based on sex and childhood trauma in persons with OUD. They underscore that lower THC doses might alleviate pain in women, albeit at the risk of more pronounced verbal learning deficits. Interestingly, THC's abuse potential remained unaffected by sex. The observation of a blunted response to THC's aversive effects associated with childhood trauma also warrants further exploration, as fewer aversive effects may increase the risk of non-medical cannabinoid use. These results can guide future investigations into the role of sex and childhood trauma in modulating cannabinoids' potential opioid-sparing effects among individuals undergoing opioid agonist therapy for OUD. Furthermore, they raise questions about the risk/benefit ratio of cannabinoids in this population and their potential to mitigate opioid-related harm. Understanding individual variability in the cannabinoid response can be instrumental in formulating effective strategies to reduce opioid overdose deaths.

Keywords: Opioid-Sparing Effects, Opioid Addiction, Cannabinoids, Methadone, Pain

Disclosure: Nothing to disclose.

P686. Ketone Supplementation Reduces Subjective and Objective Responses to an Alcohol Challenge in Humans and Rats

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Background: Previous preclinical and clinical studies have shown the efficacy of a high-fat ketogenic diet and beta-hydroxybutyrate ketone supplementation in curbing alcohol craving, alcohol consumption, and signs and symptoms of alcohol withdrawal. We previously reported that a 6-week high-fat ketogenic diet compared to a regular chow diet leads to significantly higher blood alcohol levels in response to an alcohol challenge. Here, we tested the effects of a single dose ketone supplement on subjective and objective responses to an alcohol challenge in healthy, non-dependent humans and rats.

Methods: In this single-blinded, cross-over study, healthy participants ($n = 10$, 3 female) were administered a single, oral dose of the ketone supplement Kenetik (Vitanav, Inc, which contains 25 g of ketones from D-beta-hydroxybutyrate acid and R-1,3 butanediol, and the sweetener allulose) or a placebo 30 minutes prior to an oral dose of alcohol that was weight- and sex-adjusted to achieve a breath alcohol concentration of 0.05%. Assessments of breath and blood alcohol levels and the Drug Effect Questionnaire were repeatedly administered for 180 minutes after alcohol consumption. In a parallel preclinical study, $n = 4$ male Wistar rats received oral gavages of Kenetik (0.42 g ketones/kg), allulose (0.58 g/kg, Wholesome Sweeteners Inc., Sugar Land, TX), and water on three different study days, followed by the oral consumption of alcohol (8g/kg), and blood alcohol levels were measured for 240 minutes after alcohol exposure.

Results: In humans, the intake of Kenetik prior to alcohol significantly blunted breath ($F1,78.0 = 27.6$, $p < 0.001$) and blood alcohol responses ($F1,37.3 = 27.9$, $p < 0.001$). While subjective ratings of alcohol intoxication did not differ between interventions, participants reported lower liking of alcohol ($F1,53.4 = 5.7$, $p = 0.02$), and disliked alcohol more with Kenetik than placebo ($F1,58.8 = 8.8$, $p = 0.004$). In rats, Kenetik similarly demonstrated lower blood alcohol levels ($F3,42 = 45.1$, $p < 0.001$) compared to both allulose and water. There were no detectable differences between water and allulose on blood alcohol levels.

Conclusions: Our study indicates that ketone supplementation reduces the intoxicating and rewarding effects of alcohol, which may have beneficial implications for novel treatment of alcohol use disorder.

Keywords: Ketones, Alcohol Sensitivity, Pharmacokinetic and Pharmacodynamic

Disclosure: Nothing to disclose.

P687. Increased Dopamine Level Enhance Perception by Augmenting the Segregation Within Somatomotor Network

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Background: Stimulant medications, such as methylphenidate (MP), which are effective treatments for attention deficit hyperactivity disorder (ADHD), enhance brain dopamine signaling. However, the precise mechanisms of how MP boosts cognitive efforts in healthy populations remain debated. Establishing the connection between dopamine levels and functional brain activity is essential for elucidating the mechanisms.

Methods: 37 healthy adults, aged 22-64 (13 females) participated in a single-blind, randomized, placebo-controlled crossover study involving two sessions with oral administration of either 60mg MP or placebo, in a counterbalanced design. Two [11C] raclopride PET scans were done one after 60 minutes of oral placebo for measuring baseline D2R availability, and the other after 60 min of oral MP for measuring dopamine release. Following each PET scan, 8 min-long eye-open resting-state fMRI scans were collected. Graph, dynamic, and gradient analyses were first conducted on whole-brain functional MRI for quantifying the global efficiency, network segregation and integration, and dynamic characteristics between placebo and MP administration. Subsequently, the correlation between changes in striatal dopamine levels and functional network features was quantified.

Results: In comparison to placebo, after MP administration, graph analysis results revealed a significant decrease in global efficiency of whole-brain connectivity (2-sample t-test, $p < 0.01$), and a significant increase in modularity within somatomotor (SM) network (2-sample t-test, $p < 0.01$). These changes may underlie the enhanced perception function but weakened global function after MP administration. Furthermore, dynamic and gradient analysis results showed the brain activity was more likely to dwell in SM-dominated state, characterized by greater segregation within SM network compared to other meta-states. Regression analyses further showed that the increment of dopamine level (around 10%) was significantly correlated with increased fractional occupancy ($r = 0.41$, $p = 0.01$) and dwell time ($r = 0.37$, $p = 0.03$) in SM-dominated states.

Conclusions: Our findings demonstrate that increased dopamine levels enhance perception by augmenting the segregation within SM network. However, the decline in global efficiency suggests that MP may not benefit the performance in tasks that demand high cognitive function.

Keywords: Methylphenidate, Dynamic Functional Connectivity, Connectivity Gradients, Dopamine

Disclosure: Nothing to disclose.

P688. Chronic Pain Intensity is Associated With Increased Cue-Induced Alcohol Craving After a Practice Quit Attempt Among People With Alcohol Use Disorder

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Background: Chronic pain and alcohol use have a well-known bidirectional relationship; however, the influence of chronic pain on relevant alcohol-related endpoints in the human laboratory is not often considered. Our laboratory has previously shown that higher levels of self-reported chronic pain intensity is associated with tonic (unprovoked) alcohol craving and alcohol consumption. It is currently unknown whether chronic pain intensity is associated with cue-induced craving in the human laboratory and whether pain intensity influences drinking during a practice quit attempt wherein participants are explicitly asked to abstain from alcohol.

Methods: This is a secondary data analysis from a recent study from our laboratory testing a novel human laboratory model in which individuals with intrinsic motivation to change their drinking engage in a "practice quit" attempt consisting of 6 days of complete abstinence from alcohol. Individuals with current AUD completed a randomized, double-blind, placebo-controlled study of naltrexone (50 mg QD), varenicline (1 mg BID), or matched placebo. Participants ($N = 45$) were assessed for chronic pain intensity and disability, and then titrated onto the study

medication for one week prior to starting the 6-day practice quit attempt. All participants completed an alcohol cue-exposure paradigm before starting the study medication and after 2 weeks of study medication.

Results: Participants ($N = 45$) self-reported experiencing chronic pain for an average of 30 days in the past 6 months. General linear models showed a significant effect of pain intensity but not pain disability ($p = .13$) on change in alcohol-cue reactivity such that pain intensity predicted increased cue-induced craving after the practice quit attempt ($B = .02$, $SE = .001$, $p = .02$) controlling for medication effects. Zero-inflated negative binomial regression models showed that neither pain intensity or pain disability predicted the number of drinking days during the practice quit attempt (p's = .85 and .48, respectively) or during the entire medication period (p's = .28 and .23, respectively) controlling for baseline drinking days and medication effects. Negative binomial regression models showed that neither pain intensity or pain disability did not significantly predict the number of days abstinent during the practice quit attempt (p's = .60 and .43, respectively) or during the entire medication period (p's = .76 and .39, respectively) controlling for number of baseline abstinent days and medication effects.

Conclusions: These results show that pain intensity but not pain disability is associated with increased cue-induced alcohol craving after a practice quit attempt with no significant effect on drinking outcomes. While the overall sample self-reported low intensity chronic pain, it is possible that participants with higher levels of chronic pain intensity may benefit from therapeutic interventions that reduce alcohol craving during periods of abstinence and for a treatment duration longer than the medication period used in the current study.

Keywords: Alcohol Use Disorder - Treatment, Chronic Pain, Cue-Induced Craving, Alcohol Drinking

Disclosure: Nothing to disclose.

P689. Reciprocal Connections Between Smoking and Pain During Ad Lib Smoking Using Ecological Momentary Assessment

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Background: Tobacco smoking and chronic pain are highly comorbid conditions, and have been proposed to influence each other through a reciprocal positive feedback loop, in which pain motivates smoking, and smoking, in turn, leads to worsening pain over time. Nicotine has been shown in both human and animal laboratory studies to have acute antinociceptive effects, although these may diminish as tolerance develops. Conversely, initial smoking abstinence (12-24 hrs) is associated with increased pain sensitivity (i.e., hyperalgesia), as measured by both self-report and laboratory pain assessments, that is correlated with severity of withdrawal and urge to smoke. Moreover, although the time course of early withdrawal-related hyperalgesia is unknown, evidence suggests that other common symptoms of withdrawal, such as craving and irritability, can begin to emerge as soon as 30 minutes after the last cigarette. Thus, it is possible that over time, the "analgesic" effects of nicotine become increasingly dominated by short-term relief of a hyperalgesic state resulting from fluctuating nicotine levels. However, the extent to which analgesic or hyperalgesic effects of nicotine are present and impact smoking in daily life among people with chronic pain is unclear and difficult to ascertain in a laboratory setting. Ecological momentary assessment (EMA) provides an opportunity to examine antecedents and consequences of smoking in relation to pain in real time, but only one prior study has applied this to people with chronic pain under ad lib smoking conditions. The present analyses leveraged baseline data from an ongoing trial of people who smoke (PWS) with chronic pain who completed EMA for one week in order to examine interactions between smoking and pain over time.

Methods: Twenty participants with chronic back pain who reported smoking at least 10 cigarettes per day completed one week of EMA using the MetricWire platform installed on their smartphone while smoking their usual brand of cigarettes. Participants were prompted at 5 random times during their waking hours each day to rate their level of pain intensity and unpleasantness, positive and negative affect, and urge to smoke, as well as how long ago they smoked their last cigarette, if they are "about to smoke", or if they are "smoking right now." Participants were incentivized for responding to at least 4 out of 5 prompts each day. Data from 3 participants were excluded due to low completion rates and/or problems with the app. For the remaining 17 participants, mixed models with random intercepts were used to examine the following associations: 1) differences in pain before, during, and after smoking (i.e., times when participants indicated they were "about to smoke" versus "smoking right now" versus smoking within the past 30 minutes; 2) pain as a predictor of smoking (i.e., differences in level of pain between times when participants indicated they were "about to smoke" versus not about to smoke); and 3) pain as a function of recency of smoking (i.e., differences in level of pain when participants indicated they had smoked within the past 30 minutes compared with a longer period of time).

Results: Average completion rate for participants included in the analyses was 89.6%, with each person contributing an average of 30.4 responses. Overall, participants indicated that they were about to smoke on 55 occasions (10.7% of responses), that they had smoked within the past 30 minutes on 157 occasions (30.4% of responses), and that they were currently smoking on 129 occasions (25.0% of responses). For Model 1, both pain intensity and interference were significantly lower when participants had smoked within the past 30 minutes versus "smoking right now" (both p's < .001), suggesting a reduction in pain after finishing smoking. By contrast, neither pain intensity nor interference differed between occasions when participants were "about to smoke" versus "smoking right now", suggesting that initiating smoking did not immediately impact pain levels. For Model 2, pain unpleasantness ($p = .024$), but not pain intensity ($p = .07$), was significantly associated with being about to smoke. For Model 3, neither pain intensity nor unpleasantness were associated with having smoked within the past 30 minutes compared with longer durations since the last cigarette.

Conclusions: Overall, these results reflect a pattern in which both pain intensity and unpleasantness decrease significantly after finishing smoking compared with levels that were present before and during smoking. Moreover, increased pain unpleasantness preceded smoking events, suggesting that pain unpleasantness may serve as a motivator to smoke. Thus, smoking appears to attenuate a potential withdrawal-related increase in pain that precipitates smoking, without providing any decrease from a baseline level of pain. These findings provide real-world support for the reciprocal positive feedback loop that is likely to perpetuate continued smoking among individuals with chronic pain. Future research is needed to identify strategies for disrupting this feedback loop to improve health outcomes in this population.

Keywords: Chronic Pain, Nicotine, Ecological Momentary Assessment, Tobacco Smoking

Disclosure: Nothing to disclose.

P690. Serotonin is Released in the Dorsal Striatum in Anticipation of a Reward

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Background: Serotonergic signaling throughout the brain is involved in reward processing, motivation, and behavioral inhibition. In the dorsal striatum (DS), pharmacological manipulation of the serotonin system disrupts behavioral control and the prospective encoding of rewards. In order to better understand the involvement of serotonin in striatal reward processing, we monitored serotonin levels using a G-Protein Activation Based (GRAB) fluorescent serotonin sensor (GRAB-5-HT) in the DS of adult mice during both consummatory and Pavlovian tasks.

Methods: We used GRAB-5HT to measure serotonin levels in the DS in male and female mice. In one set of experiments we measured serotonin release in the context of varying concentrations of evaporated milk reward ($N = 10$) or under varying satiety states ($N = 7$) in the Davis Lickometer. In a second set of experiments, male and female mice ($N = 16$) were trained in a Pavlovian delay conditioning paradigm in Bussey-Saksida touch screen operant chambers. Mice were presented with 8s long tones (CS+: white noise or 2 kHz tone counterbalanced) preceding delivery of evaporated milk reward, and an equal number of 8s long CS- tones (opposite of assigned CS+ cue) presentations that were not associated with reward delivery.

Results: We saw a robust serotonin release in the DS that began one to two seconds prior to the onset of reward consumption, and peaked during the first lick ($t(9) = 4.68$, $p = 0.001$). The rise in DS serotonin also encoded the value of the reward, with higher reward concentrations being associated with larger and more prolonged rises in serotonin ($F(5, 45) = 9.15$, $p = 0.002$). Mice were also given intermittent access to evaporated milk reward or water under different homeostatic states including sated, food restricted, and water restricted conditions. Relative to the food restricted condition, water restriction increased the amount of water, but not evaporated milk, consumed during access periods ($F(1, 6) = 8.95$, $p = 0.024$), however serotonin release was only associated with the reward, but not water consumption ($F(1, 6) = 6.4251$, $p = 0.044$). This suggests that the serotonin release in the dorsal striatum can be dissociated from the motoric aspects of liquid consumption. Next, in order to determine how serotonin encodes cued reward, we used a Pavlovian paradigm. Given the similarity of the CS+ and CS- cues that were used, after ten training sessions only half of the animals displayed significant CS +/CS- discrimination as measured by anticipatory approach during the CS. In these discriminating mice, serotonin release during CS+ trials was significantly higher than during CS- trials. In contrast, the non-discriminating mice did not show a difference in the serotonin release between CS+ and CS- trials (interaction: $F(1, 14) = 10.58$, $p = 0.006$).

Conclusions: Overall, these data suggest that striatal serotonin is linked to valuation and reward approach and/or anticipation. DS serotonin rises in anticipation of a reward, encodes the relative value of a reward, and seems to reflect the hedonic, rather than the consummatory aspects of a liquid reward. Additionally, the serotonin signal is associated with a cue that predicts a reward, rather than the sensory aspects of the cue. Ongoing studies are focused on establishing causality between serotonin release and behavioral processes through simultaneous GRAB-5HT monitoring and optogenetic inhibition of serotonin terminals in the DS. Understanding how serotonin is involved in learning and representing reward in goal-directed behavior is important for investigating the pathology of psychiatric disorders which include disordered reward processing.

Keywords: Serotonin, Reward Anticipation, Pavlovian Conditioning, GRAB-5HT, Dorsal Striatum

Disclosure: Nothing to disclose.

P691. Predator Odor (TMT) Exposure Potentiates Alcohol Interoceptive Sensitivity Through GABA-A Receptor Adaptations in the Prelimbic Cortex in Males Rats

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Background: Post-traumatic stress disorder (PTSD) increases vulnerability to develop alcohol use disorder (AUD) co-morbidity. Alcohol produces distinct interoceptive (subjective) stimulus effects that can influence drinking. Traumatic stress may increase susceptibility to develop AUD by altering interoceptive sensitivity to alcohol. The prelimbic cortex (PrL) and the anterior insular cortex (aIC) are involved in stress response and interoceptive sensitivity to alcohol. The goal of the present study was to determine the long-term effects of a traumatic stressor on interoceptive sensitivity to alcohol.

Methods: The present animal studies used exposure to the predator odor 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) to model a traumatic stressor and assessed whether subsequent sensitivity to the interoceptive effects of alcohol was changed. Male and female, Long-Evans rats ($N = 85$) were trained to discriminate the interoceptive effects of alcohol (2.0 g/kg, i.g.) from water using a Pavlovian drug discrimination procedure, which serves as an index of interoceptive sensitivity to alcohol. Rats underwent TMT exposure (15-min) and then remained undisturbed in their home cage for 2 weeks prior to alcohol sensitivity testing (dose response: 0.0, 0.5, 1.0, 2.0 g/kg, i.g.) and alcohol substitution testing with systemic and site-specific (PrL and aIC) GABA-A receptor agonism. TMT exposures were video recorded to quantify the behavioral stress response of each rat. In order to capture the clinical observation of individual heterogeneity in vulnerability to develop stressor-related disorders, measures of behavioral stress reactivity during the TMT exposure were used to create two sub-groups within the TMT exposure group. These two sub-groups consisted of (1) rats that engaged in less digging behavior and more immobility behavior ("TMT-1") and (2) rats that engaged in more digging behavior and less immobility behavior ("TMT-2"). Data for alcohol sensitivity was re-analyzed using these 3 groups (TMT-1, TMT-2, CTRL). Finally, follow-up experiments examined locomotion, acoustic startle response, and brain c-Fos expression in the PrL and aIC following TMT exposure and acute alcohol administration (2.0 g/kg, i.g.) in a separate cohort of male and female rats (c-Fos: $N = 64$; behavior: $N = 96$).

Results: TMT exposure produced increased defensive digging and immobility behavior during the TMT exposure in both males and females. TMT exposure potentiated the interoceptive effects of alcohol in males (2-WAY ANOVA; main effect of TMT: $F(1, 23) = 9.69$, $p = 0.0049$), but not in females when tested 2 weeks after the stressor. Additionally, in males, TMT exposure potentiated alcohol substitution with systemic pentobarbital and site-specific PrL muscimol ($p < 0.05$), but not aIC muscimol. Again, these effects were not observed in females. Interestingly, females appeared to have greater baseline (control group) sensitivity for alcohol substitution with GABA-A receptor agonism compared to males. In subgroup analyses, TMT-2 showed greater interoceptive sensitivity to alcohol compared to both TMT-1 and CTRL groups in males ($p < 0.05$), but not in females. In males, TMT exposure

increased c-Fos expression in the PrL ($p < 0.05$), but alcohol administration blocked this effect. In the aIC, c-Fos was not affected by TMT exposure or alcohol in males. TMT exposure and alcohol did not affect c-Fos in the PrL or aIC in females. For behavioral sensitivity experiments, the control group showed blunted locomotion and acoustic startle response to alcohol administration in males ($p < 0.05$), but the TMT group failed to show these effects of alcohol.

Conclusions: These data demonstrate that TMT exposure potentiates the interoceptive effects of alcohol through PrL GABA-A receptor adaptations in males. Furthermore, greater behavioral stress-reactivity during the TMT exposure was associated with the most potentiated interoceptive sensitivity to alcohol. Follow-up data suggest that this effect may be driven by potentiated sensitivity to the stimulatory effects of alcohol through a GABAergic disinhibition mechanism in the PrL. Together, these data may improve our understanding of PTSD-AUD comorbidity as increased sensitivity to alcohol may prime more drinking.

Keywords: Drug Discrimination, Alcohol Sensitivity, Traumatic Stress, Predator Odor, Sex Differences

Disclosure: Nothing to disclose.

P692. Ethanol Potentiation of Fentanyl-Induced Respiratory Depression: Sex Differences and Mortality

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Background: The number of drug overdose deaths involving opioids in the United States continues to rise, surpassing 100,000 annually (Centers for Disease Control, 2021). Opioid overdose deaths are primarily attributed to the respiratory depressant effects of opioids, which inhibit both peripheral and central areas responsible for maintaining respiratory rhythm and flow. Alcohol misuse, which can also lead to respiratory depression and death at high doses, is also frequently reported in fatalities involving heroin and fentanyl, with an estimated co-involvement rate of approximately 30%. This is clinically relevant because while naloxone has proven effective in reversing opioid-induced overdoses, its efficacy may diminish for overdoses from the combined use of alcohol and fentanyl. Understanding these interactions can aid in developing targeted interventions for co-occurring alcohol/opioid overdoses.

Aim: To characterize the effects of concomitant administration of fentanyl and ethanol on ventilation measures and naloxone effectiveness in reversing the hypoventilation caused by the combination of fentanyl and ethanol.

Methods: Using whole-body plethysmography, we analyzed ventilation parameters on a breath-by-breath basis. Four cohorts of female and male Long-Evans rats underwent intravenous catheter surgery. After habituation to plethysmography chambers, the rats were tested in a within-subjects, Latin-square design with tests one week apart. Cohort 1 received water, ethanol 1.18 g/kg (EtOH 1.18), fentanyl 25 µg/kg (FTN 25), and the combination of ethanol and fentanyl. Cohort 2 received water, ethanol 0.59 g/kg (EtOH 0.59), FTN 25, and the combination of ethanol and fentanyl. Cohort 3 received water, EtOH 0.59, fentanyl 3 µg/kg (FTN 3), and the combination of ethanol and fentanyl. After the initial Latin-square design, rats from cohort 3 received the combination of FTN 25 and EtOH 0.59 followed, 5 min later, by naloxone (0 or 100 µg/kg). In cohort 4 the effects of FTN 25, EtOH 0.59, and their combination were compared between non-dependent rats and ethanol- and fentanyl-dependent groups. All drugs were administered intravenously.

Results: Only the combination of FTN 25 and EtOH 1.18 resulted in mortality (~42% females, ~33% males). 1) Compared to water, FTN 25, EtOH 1.18, and their combination reduced minute ventilation ($F3,30 = 15.73$, $p < 0.0001$) and increased apneic pauses ($F3,30 = 10.04$, $p < 0.0001$). The fentanyl and ethanol combination led to a 65% reduction in minute ventilation from baseline compared to a 42% reduction caused by fentanyl alone ($p = 0.007$) and an 80% increase in apneic pauses compared to 50% from fentanyl alone ($p = 0.03$) or 60% from ethanol alone ($p = 0.02$). There were no significant sex differences in these effects. 2) Compared to water, FTN 25, EtOH 0.59, and their combination reduced minute ventilation ($F3,51 = 16.69$, $p < 0.0001$) and increased apneic pauses ($F3,45 = 34.95$, $p < 0.0001$). The fentanyl and ethanol combination led to a 96% increase in apneic pauses compared to an 86% increase after fentanyl alone ($p = 0.0004$) or a 58% increase following ethanol alone ($p < 0.0001$). Female rats had greater reductions in minute ventilation than males ($p = 0.0005$) with FTN 25. 3) Compared to water, EtOH 0.59 and FTN 3 + EtOH 0.59 combination mildly reduced minute ventilation ($F3,57 = 7.16$, $p = 0.0004$) and mildly increased apneic pauses ($F3,57 = 2.94$, $p = 0.04$). The fentanyl and ethanol combination led to a 35% reduction in minute ventilation compared to 26% following fentanyl alone ($p = 0.0006$). There were no significant sex differences in these effects. Naloxone 100 µg/kg transiently (< 5 min) reversed the reduction on minute ventilation ($F15,225 = 13.61$, $p < 0.0001$) from FTN 25 + EtOH 0.59, but did not affect apneic pauses ($F15,180 = 1.29$, $p = 0.21$). 4) The fentanyl-dependent group showed partial tolerance to FTN 25 ($F30,555 = 4.75$, $p < 0.0001$) and FTN 25 + EtOH 0.59 ($F30,495 = 2.53$, $p < 0.0001$), which was characterized by a shorter lasting reduction of minute ventilation (20 vs. 10 min).

Conclusions: The combination of fentanyl and ethanol leads to severe apnea that is not fully reversed by naloxone. This research contributes to an understanding of the contribution of alcohol-opioid combinations in overdoses, which is relevant to research aimed at preventing and reversing overdoses from drug combinations.

Keywords: Opioid Overdose, Respiratory Depression, Alcohol, Fentanyl, Naloxone

Disclosure: Nothing to disclose.

P693. Unique Pharmacodynamic Properties and Low Abuse Liability of the µ-Opioid Receptor Ligand (S)-Methadone

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Background: (R,S)-methadone (MTD) is a µ-opioid receptor (MOR) agonist that is comprised of equal amounts of (R)-MTD and (S)-MTD enantiomers. The therapeutic properties of (R,S)-MTD are believed to be mediated by the actions of (R)-MTD. (S)-MTD is now under clinical development as a treatment for depression. Although its precise in vivo pharmacology is not well understood, (S)-MTD's antidepressant mechanism of action is attributed to N-methyl-D-aspartate receptor (NMDAR) antagonism.

(R,S)-MTD produces weaker activation of midbrain dopamine systems and has lower abuse liability when compared to other

opioids. The reduced dopaminergic effects of (R,S)-MTD are dependent on its unique, weak interaction with MOR-galanin 1 receptor (Gal1R) heteromers specifically expressed in the ventral tegmental area (VTA).

In order to explore the analgesic and abuse liability profiles of (R,S)-MTD and its enantiomers, and to address the gaps in knowledge about these compounds, we performed an in-depth in vitro, in vivo and in silico pharmacological characterization of (R,S)-MTD, (R)-MTD and (S)-MTD.

Methods: We investigated the binding profile of (R)-MTD and (S)-MTD. Each enantiomer (100 nM and 10 μM) was tested for its ability to competitively inhibit binding or activity at a panel of 98 receptors and enzymes that are known targets for medications. The MOR affinities for (R,S)-MTD and its enantiomers were determined via [3H]DAMGO binding in rat brain tissue homogenate.

We next tested the analgesic, cataleptic, and abuse liability profiles of (R,S)-MTD and its enantiomers. We performed a dose-response time course for (R,S)-MTD, (R)-MTD, and (S)-MTD in rats (n = 5 per dose group) on catalepsy score and hot plate latency. Separate groups of rats (n = 8-9) underwent IVSA training for (R,S)-MTD (100 μg/kg/infusion), (R)-MTD (50 μg/kg/infusion), or (S)-MTD (500 μg/kg/infusion), initially on FR1 for 10 days then increased to FR5 for the remainder, and their intravenous self-administration (IVSA) dose-response curves were determined. We also tested the ability of (R,S)-MTD (4 mg/kg, s.c.; n = 6), (R)-MTD (2 mg/kg, s.c.; n = 6), and (S)-MTD (30 mg/kg, s.c.; n = 6) to occupy MORs ([3H]DAMGO, 5nM) or NMDARs ([3H]MK-801, 5 nM).

We next examined the effects of (R,S)-MTD and its enantiomers on VTA dopamine signaling. First, we looked at the effects on locomotor activity in mice (n = 8-12). We also tested to see if pretreatment with (S)-MTD prevented (R)-MTD-induced locomotor activity increases in mice (n = 7-8). Next, we studied the effects of (R)-MTD (3 μM and 10 μM) and (S)-MTD (10 μM and 100 μM) perfusion into the rat VTA (n = 7-9) on dopamine release, using in vivo microdialysis. We also tested whether perfusing (S)-MTD (100 μM) into the VTA prior to (R)-MTD (10 μM) prevented dopamine release.

We next investigated the possibility of divergent pharmacodynamic effects MTD enantiomers at MOR-Gal1R. BRET experiments (n = 6 per drug) were performed to evaluate differences in the intrinsic efficacy of (R,S)-MTD, (R)-MTD and (S)-MTD at the MOR. CODA-RET experiments (n = 6 per drug) were then performed to determine whether MOR-Gal1R heteromerization might determine the specific pharmacodynamic profile of (S)-MTD. Lastly, we performed molecular dynamics (MD) simulations to predict docking position of (R)-MTD and (S)-MTD in MOR and MOR-Gal1R.

Results: At 100 nM, (S)-MTD inhibited binding only at MOR (79%), and (R)-MTD was similar. The Ki values obtained for MOR were 15.6 ± 0.1 nM for (R,S)-MTD, 7.5 ± 0.1 nM for (R)-MTD and 60.5 ± 0.1 nM for (S)-MTD.

(R,S)-MTD, (R)-MTD, and (S)-MTD demonstrated full agonistic activity in the hotplate test, with ED50 values of 1.2, 0.5 and 17.9 mg/kg, respectively. The cataleptic ED50 values were 2.1, 0.9 and 59.4 mg/kg, respectively. For IVSA, whereas rats trained on (R)- and (R,S)-MTD adjusted lever press rates to maintain stable infusion rates, rats trained on (S)-MTD did not and they showed no evidence of dose response. Furthermore, we found that (R,S)-MTD, (R)-MTD, and (S)-MTD produced near total (99%, 91%, and 79% respectively) occupancy of MORs 30 min after injection. However, none of the drugs produced any NMDAR occupancy.

Both (R,S)-MTD and (R)-MTD produced hyperlocomotion with an inverted U-shaped curve in mice. However, (S)-MTD never increased locomotion. Importantly, (S)-MTD pretreatment dose dependently prevented (R)-MTD-induced hyperlocomotion. (R)-MTD lead to an increase in somatodendritic dopamine release at 3 μM and 10 μM in the rat VTA. In contrast, (S)-MTD did not produce any change in dopamine release even at 100 μM. Additionally, like the locomotor data, (S)-MTD pre-perfusion prevented (R)-MTD-induced dopamine release.

In cells only expressing MOR, all three compounds acted as agonists; however, (S)-MTD was less potent and behaved more as a partial agonist compared to (R,S)-MTD and (R)-MTD. At MOR-Gal1R heteromers, (R,S)-MTD and (R)-MTD remained agonists, in contrast, (S)-MTD showed a loss in efficacy. MD simulations revealed that when (S)-MTD is docked in MOR-Gal1R, the receptor is locked in an inactive state which explains its conversion to an antagonist at this heteromer.

Conclusions: (S)-MTD, like (R)-MTD, binds to and activates MORs in vitro, but (S)-MTD antagonizes the MOR-Gal1R heteromer, decreasing its abuse liability. In sum, we report novel and unique pharmacodynamic properties of (S)-MTD that are relevant to its potential mechanism of action and therapeutic use.

Keywords: Mu Opioid Receptor, Receptor Heteromerization, Abuse Liability

Disclosure: Nothing to disclose.

P694. Using Rat Models of Alcohol-Opioid Polysubstance Use to Test the Ability of Oxytocin to Reduce Drug Intake and Seeking

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Background: Opioid use remains at crisis levels in the US. While effective treatments for opioid use disorder exist, co-morbid alcohol use worsens treatment outcomes and increases the likelihood of overdose. Despite this evidence and the prevalence of opioid-alcohol co-use in humans, there is a paucity of research at the preclinical level. Here we developed models of opioid-alcohol polysubstance use (PSU) that were used to test the ability of the neuropeptide oxytocin to reduce drug intake.

Methods: In the oral co-use model, male and female rats (n = 8-9/group) were given 2-bottle choice access to oral oxycodone and/or water for 7 days, followed by 2-bottle choice access to alcohol (20% v/v) and/or water for five 24-hr sessions. Next, monosubstance (oxycodone- or alcohol-alone) groups continued to have access to only one drug while PSU rats had 3-hr access to 2-bottle choice for oxycodone followed by 6-hr access to 2-bottle choice for alcohol. After 14 days, oxytocin (0 or 1.0 mg/kg IP) was administered 30 minutes prior to the 2-bottle choice access to alcohol and intake and blood alcohol level assessed. For the intravenous self-administration (IVSA) model, male and female rats (n = 9/sex/group) were given 3-hr access to oxycodone IVSA prior to 6-hr access to 2-bottle choice for alcohol. IVSA was conducted on an FR-1 schedule for 6 days followed by an FR-3 schedule for 6 days. Next, rats underwent economic demand curve analyses for intravenous oxycodone in which FR requirements were increased every 2 days until 0 infusions were attained. Oxytocin (0 or 1.0 mg/kg IP) was administered 30 minutes prior to demand curve sessions. Blood alcohol levels will be compared between PSU and alcohol-only conditions.

Results: In the oral consumption model, there were no effects of co-use on intake of or preference for either alcohol or oxycodone. Oxytocin decreased alcohol intake in both the alcohol-only condition and PSU condition. In the IVSA model, male and female PSU rats displayed reduced intake of both oxycodone and alcohol relative to monosubstance use conditions during the FR-3 portion of the experiment. However, demand for intravenous oxycodone was increased by access to alcohol only in females. In the oxycodone-only condition, oxytocin increased

demand elasticity for IVSA oxycodone in males with no effect in females.

Conclusions: Route of administration and sex influence the ability of alcohol to alter the motivation to seek oxycodone. Intravenous oxycodone reduces alcohol intake and preference while orally-consumed oxycodone does not, likely due to higher bioavailability of intravenous oxycodone. Oxytocin reduces alcohol intake in rats with both alcohol-only and oxycodone-alcohol histories but is less effective at increasing elasticity of demand for intravenous oxycodone in female rats. These data indicate that oxytocin may remain effective at reducing alcohol consumption in PSU conditions but is less effective at reducing opioid-seeking in females.

Keywords: Opioid Addiction, Alcohol, Oxytocin

Disclosure: Nothing to disclose.

P695. The Nociceptin Receptor System as a Target for Novel Opioid Use Disorder Therapeutics

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Background: A novel mechanistic opportunity to treat opioid use disorder (OUD) is to employ small molecules that target the nociception receptor (NOPr) axis. The "non-classical" NOPr binds the endogenous neuropeptide nociceptin/orphanin FQ (N/OFQ), named for its efficacy to evoke nociception. The N/OFQ-NOPr axis is an opioid-like system which is well represented in reward-processing circuits of the brain. The NOPr antagonist LY2940094 (formerly BTRX-246040) exhibits high NOPr occupancy and target engagement in rodents. Initial clinical trials indicated that LY2940094 is safe and well-tolerated in clinical populations. In the present studies, we assessed the efficacy of LY2940094 to alter opioid intake and relapse vulnerability as well as physiological measures, naloxone-induced fentanyl withdrawal symptoms and fentanyl-induced analgesia in preclinical models.

Methods: Male, Sprague-Dawley rats (n = 6-18/group) were trained to stability on fentanyl self-administration (3.2 µg/kg/inf, i.v.) prior to pretreatment with acute or repeated doses of LY2940094 (0 or 30 mg/kg, i.p.; 15 min) and reassessment of fentanyl intake. Responding for fentanyl-associated cues during abstinence following LY2940094 (0-30 mg/kg, i.p.; 15 min) and the abuse liability of LY2940094 (2 mg/kg/inf, i.v.) was assessed in the fentanyl self-administration assay. The fentanyl (0.02 mg/kg, s.c., 20 min) vs. saline drug discrimination paradigm was employed to inform mechanisms of LY2940094 (0-30 mg/kg, i.p., 20 min) and additionally assess abuse liability. Rats were implanted with an osmotic minipump which delivered fentanyl (0.6 mg/kg/day; s.c.); on day 10, LY2940094 (0 or 30 mg/kg, i.p. 15 min) was administered prior to saline or naloxone (0.03 mg/kg, s.c.) and withdrawal symptoms measured for 60 min. Employing the MouseOx™ system, we assessed the effects of escalating fentanyl dosing (12.5-50 µg/kg; s.c.) to evoke quantifiable physiological responses following administration of vehicle or LY2940094 (30 mg/kg, i.p., 15 min). The incremental hot plate test was performed to measure analgesia and nociception following fentanyl (50 µg/kg, s.c.) in rats pretreated with LY2940094 (0-30 mg/kg, i.p., 20 min).

Results: LY2940094 suppressed fentanyl intake and fentanyl-seeking ($p < 0.05$). Repeated treatment with LY2940094 for either two- or three-days during abstinence reduced reinstatement of intake by over 50% ($p < 0.05$). The interoceptive effects of LY2940094 were not perceived as similar to fentanyl, but LY2940094 dose-dependently suppressed the stimulus properties of fentanyl ($p < 0.05$). LY2940094 did not sustain intake in rats trained to self-administer fentanyl, indicating its lack of abuse liability. LY2940094 did not alter physiological measures associated with fentanyl-evoked respiratory depression. Neither fentanyl-evoked anti-nociception nor naloxone-precipitated fentanyl withdrawal symptoms were impacted by LY2940094.

Conclusions: Our findings implicate the N/OFQ-NOPr axis as a biological mediator of opioid-related reward and cue-evoked drug-seeking behavior in rodents. A greater understanding of the role of the N/OFQ-NOPr system in these behaviors is an active area of research as NOPr agonists and antagonists have been reported to paradoxically evoke overlapping outcomes, and may be explained by N/OFQ activation of N/OFQ-sensitive neural circuits that compete for behavioral control or perhaps NOPr desensitization factors. Nonetheless, our preclinical data position the NOPr antagonist LY2940094 as a potential therapeutic strategy to mitigate opioid intake and relapse vulnerability without inherent abuse liability and with a limited side effect profile.

Keywords: Nociceptin/Orphanin FQ, Fentanyl, Opioids

Disclosure: Delix: Consultant (Self).

P696. The Dual Orexin Receptor Antagonist Suvorexant Reduces Cocaine Motivation and Normalizes Sleep Disturbances During Abstinence

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Background: The transition to uncontrolled cocaine use in rats is accompanied by an increase in the number and activity of orexin neurons. Orexin neurons drive reward and wakefulness, both of which are perturbed in cocaine use disorder (CUD), raising the possibility that medications that reduce orexin signaling might offer multifaceted therapeutic benefit. Here, we tested if the FDA-approved dual orexin receptor antagonist (DORA), suvorexant (BelsomraTM), can be repurposed to normalize cocaine motivation during the active period, and to ameliorate abstinence-associated insomnia during the inactive period.

Methods: Male (n = 12) and female (n = 14) rats were trained during the active period to self-administer cocaine on an intermittent access schedule and then tested for cocaine demand. Prior to demand testing, they received acute treatment with suvorexant (0, 3, 10, 30mg/kg, p.o., counterbalanced). To test for any soporific effects, a subset of rats (male n = 6; female n = 7) were subsequently tested during the active period on the rodent psychomotor vigilance task (rPVT), which requires rats to maintain attention for 30min to earn sucrose rewards; rats received suvorexant prior to testing, as above. A second group of rats (male n = 9; female = 5) underwent extinction training for 7d and received suvorexant (0, 30mg/kg, p.o.) 1h prior to the onset of the inactive period. In a third group of rats (male n = 9; female = 5), we induced a conditioned place preference (CPP) to cocaine injections (10mg/kg). CPP was extinguished in daily sessions over 5d. During this time, rats were treated with suvorexant (0 vs. 30mg/kg; p.o.) 1h prior to the onset of the inactive period, and in a subset of rats, sleep was monitored via EEG/EMG recordings. Cocaine demand and rPVT accuracy data were analyzed using 2 sex (male, female) x 4 dose (0, 3, 10, 30mg/kg) ANOVA with Tukey post-hoc tests. Time spent in REM was calculated as a percent of pre-drug baseline; these data were compared across suvorexant doses (0, 30mg/kg) using ANOVA. CPP data were analyzed using 5 day (days 1-5) x 2 dose (0, 30mg/kg) ANOVA. Significance for all tests was set at $p < 0.05$.

Results: For demand, there was no main effect of sex ($F = 2.576$, $p = 0.112$) or sex x dose interaction ($F = 1.415$, $p = 0.244$). For rPVT, there was no main effect of sex ($F = 0.529$, $p = 0.482$) or sex x dose interaction ($F = 0.163$, $p = 0.956$). Acute treatment with suvorexant (10mg/kg) during the active period decreased cocaine demand ($p = 0.046$) without affecting performance on the rPVT ($p = 0.189$). Repeated dosing with suvorexant during the inactive period increased the amount of rapid eye movement sleep ($p = 0.034$) during cocaine abstinence and facilitated extinction of cocaine seeking ($p = 0.034$) and CPP ($p = 0.048$).

Conclusions: At low, non-sedating doses, suvorexant administered during the active period reduces cocaine motivation without promoting sedation. Higher doses administered in the inactive period normalize sleep and reduce subsequent drug seeking. These data support different daytime vs. nighttime dosing regimens with DORAs for the management of CUD.

Keywords: Sleep Disturbances, Cocaine Use Disorder, Dual Orexin Receptor Antagonists, Orexin, REM Sleep

Disclosure: Nothing to disclose.

P697. Morphine-Induced Antinociception, Tolerance and Withdrawal in Rats Eating a High Fat/High Carbohydrate or Ketogenic Diet

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Background: Eating a high fat diet leads to negative health consequences such as obesity and type 2 diabetes. Recent evidence also suggests that eating a high fat diet can impact drug sensitivity. For example, eating a high fat/ high carbohydrate laboratory chow enhances the sensitivity of rats to the behavioral effects of cocaine and methamphetamine. While high fat/high carbohydrate diets are associated with weight gain, ketogenic diets that are high in fat, but very low in carbohydrates have been used for the treatment of epilepsy and have been investigated for weight loss. We hypothesized that diet would increase sensitivity of rats to some of the effects of morphine (e.g., tolerance and withdrawal).

Methods: Female Sprage Dawley rats ($n = 48$) were given access to either a high fat/high carbohydrate chow (60% kcal from fat), a ketogenic chow (90.5% kcal from fat) or standard chow (17% kcal from fat) for several weeks prior to once weekly testing with cumulative doses of morphine in two different antinociception assays. In experiment 1, approximately 24-hours before the first morphine test, unilateral hindpaw inflammation was induced by injecting 0.1ml of complete freund's adjuvant (CFA) or saline into the footpad. A saline injection, followed by 4 cumulative injections of morphine (1.0-17.8 mg/kg IP) were administered every 15 minutes, and 13 minutes after each injection, the force that resulted in paw withdrawal was recorded. Morphine tests were repeated once per week, for 4 consecutive weeks. In experiment 2, morphine-induced antinociception was measured using the warm water tail withdrawal procedure. A saline injection, followed by 4 cumulative injections of morphine (0.32-17.8 mg/kg IP) were administered every 15 minutes and 13 minutes after each injection, the latency for rats to remove their tail from hot water baths (40, 50, or 55°C) was recorded. Additionally, to study the effects of chronic morphine exposure, morphine was administered twice daily for 19 days (3.2-56 mg/kg IP) increasing in ¼ log doses every 3 days, followed by an additional warm water tail withdrawal assessment with morphine (3.2-56 mg/kg IP). After chronic morphine administration, non-precipitated withdrawal was measured by examining observational signs of withdrawal and changes in body weight following morphine discontinuation. It was hypothesized that rats eating high fat chow would be more sensitive to the acute effects of morphine in both the inflammatory pain assay (experiment 1) and the thermal pain assay (experiment 2) than rats eating other diets. It was further hypothesized that rats eating high fat chow would also be more sensitive to the development of tolerance to the antinociceptive effects of morphine and withdrawal symptoms following the discontinuation of chronic morphine administration, as compared to rats eating a ketogenic or standard chow. Paw withdrawal threshold (in grams of force; experiment 1) and warm water tail withdrawal latencies (in seconds; experiment 2) were analyzed using two-way repeated measures ANOVAa with diet and dose as factors. Body weight, and observable signs of withdrawal were also analyzed using two-way repeated measures ANOVAs with day and diet as factors. Finally, effective dose (ED)50 values determined from individual antinociception dose-response curves were also examined and analyzed using a two-way repeated measures ANOVA with diet and week as factors.

Results: Morphine-induced antinociception was comparable among rats eating different diets when tested under acute conditions in both experiment 1 (assessing inflammatory pain) and experiment 2 (assessing thermal pain). After chronic morphine administration, all rats developed tolerance to morphine-induced antinociception in experiment 2, and this effect was comparable between groups. Finally, morphine discontinuation resulted in observable withdrawal signs among all rats. There were no group differences in the observable withdrawal signs; however, rats eating ketogenic chow experienced less withdrawal-related weight loss as compared to the other groups.

Conclusions: These results suggest that while morphine-induced antinociception appears to be unaltered by dietary manipulation, withdrawal symptoms following discontinuation of chronic morphine administration might be blunted by consuming a ketogenic diet. Future studies will examine these and other effects of morphine in studies that include both male and female rats to explore potential sex differences.

Keywords: High Fat Diet, Ketogenic Diet, Morphine Sensitivity, Antinociception, Opioid Withdrawal

Disclosure: Nothing to disclose.

P698. Evaluation of Chronic Oral Cannabidiol on Multidimensional Opioid Withdrawal Symptoms in Rats

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Background: The endocannabinoid system has shown promise as a target for opioid use disorder (OUD) and symptoms associated with opioid withdrawal; cannabinoid receptors are co-localized with opioid receptors and are key regulators of symptoms such as emotion regulation (anxiety, irritability), pain sensitivity (hyperalgesia). The non-intoxicating cannabis constituent cannabidiol (CBD) is currently under exploration for potential therapeutic benefits for OUD and other substance use disorders. This research aimed to evaluate the use of chronic oral CBD for alleviation of multidimensional withdrawal symptoms in a preclinical model of opioid dependence.

Methods: Adult Sprague-Dawley rats ($N = 72$, 12 per drug group; 50% female) were given morphine sulfate (ascending doses, 10-50 mg/kg, IP) or vehicle (0.09% saline) twice daily for 10 days. Following morphine discontinuation, CBD isolate (10, 30

mg/kg) or vehicle (USP sesame oil) was administered via oral gavage at 1 ml/kg. Oral CBD administration began 12 hrs following the last morphine injection and continued daily for 7 days. Body weight (g) and food intake (g) were measured daily throughout morphine administration and withdrawal. To assess somatic symptoms of opioid withdrawal, rats were placed in clear plastic containers and video recorded 36h, 4 days, and 7 days following opioid discontinuation. Physical symptoms of opioid withdrawal (e.g., escape jumps, wet-dog shakes, abnormal posture, etc.) were later scored by two independent, blinded observers. The von Frey test of mechanical sensitivity was also conducted at the same time points. Irritability-like behavior (bottle-brush test) and anxiety-like behavior (the elevated plus maze and open field test) were assessed on days 4 and 7 of protracted withdrawal. Data were analyzed using two-way analysis of variance (ANOVA), with dependence group and CBD dose as between-subjects factors. Time course evaluations (e.g., somatic symptoms, pain sensitivity) were evaluated over days 1, 4, and 7 of withdrawal using repeated measures ANOVAs.

Results: CBD did not mitigate body weight loss or decreased food intake observed during opioid withdrawal (p's>0.05). No effects of CBD were observed on pain sensitivity in opioid-dependent rats or controls (p's>0.05). Somatic symptoms of opioid withdrawal were increased on day 1 in opioid-dependent rats, and not alleviated by CBD treatment (p>0.05). CBD did not affect irritability- or anxiety-like behavior of opioid-withdrawn rats or controls (p's>0.05).

Conclusions: Twice daily injections of morphine produced a robust spontaneous withdrawal syndrome in male and female rats. CBD did not reduce the peak severity of acute withdrawal symptoms, nor the time course of protracted withdrawal in opioid-dependent rats. These data do not support the use of CBD for alleviation of physical or psychological withdrawal symptoms in an acute nor post-acute opioid withdrawal phase. Studies are ongoing to determine any differential effects of CBD between sexes, and with other medications that target the endocannabinoid system for comparison.

Keywords: Cannabidiol, Opioid Withdrawal, Physical Dependence, Animal Models

Disclosure: Nothing to disclose.

P699. The Effects of THC on Oxycodone-Induced Motivational and Neural Changes in a Novel Rat Model

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Background: Opioid use disorder (OUD) lacks an effective, broad-spectrum treatment. Limited clinical evidence suggests that co-use of cannabinoids can reduce the rates of opioid dependence and severity of withdrawal, though at the cost of elevated anxiety and depression. Despite incomplete understanding of risks vs. benefits of opioid-cannabinoid interactions, OUD is currently considered a qualifying condition for the use of medical marijuana in several US states. Thus, there is an urgent need to advance well-controlled, translational animal research to investigate the neural and behavioral consequences of cannabinoid-opioid co-use. Here we investigated the effects of daily oral Δ9-tetrahydrocannabinol (THC) consumption on several key neurobehavioral variables associated with oxycodone seeking, including behavioral economic demand for intravenously self-administered oxycodone, withdrawal-associated anxiety, reinstatement of oxycodone seeking, and finally on changes in opioid and cannabinoid receptors in rat brain.

Methods: Male and female adult Sprague-Dawley rats (n = 48) were first trained to self-administer oxycodone or sucrose under a FR (fixed ratio)-1 schedule for 6 days followed by a FR-3 schedule for 6 days. After reaching stable intake during training, rats began economic demand procedures in which the FR requirement to earn a reinforcer was increased in quarter log unit increments until zero reinforcers were attained for a given FR. Throughout the demand procedures, rats also received access to unsweetened gelatin containing either THC or vehicle in the home cage for one hour following the oxycodone/sucrose sessions. Somatic signs of withdrawal were assessed, and the light-dark box test was used to assess anxiety-like behavior at 22 hr withdrawal early in the demand portion of the experiment. Following completion of economic demand procedures, self-administration was re-established prior to 14 days of home cage abstinence. On Day 15, oxycodone rats underwent a cue-primed reinstatement test. In addition to the demand experiments, a subset of rats self-administered sucrose on a FR-1 schedule for 4 weeks, with THC/placebo gelatin access beginning in week 3. The effects of oxycodone challenge on μ-opioid (MOR) and CB1 receptor activity were assessed using phosphosite-specific antibodies across a network of brain regions known to regulate opioid dependence.

Results: Ongoing analysis revealed no effects of sex on oxycodone self-administration. Importantly, THC consumption led to increased demand elasticity for oxycodone IVSA and decreased cue-primed relapse, with no effects on sucrose intake. Tissue analysis in progress is aimed to collect data on MOR and CB1R phosphorylation in response to an acute oxycodone challenge in the prefrontal and orbitofrontal cortex, basolateral amygdala, and nucleus accumbens.

Conclusions: Voluntary oral THC consumption reduces motivation to seek intravenous oxycodone but not sucrose in male and female rats.

Keywords: Oxycodone, THC, Behavioral Economics, Drug Relapse, Opioid Receptor

Disclosure: Nothing to disclose.

P700. Effects of Novel GLT-1 Modulator, MC-100093, on Neuroinflammatory and Neurotrophic Biomarkers in Mesocorticolimbic Brain Regions of Alcohol Preferring Rats Exposed Chronically to Ethanol

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Background: Chronic ethanol consumption may cause alteration in several neurotransmitters, including the excitatory amino acid, glutamate. Indeed, chronic ethanol consumption may cause an increase in extracellular glutamate concentrations in key reward brain regions, including prefrontal cortex (PFC) and nucleus accumbens (NAc), and consequently leading to oxidative stress and neuroinflammation. Previous studies from our laboratory demonstrated that beta-lactams, ampicillin/sulbactam, decrease ethanol consumption in alcohol preferring rats (P rats), and this behavioral effect was associated with upregulation of major glutamate transporter, GLT-1, in NAc shell. Furthermore, this previous study showed that upregulation of GLT-1 by beta-lactams attenuated ethanol-induced increase in Tumor Necrosis Factor alpha (TNF-alpha) expression in NAc shell. Recent study from our laboratory tested a novel beta-lactam, MC-100093, which does not have any antibiotic action, and this drug was effective in reducing ethanol intake. This effect was associated with normalization of GLT-1 expression in NAc shell. In this present study, we

investigated whether the normalizing effect of MC-100093 on GLT-1 expression might be associated with attenuation of ethanol-induced increase in neuroinflammation and decrease in neuroprotection. Thus, our study focused on the effects of chronic ethanol consumption and MC-100093 in the expression of brain derived neurotrophic factor (BDNF) as a neuroprotective marker and neuroinflammatory factor such as TNF-alpha in subregions of the NAc (shell and core) and medial PFC (Infralimbic, IL; and Prelimbic, PL) in P rats.

Methods: Male P rats (n = 16) were divided into three groups: a) Control group was exposed to water for five weeks and received saline vehicle injection (i.p.) every 24 hours during Week 6 for a total of five days; b) Alcohol group was exposed to free choice of ethanol (15% and 30%) as well as water for five weeks and received saline vehicle (i.p.) every 24 hours during Week 6 for a total of five days; c) MC-100093 group was exposed to free choice of ethanol (15% and 30%) as well as water for five weeks and received MC-100093 (100 mg/kg, i.p.) every 24 hours during Week 6 for five days.

Results: Behavioral drinking analysis showed that MC-100093 reduced ethanol intake from Days 2-5. Importantly, Western blot analysis showed that MC-100093 attenuated ethanol-induced downregulation of BDNF expression in the IL, however, we did not see any changes in BDNF expression in PL. In addition, MC-100093 attenuated ethanol-induced decrease in BDNF expression in NAc shell. Although chronic ethanol consumption did not change BDNF expression in NAc core, MC-100093 increased BDNF expression in this brain region. This indicates that MC-100093 has a neuroprotective effect mediated through upregulation of BDNF. Furthermore, we investigated TNF-alpha as a neuroinflammatory marker in the subregions of NAc and PFC in this ethanol drinking regimen with the use of MC-100093. Western blot analysis revealed that chronic ethanol consumption increased TNF-alpha expression in NAc core and shell, and MC-100093 normalized the expression of TNF-alpha in both NAc subregions. Additionally, chronic ethanol consumption increased TNF-alpha expression in subregions of the medial PFC such as IL and PL, and MC-100093 normalized the expression of TNF-alpha in both PFC subregions. Importantly, we tested MC-100093 in female P rats, and we found that the drug decreased ethanol intake. We are currently investigating the effects of MC-100093 in the expression of BDNF and TNF-alpha.

Conclusions: MC-100093 attenuated neuroinflammation caused by ethanol intake as well as increased neurotrophic factor in mesocorticolimbic brain regions of P rats. MC-100093 is considered a small molecule that may have potential therapeutic effects for the treatment of alcohol dependence.

Keywords: Neuroinflammation, Ethanol Intake, MC-100093, BDNF, TNF-Alpha

Disclosure: Nothing to disclose.

P701. Ca2+-Permeable AMPA- Type Glutamate Receptors in the Nucleus Accumbens Core Mediate the Development of an Enhanced Motivation for Cocaine and the Faster Time-Course for its Development in Females Than Males

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Background: Women meet the criteria for substance use disorder and seek treatment after fewer years of drug use as compared to men. This accelerated disease progression has been termed the telescoping effect. It is a robust phenomenon that has been consistently observed in humans for multiple addictive drugs, including cocaine, alcohol, opioids, and tobacco, as well as, in preclinical studies for cocaine, indicating the effect observed in humans is likely biologically based. There is accumulating evidence that the role of nucleus accumbens core (NAc) AMPA receptor signaling increases with the development of addiction. Most notable is the large body of work showing the incubation, or increasing levels, of drug-seeking over withdrawal from extended-access drug self-administration (a key feature of an addiction-like phenotype in rodents) is mediated by the accumulation of synaptic, Ca2+-permeable AMPA- type glutamate receptors (CP-AMPAR) in the NAc. Given the importance of NAc CP-AMPAR for drug-seeking, here we examine its role in the development of an enhanced motivation for cocaine, another key feature of an addiction-like phenotype, and determine whether the accumulation of NAc CP-AMPAR occurs sooner during withdrawal in females and underlies the telescoping effect.

Methods: Motivation for cocaine was assessed under a progressive-ratio schedule before and after extended-access cocaine self-administration (24 h/day, 96 infusions/day, 10 days) and a 7-day withdrawal period, which are conditions that have been shown to induce an addiction-like phenotype (defined as a greater than 15% increase in motivation) in females, but not males. During the withdrawal period, females received intraperitoneal injections of the mGluR1 positive allosteric modulator (PAM) SYN119 (0 or10 mg/kg), which has been shown to prevent the recruitment of CP-AMPAR in the NAc; while males received intraperitoneal injections of the mGluR1 antagonist JNJ16259685 (0 or 5 mg/kg), which has been shown to accelerate the recruitment of NAc CP-AMPAR. Once a stable level of motivation was established after the withdrawal period, the effect of NAc-infusions of the CP-AMPAR antagonist naspm (40 µg/side) were examined.

Results: Preliminary findings indicate that similar to our previous studies, motivation for cocaine was increased from baseline (or prior to extended-access cocaine self-administration) in vehicle-treated females, but not males, following extended-access cocaine self-administration and a 7-day withdrawal period. As expected, this motivational shift was blocked in females by systemic administration of SYN119 and induced in males by systemic administration of JNJ16259685. Additionally, NAc-infused naspm reduced motivation for cocaine in males that developed an enhanced motivation for cocaine.

Conclusions: These results indicate that CP-AMPA receptors in the NAc underlie the development of an enhanced motivation for the cocaine and the faster time-course for its development in females versus males.

Keywords: Telescoping Effect, Sex Differences, Cocaine, Extended-Access Self-Administration, Ca2+-Permeable AMPA-Type Glutamate Receptors

Disclosure: Nothing to disclose.

P702. Ghrelin Receptor Blockade, but not Depletion of Peripherally Circulating Ghrelin via Blockade of β1 Adrenergic Receptors, Decreases Binge-Like Drinking in Mice

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Background: Studies have linked the adrenergic system to alcohol-related behaviors in rodents and humans. Yet, most studies have used male subjects only and the results are, in some cases, incongruent. The adrenergic system modulates the release of ghrelin, another system linked to AUD, via β1

adrenergic receptors (β1AR). Ghrelin receptors (GHSR) are expressed both in the brain and the periphery. We and other groups have shown that GHSR blockade decreases alcohol consumption in rodents. Specifically, we found that both intraperitoneal and intracerebroventricular administration of GHSR antagonists reduced alcohol binge drinking in mice, whereas a vaccine-based sequestration of circulating ghrelin did not. Thus, our hypothesis is that binge-like drinking is reduced by central GHSR antagonism by a mechanism that is independent of peripherally circulating ghrelin. Thus, we aimed to investigate the relationship between ghrelin and β1ARs on binge-like drinking in male and female mice. We tested the hypothesis that β1AR blockade will reduce blood ghrelin levels, but this will have no effect on binge-like alcohol drinking.

Methods: "Drinking-in-the Dark" (DID) is a model of binge drinking validated in C57Bl6 mice. Mice have access to alcohol for 2 h or 4 h sessions and reach pharmacologically significant blood alcohol levels. We used 18 male and 18 female C57Bl6 mice 8-11 weeks of age. We administered two β1AR blockers intraperitoneally: atenolol (AT, peripherally restricted) and metoprolol (MT, brain permeable) in separate groups of mice. These drugs were administered 1.5, 13.5, and 25.5 h prior to the drinking session; the doses and treatment schedule were chosen from prior literature. Alcohol intake was measured in g/kg of body weight and results were analyzed by ANOVA or mixed-effects model with sex and dose as factors. To measure blood ghrelin levels, we collected blood from the submandibular vein into a tube coated with a protease inhibitor and EDTA. Blood was placed on ice and centrifuged for 15 min at 1600 g. Plasma ghrelin levels were measured by ELISA and analyzed by ANOVA. For behavioral experiments, JMV2959, a GHSR antagonist, and PF-5190457, a GHSR inverse agonist, were co-administered with vehicle, AT, or MT. JMV2959 was administered 20 min prior to drinking sessions and PF-5190457 was administered 60 min prior to drinking sessions.

Results: Blood measurements indicated that both MT ($p = 0.0076$) and AT ($p < 0.0001$) significantly decreased blood ghrelin levels. When administered alone, MT but not AT decreased binge-like alcohol intake ($p = 0.0096$). JMV2959 decreased alcohol intake alone and when co-administered with AT or MT ($p < 0.0001$). There was an additive effect seen when JMV2959 was co-administered with MT. PF-5190457 decreased alcohol intake alone and also when co-administered with AT or MT ($p < 0.0001$). We observed no significant sex differences in the results.

Conclusions: Results suggest that the blockade of central but not peripheral β1ARs reduces binge-like alcohol drinking in mice of both sexes. Although β1AR blockade significantly decreased blood ghrelin levels, it does not prevent GHSR blockers from reducing binge-like alcohol drinking. The combination of GHSR blockade and central β1AR blockade has additive effects in reducing drinking. In conclusion, the peripherally circulating ghrelin peptide itself may not drive binge-like drinking, but both β1AR and GHSR are possible therapeutic targets for AUD intervention. This work has translational relevance as PF-5190457 is the first GHSR blocker that has progressed to clinical development. We addressed a gap in the literature by providing novel information and physiologic mechanisms that support the role of both the β1AR and the ghrelin system in binge drinking, and an intersectional link between the ghrelin system, stress systems, and AUD. Future studies should elucidate the biological mechanisms by which these systems affect alcohol intake, and the potential additive effects of MT and GHSR blockade should be further investigated in AUD.

Keywords: Binge Drinking, Ghrelin, Noradrenergic System, Consummatory Behavior, Reward

Disclosure: Nothing to disclose.

P703. Gabapentin Enhances the Reinforcing and Unconditioned Behavioral Effects of Opioids

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Background: Recent epidemiological studies suggest significant misuse of gabapentinoids (gabapentin and pregabalin) in people with opioid use disorder and that co-use of gabapentinoids and opioids increases the risk of opioid-related death. Post-mortem studies have identified gabapentinoids in up to 40 percent of fatal drug overdoses, almost exclusively those involving opioids. The number of gabapentinoid prescriptions is also on the rise, with the majority being for off-label indications. Despite these alarming trends, little research has evaluated potentially harmful interactions between gabapentinoids and opioids. Individuals with opioid use disorder that use gabapentinoids often report that gabapentinoids enhance the euphoric/positive reinforcing effects of opioid drugs. This study characterized the behavioral effects of gabapentin alone and in combination with heroin, and the effects of gabapentin on self-administration of heroin, buprenorphine, and cocaine in rats.

Methods: Male Sprague Dawley rats were trained to self-administer heroin (0.01 mg/kg/infusion), buprenorphine (0.1 mg/kg/infusion), or cocaine (0.32 mg/kg/infusion) first under a fixed ratio 1 timeout 5 seconds schedule of reinforcement where one lever press resulted in an infusion of drug followed by a 5-second timeout, and then under a progressive ratio schedule of reinforcement in which the response requirement increased for each infusion earned. Following acquisition of drug self-administration and stable responding under the progressive ratio schedule of reinforcement saline was substituted for the training drug until behavior stabilized at fewer than 5 infusions per session. Rats were then randomized to a dose of the training drug (heroin: 0.0032-0.1 mg/kg/infusion, buprenorphine: 0.0032-0.1 mg/kg/infusion, cocaine: 0.01-1 mg/kg/infusion). Rats self-administered a dose of drug for at least 3 days until consecutive days differed by no more than 1 infusion earned. The following day animals were pretreated with gabapentin (1-32 mg/kg) i.v. 15 minutes prior to the self-administration session. Self-administered drug unit dose and pretreatment condition were evaluated in a pseudo-random order until dose-effect curves were determined under each pretreatment condition. The number of infusions earned and the breakpoint (the number of responses required for the last infusion earned) at each dose were compared across pretreatment condition.

In a second experiment 7 male and 7 female Sprague Dawley rats received gabapentin (32-230 mg/kg), heroin (0.56-1.78 mg/kg) or a combination of gabapentin and heroin s.c. All rats received all test conditions in a pseudo-random order. At 1, 2, 3, 5, and 7 hours following administration rats underwent 5 minutes of behavioral observation followed by a series of behavioral tests including the rotarod and horizontal bar tests. During the observation periods rats were scored for 26 common behaviors. Scores for each behavior and latencies on the rotarod and horizontal bar were compared across treatment conditions.

All procedures were approved by the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee.

Results: Animals self-administered heroin, buprenorphine, and cocaine in a dose-dependent fashion with breakpoints significantly greater than for saline. The heroin dose-effect curve peaked at 0.032 mg/kg/inf with a maximum of ~9 infusions earned. Pretreatment with 32 mg/kg gabapentin did not alter the maximum number of infusions earned of heroin but shifted the

curve leftward such that this maximum occurred at the 0.01 mg/kg/inf unit dose. Buprenorphine maintained similar levels of responding (~6 infusions) across doses spanning 0.01-0.1 mg/kg/inf. Pretreatment with gabapentin increased the number of infusions earned at each dose of buprenorphine tested. Rats self-administered a maximum of ~15 infusions of 1 mg/kg/inf cocaine and pretreatment with gabapentin did not alter the potency of cocaine or the maximum number of infusions earned.

Gabapentin dose and time dependently reduced latency to fall from the rotarod but did not alter latency to move from the horizontal bar. Heroin did not alter rotarod performance but increased latency to move from the horizontal bar. Heroin dose and time dependently suppressed overall activity of rats during observation periods and increased immobility and presence of flat body posture. These duration and magnitude of the effects of heroin were enhanced when given together with gabapentin. No significant sex differences were observed in the effects of heroin or gabapentin.

Conclusions: Gabapentin enhanced the reinforcing effects of both heroin and buprenorphine, consistent with reports in humans that gabapentinoids can enhance the euphoric/positive reinforcing effects of opioids. That gabapentin did not alter the reinforcing effects of cocaine is consistent with the low prevalence of gabapentin co-use with cocaine in humans. Gabapentin enhancing the sedative effects of opioids is also consistent with clinical reports describing increased risk of falls or other adverse events when gabapentinoids are co-prescribed with opioids. The current study highlights significant interactions between gabapentinoids and opioids related to both opioid overdose and substance misuse that warrant further investigation.

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Keywords: Opioids, Gabapentin, Polysubstance Abuse, Self-Administration

Disclosure: Nothing to disclose.

P704. New Developments on Fluornitrazene; a Novel Synthetic Mu Opiate Agonist

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Background: Mu opioid receptor (MOR) agonist medications are the most effective analgesics available. However, such medications have known adverse effects including respiratory depression and abuse liability. As such, there is a concerted effort to develop efficacious MOR agonists with high therapeutic indices with biased agonist properties. A tool for such an endeavor involves the development of a MOR-selective radiotracer for in vivo target engagement studies using positron emission tomography (PET). [11C]Carfentanil is the only MOR-selective agonist PET radiotracer available, but it has high potency and restricted accessibility, making an [18F]-labeled MOR-selective agonist radiotracer with lower potency and longer half-life a preferable alternative. The aim of these studies was to characterize a novel fluorinated etonitazene analog (aka, fluornitrazene (FNZ)) and assess its potential to be radiolabeled and used as a MOR selective in vitro [3H]-labeled radioligand and in vivo [18F]-labeled PET radiotracer.

Methods: To assess selectivity and propensity for brain entry, FNZ was screened against a panel of >100 receptors/enzymes and its drug transporter inhibition activity at 100 nM and 10 μM. FNZ and [3H]FNZ were studied via competitive binding assays using rat brain membranes incubated in buffer solutions of 10 nM [3H]DAMGO (46 Ci/mmol) or 1 nM [3H]FNZ (43 Ci/mmol) with increasing concentrations of unlabeled DAMGO and FNZ. Non-specific binding was determined in the presence of 100 μM naloxone. One-site competition curves were fitted, and Ki values were calculated using the Cheng-Prusoff equation. FNZ was also evaluated in its propensity to stimulate cAMP and β arrestin-signaling using HEK293 cells transfected with hMOR cDNA and the respective genetically-encoded sensors. To determine in vivo binding kinetics, [18F]FNZ was synthesized (specific activity: 17,500 mCi/μmole) and injected intravenously into rats and squirrel monkeys and scanned with PET for 60-minutes. To further validate the in vivo kinetics, ex vivo experiments were designed to inject [3H]FNZ (i.v.) and collect tissue 7-minutes post-injection for autoradiography visualization. The behavioral and physiological effects of FNZ were assessed using intravenous self-administration (IVSA) and by testing antinociception, catalepsy, body temperature, and oxygen levels.

Results: The screening data found that at 100 nM, FNZ inhibited binding of only MOR. In the efflux transporter panel, at 100 nM, FNZ inhibited the activity of only OCT2 and MATE2-K, which are peripheral efflux transporters. Competitive binding assays against [3H]DAMGO showed that FNZ had a Ki = ~1.0 nM. Similarly, [3H]FNZ showed a Kd = ~1.3 nM. FNZ showed an EC50 of ~0.1 nM and Emax ~100% for cAMP and EC50 of ~10 nM and Emax ~100% for β arrestin. The time activity analysis in rats and squirrel monkeys found that [18F]FNZ rapidly enters the brain and exits within 20-30 minutes. While in the brain, [18F]FNZ accumulates in MOR-rich areas and this accumulation can be blocked by pretreatment with naltrexone. The ex vivo data found [3H]FNZ in MOR-rich areas with the signal blocked by pretreatment with naloxone. Self-administration data showed that FNZ is reinforcing, however, priming with FNZ does not reinstate active lever pressing after extinction. Additionally, pretreatment with FNZ decreased heroin self-administration at doses lower than those that decrease food self-administration. FNZ produces max antinociception at 10 μg/kg with minimal catalepsy and no temperature decrease. Changes in oxygen responses induced by FNZ and fentanyl were similar in both the brain and periphery.

Conclusions: We show how a novel drug can be characterized by using several in vivo / vitro radiometric and behavioral assays to gain an understanding of its mechanism as a potential pharmaceutical. The results show that FNZ is a selective MOR agonist and [3H]FNZ exhibits favorable properties as an in vitro radioligand. FNZ exhibits minimal interaction with efflux transporters and both [18F]- and [3H]- FNZ showed rapid uptake and accumulation in MOR-rich brain areas, consistent across species, which bolsters its translational potential. Although reinforcing, once FNZ self-administration is terminated, rats do not reinstate after FNZ priming. The effective dose of FNZ to promote pain suppression was in a range insufficient to induce adverse effects such as catalepsy and hypothermia. Due to the similarity in oxygen responses between FNZ and fentanyl, we aim to complete the safety profile of FNZ to determine toxicity and long-term usage.

Keywords: Mu-Opioid Receptor Agonist, PET Imaging, Drug Development

Disclosure: Nothing to disclose.

P705. Adolescent Delta-9-Tetrahydrocannabinol (THC) Exposure Impacts Cortical Contributions to Working Memory in Male and Female Rats

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Background: The ability to maintain and manipulate information within working memory is crucial to cognition and control over behavior, and the prefrontal cortex (PFC) is necessary for this process. THC exposure that occurs during vulnerable timepoints, such as adolescence, can produce divergent effects on neuronal development and relevant behaviors that can be measured well into adulthood. Multiple factors can influence the extent of these effects, including the magnitude of drug exposure and sex. Adolescent THC exposure can influence working memory performance in adulthood and may promote or impair performance based on dose and sex. THC exposure during adolescence can also promote long-lasting changes to the development of the PFC which may impact working memory. We investigated how adolescent THC exposure impacts the contribution of prefrontal cortical neurons to working memory task performance.

Methods: Male and female rats were chronically implanted with indwelling jugular catheters for intravenous self-administration of up to 0.1 mg/kg/infusion THC or a control vehicle solution during adolescence (P31-P52). Rats remained in the homecage until reaching adulthood (P70+), where they were then trained on a delayed-match-to-sample working memory task. Rats were trained to respond into an illuminated aperture and wait throughout a variable delay phase ranging from 2-24 seconds. After the delay phase, three adjacent apertures were illuminated, and the rat responded into the previously selected aperture to receive a sucrose pellet reward. Behavioral testing was conducted with concurrent single photon calcium imaging recordings. Rats expressed the genetically encoded calcium sensor, AAV1.CaMKII.GCaMP6f in the prelimbic PFC and imaging was conducted during working memory test sessions where animals performed the task under increasingly difficult delay periods.

Results: Adolescent male and female rats successfully completed THC self-administration with no significant differences between sex emerging for active lever presses, inactive lever presses, or the number of infusions received ($p>0.1$ for all comparisons). Adult rats ($N = 9$ female, $N = 6$ male) were then imaged throughout a total of 9 sessions while performing increasing difficult versions of the working memory task. $N = 51$-207 cells/animal were recorded from the prelimbic prefrontal cortex of THC-exposed or control animals. Adolescent THC exposure resulted in a reduction in calcium events only in females compared to unexposed controls ($p < 0.05$). THC-exposed animals exhibited a distinct pattern of activity from significantly modulated neurons during the delay preceding correct responses, while no difference emerged between drug-exposed animals and controls prior to an incorrect response ($p < 0.05$). Additionally, the proportion of neurons recruited immediately prior to a correct response was significantly higher in THC-exposed animals, regardless of sex ($p < 0.05$). **Conclusions:** These results indicate that adolescent THC exposure can produce long-lasting changes in PFC contributions to working memory performance, and that sex may interact with THC exposure to influence how cells are recruited to maintain information within working memory.

Keywords: Cannabinoid, Adolescent, Prefrontal Cortex

Disclosure: Nothing to disclose.

P706. Metabotropic mGlu1 Receptor Regulation of Cortical Inhibition and Cognitive Function: Implications in Adolescent Cocaine Exposure

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Background: Exposure to psychostimulants, such as cocaine, during adolescence produces persistent changes in the prefrontal cortex (PFC) which parallel cognitive deficits seen in adulthood. Further, adolescent exposure to psychostimulants impairs inhibitory transmission in the PFC in adulthood, suggesting that enhancing PFC inhibitory transmission may be a promising strategy to reverse drug-induced cognitive deficits. Activation of the mGlu1 subtype of metabotropic glutamate receptor increases inhibitory transmission in the PFC and working memory by selective excitation of somatostatin-expressing GABA interneurons (SST-INs). Therefore, we hypothesize that repeated exposure to cocaine during a critical developmental period in adolescence disrupts PFC inhibition via SST-INs and drives working memory impairments in adulthood which can be mitigated by activation of mGlu1.

Methods: Male and female SST- and PV-Ai9 tdTomato mice were injected once daily with cocaine (20 mg/kg, i.p.) for 7 days (postnatal day 35-42). Whole-cell patch-clamp electrophysiological recordings from SST and PV interneurons within the PFC were conducted between 10-12 weeks of age. Additionally, touchscreen-based automated cognition testing was used to determine working memory performance in adult mice. Novel mGlu1 positive allosteric modulators (PAMs) were leveraged in behavioral and electrophysiology studies to determine their procognitive efficacy and mechanism of action within the PFC.

Results: We found that repeated administration of cocaine during a critical adolescent period impaired PFC SST-IN, but not parvalbumin-expressing interneuron (PV-IN), firing compared to saline-treated mice. Adolescent cocaine exposure significantly decreased the frequency of spontaneous excitatory postsynaptic currents onto SST-INs but not PV-INs. These findings were paralleled by adolescent cocaine-induced impairments in spatial working memory in adulthood. Importantly, these physiological and behavioral effects of adolescent cocaine exposure were reversed by selective mGlu1 activation. Lastly, repeated amphetamine administration during the same adolescent critical period did not result in impaired SST-IN function or spatial working memory in adulthood.

Conclusions: These studies show that: 1) cocaine, but not amphetamine, exposure during an adolescent critical period induces persistent and selective deficits in PFC SST-IN function and cognition in adulthood and 2) selective activation of mGlu1 with PAMs represents a novel strategy for reversing cocaine-induced cognitive impairments. Together, these studies provide insight to the cell type- and brain region-specific consequences of adolescent drug exposure and lasting effects on cognitive performance in adulthood while highlighting a potential therapeutic avenue to treat these deficits.

Keywords: Adolescence- Critical Period, Cocaine Use Disorder, Cognition, Electrophysiology, Working Memory

Disclosure: Nothing to disclose.

P707. Improved Pharmacokinetics of Dopamine D4 Receptor-Selective Ligands Trough Bioisosteric Replacement of Amide With 1,2,3-Triazole-Linked Analogues

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Background: The neurotransmitter dopamine signals through G protein-coupled receptors that regulate a variety of neurophysiological functions, including movement, emotional regulation,

motivation, and cognition. Dopamine D4 receptors (D4Rs) are enriched in the hippocampal and prefrontal cortical regions of the brain that are critical for attention, cognition, memory formation, and decision making. While the physiological relevance of D4R signaling in the brain is not fully understood, preclinical studies indicate that D4R-selective ligands can improve outcomes in animal models of neuropsychiatric disorders with deficits in cognition and behavioral control, including Alzheimer's disease, ADHD, and substance use disorder (SUD). There are no currently FDA-approved medications that selectively target the D4R. We recently reported a library of novel D4R-selective ligands of varying efficacies, based on a phenylpiperazine scaffold, to investigate D4R function in preclinical models. However, the in vivo utility of some of these ligands was limited by rapid phase I metabolism, which invariably cleaved an amide bond in the molecular template. The objective of this study was to develop metabolically stable ligands with high D4R binding affinity and subtype selectivity.

Methods: We extended our previous structure activity relationship studies by exploring modifications to amide bond segments of the A-412997 analogues to overcome bioavailability and metabolic shortcomings of the D4R compounds. The new analogues were designed and synthesized by using a click chemistry reaction approach to bioisosterically replace the amide with 1,2,3-triazoles. Compounds were purified and analytically characterized followed by CHN combustion elemental analysis. In vitro binding affinities were determined via [3H]N-methylspiperone radioligand binding using membranes prepared from HEK293 cells expressing dopamine D2-like receptors (D2R, D3R, D4R). The ligands were also studied in β-arrestin recruitment assays for their effects on D2R-like function. We further performed in vitro and in vivo pharmacokinetic analyses with selected compounds.

Results: We produced a new library of novel ligands featuring a bioisosteric replacement of the amide linker with a 1,2,3-triazole moiety using click chemistry methods. Compounds were profiled using radioligand binding displacement assays and β-arrestin recruitment assays. Metabolic stability in rat and human liver microsomes, followed by in vivo pharmacokinetics analyses were performed on a subset of ligands. We identified several compounds with nanomolar D4R binding affinity and excellent D2-like subtype selectivity (≥705 versus D2R and D3R). The compounds displayed D4R partial agonist or antagonist activity in functional β-arrestin recruitment assays. Based on the analysis profiles, lead compounds were selected for in vitro metabolic stability in rat and human liver microsomes where they displayed improved stability profile, versus the respective amide analogues. Of these, the most promising compound was selected for in vivo pharmacokinetic assessment in rats where it displayed good brain penetration.

Conclusions: The pharmacological analyses indicate that the 1,2,3-triazole compounds maintain binding and functional profiles like their matching amide analogues with improved metabolic stability. The 1,2,3-triazole moiety is more resistant to drug metabolism with advantageous pharmacokinetic profiles. Further in vivo analyses of these compounds may provide insights into targeted drug discovery leading to a better understanding of the role of D4Rs in neuropsychiatric disorders, such as SUD.

Keywords: Substance Abuse Disorders, Dopamine D4 Receptor, Triazole, Partial Agonist Ligands

Disclosure: Nothing to disclose.

P708. Operant Self-Administration of Oral Nicotine in Mice

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Background: Smoking is a major global health issue. Cigarettes and other tobacco products have a substance called nicotine that makes people want to keep using them, leading to addiction. While we don't fully understand how nicotine addiction works in the brain, it's crucial to study it more closely. Unfortunately, there is currently no simple and dependable way to study nicotine addiction in mice using a self-administration method. Our aim is to create a mouse model where mice can choose to take nicotine on their own, which will help us better understand how nicotine addiction happens in the brain.

Methods: We conducted a study using adult male and female mice of the C57BL/6J strain. The mice were given access to two bottles, one containing water and the other containing nicotine solutions. This went on for 4 weeks, during which the concentration of nicotine in the solutions was gradually increased from 15 to 480 micrograms per milliliter. After the two-bottle choice phase, the mice were given mecamylamine and then tested in an open field and elevated zero maze to check for possible nicotine withdrawal effects. Next, the mice were trained to perform an operant self-administration task to get oral nicotine rewards.

Results: We observed that the mice consumed more of the 30 micrograms/ml nicotine solution compared to higher concentrations. However, they ended up consuming the highest total amount of nicotine (measured in milligrams per kilogram of body weight per day) when given the highest concentration of 480 micrograms/ml. Female mice consumed more nicotine than male mice, especially in the concentration range of 60 to 120 micrograms/ml. Based on these results, we focused on female mice for the subsequent experiments.

After the mecamylamine treatment, the mice given nicotine showed more activity in their movement compared to the control group, which only had water in their home cages. However, there was no significant difference between the two groups in the elevated zero maze test.

The mice learned to increase lever responses for oral nicotine rewards during 2-hour sessions. They responded more to the active lever than the inactive one when using a fixed-ratio 2 schedule. Changing the schedule or increasing the nicotine concentration did not significantly affect their response patterns. We also tried a progressive-ratio schedule with different nicotine concentrations and a varenicline treatment, but these didn't lead to significant changes in operant responses either. However, the mice failed to differentiate between active and inactive levers when the nicotine reward or conditioned cues were removed, although their responses didn't decrease significantly.

Conclusions: The C57BL/6J mice readily consumed nicotine solutions without any coercion or the need to mix them with sweeteners. Female mice consumed more nicotine than males. The mecamylamine-precipitated withdrawal resulted in increased locomotor activity in the mice given nicotine, but it didn't produce noticeable differences in the elevated zero maze test. The results suggest that the withdrawal symptoms may have been relatively mild.

During the 4-week study, the mice that consumed nicotine learned to respond to the lever that delivered the nicotine solution and could differentiate between the active and inactive levers. On the other hand, the mice that were rewarded with water didn't reliably respond to the levers or distinguish between them. However, after acquiring lever responses for oral nicotine, the mice didn't show significant changes in response to different nicotine concentrations or varenicline treatments. This indicates that oral nicotine may lead to strong habitual responses that are not easily influenced by manipulations of nicotinic receptors. The operant oral nicotine self-administration model could still be useful for studying the neural mechanisms involved in acquiring nicotine self-administration and habit formation.

Keywords: Nicotine Addiction, Oral Self-Administration, Habit Formation

Disclosure: Nothing to disclose.

P709. Interactions Between Fentanyl and Alpha-2 Adrenergic Agonists in Assays of Drug Self-Administration in Rats

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Background: Illicit fentanyl is increasingly being adulterated with alpha-2 agonists including xylazine. Unfortunately, there are a paucity of studies that have examined opioid and alpha-2 agonist interactions on measures of drug reinforcement. Preclinical drug self-administration procedures have demonstrated utility to improve our basic understanding of drug-drug interactions on measures of reinforcement.

Methods: Male (n = 7) and female (n = 7) Sprague-Dawley rats were implanted with jugular catheters and trained to respond for fentanyl infusions under a terminal fixed-ratio (FR5) schedule of reinforcement during daily 2h sessions. Experiment 1 determined the dose-effect function of fentanyl (0.32-10 ug/kg/infusion), xylazine (1-320 ug/kg/infusion), lofexidine (0.32-32 ug/kg/infusion), and clonidine (3.2-32 ug/kg/infusion) using a substitution procedure. Experiment 2 determined fentanyl and alpha-2 agonist interactions where FR values (1,3,6,10,18,32,56,100,180,320,560, 1000) were increased every day during availability of fentanyl alone (3.2 ug/kg/infusion) alone and in combination with varying fixed-proportions of xylazine (3.2 ug/kg/infusion) until the rat failed to complete the response requirement. Behavior was rebaselined at 3.2 ug/kg/infusion fentanyl before testing another fentanyl/xylazine fixed-proportion mixture. Behavioral economic demand curves related drug or drug mixture intake to FR price, and reinforcement measures were derived. Animal maintenance and research were conducted in accordance with the 2011 Guidelines of the National Institutes of Health Committee on Laboratory Animal Resources. Both enrichment and research protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Results: Fentanyl alone functioned as a reinforcer in both male and female rats. Xylazine, lofexidine, and clonidine did not function as reinforcers up to doses that produced motor incoordination. Behavioral economic measures of reinforcement including alpha and essential value were significantly decreased when 10 ug/kg/infusion xylazine was combined with 3.2 ug/kg/infusion fentanyl compared to fentanyl alone. Studies are ongoing with larger and smaller fixed-proportion mixtures of xylazine and fentanyl. Preliminary analysis of sex differences did not detect any significant sex differences likely due to being underpowered to detect sex differences.

Conclusions: The present results suggest 3 main findings. Alpha-2 agonists such as xylazine, lofexidine, and clonidine do not function as reinforcers in male and female rats under a simple FR5 schedule of reinforcement. Male and female rats self-administered fentanyl under both the FR5 schedule and between-session progressive-ratio schedule of reinforcement consistent with the extant literature. Overall, these results do not suggest that xylazine adulteration of illicit fentanyl is enhancing the addictive effects of fentanyl in nonopioid-dependent subjects. Future studies will determine other fentanyl/xylazine fixed-proportions and compare results to fentanyl/lofexidine fixed-proportion mixtures.

Keywords: Fentanyl, Behavioral Economics, Drug-Drug Interaction, Drug Self-Administration, Xylazine

Disclosure: Nothing to disclose.

P710. Synthetic Contraceptive Hormones Occlude the Ability of Nicotine to Reduce Ethanol Consumption in Ovary-Intact Female Rats

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Background: Tobacco and alcohol use disorders (TUD and AUD) are tremendous health liabilities, and co-use of these substances is highly prevalent. Women are particularly at risk, as AUD is increasing at alarming rates and long-term smoking cessation is more difficult to achieve in women. Ovarian hormones can affect frequency of binge drinking and cigarette craving in women, with increases in 17β-estradiol (E2) and progesterone being associated with addiction risk and resilience. Despite clinical literature demonstrating interactions between steroid hormones and polydrug use, little research has examined how contraceptive hormones may influence motivation for alcohol and nicotine. We determined the influence of synthetic estrogen (e.g., ethinyl estradiol, EE) and/or progesterone (levonorgestrel, LEVO) on nicotine and ethanol (EtOH) consumption using a sequential use model in female rats. We hypothesized that rats treated with LEVO alone will consume less nicotine and EtOH, and rats given EE +LEVO will show higher consumption of both drugs.

Methods: 77 ovary-intact Long Evans female rats first underwent 15 daily 4-hr drinking in the dark (DID) sessions, in which a 10% EtOH or vehicle (water) bottle was inserted into the homecage, with no access to water from the lixit. Rats in the EtOH group received an additional 12 sessions of two-bottle preference testing where rats were provided a water alternative. Rats underwent morning DID sessions, followed by subcutaneous injections of either LEVO (0.6 ug/0.1 mL sesame oil), EE+LEVO (0.6 ug LEVO + 0.18 ug EE+LEVO/0.1 mL sesame oil), or vehicle (0.1 mL sesame oil) and afternoon nicotine (0.06 mg/kg/infusion) or saline self-administration (SA) sessions on a fixed-ratio (FR)-1 schedule. Nicotine SA groups then underwent within-session nicotine demand where nicotine dose was manipulated. Finally, rats underwent additional EtOH preference testing and blood ethanol concentration analysis. All experimental protocols involving animal studies were approved by the Institutional Animal Care and Use Committee.

Results: All rats demonstrated consistent preference for EtOH over water (linear mixed effects modeling (LME); $p < 0.05$). Contrary to our hypothesis, EE+LEVO did not increase nicotine or EtOH consumption above vehicle levels. However, rats receiving vehicle treatments consumed less EtOH and nicotine when these substances were sequentially self-administered, indicative of economic substitution (LME; $p's < 0.05$). This effect was occluded in rats that were exposed to either EE+LEVO or LEVO alone (LME; $p's > 0.05$). Water control rats showed high EtOH preference following nicotine SA (LME; $p < 0.05$), and saline SA rats consumed more EtOH than their nicotine counterparts (LME; $p < 0.05$). BEC was positively correlated with EtOH consumption (linear regression analysis; $p < 0.05$). Finally, rats receiving EE +LEVO while undergoing saline SA consumed less EtOH than vehicle controls, which may have important implications for women who use contraceptives and drink alcohol but do not smoke.

Conclusions: The results show that EtOH and nicotine act as economic substitutes when they are consumed sequentially, and that chronic synthetic hormone exposure impacts nicotine and EtOH co-use and EtOH consumption in females. Future studies are needed to understand how chronic use of different contraceptive

formulations alter patterns of polydrug use in women to inform women's care by determining safer hormonal contraceptive use when women either only smoke, only drink, or concurrently drink and smoke.

Keywords: Nicotine, Ovarian Hormones, Alcohol

Disclosure: Nothing to disclose.

P711. Psilocybin Reduces Heroin Seeking Behavior and Modulates Expression of Cytokine Signaling Molecules in the Brain

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Background: The number of drug-related overdose deaths exceeded 100,000 for the first time ever in the United States in 2021, with opioids contributing to more than half of all overdose deaths. A critical goal for the reduction of opioid misuse is the maintenance of abstinence and prevention of opioid relapse following successful detoxification. Recent clinical trials have begun to explore the therapeutic benefits of the psychedelic compound psilocybin for assistance in circumventing perseverant maladaptive behaviors, including craving and use of nicotine and alcohol. Psilocybin acts as an agonist at the serotonin 2A receptor (5-HT2AR) and preclinical studies have established that pharmacological activation or inhibition of 5-HT2AR can impact seeking for psychostimulants, alcohol, and nicotine. While the gene that encodes 5-HT2AR, Htr2a, has been linked to heroin dependence, some studies suggest that 5-HT2AR agonists may decrease motivation for opioids. However, the impact of psilocybin on opioid relapse and maintenance is currently unknown.

Methods: The effects of systemic administration of psilocybin or the 5-HT2AR antagonist ketanserin on opioid self-administration and relapse were evaluated in a rat model of heroin self-administration. Adult male rats underwent jugular vein catheterization and self-administration of 0.075 mg/kg/infusion heroin on an FR1 schedule. Psilocybin or ketanserin (0.1, 1.0, or 3.0 mg/kg) were administered intraperitoneally either prior to a self-administration session, or after >2 weeks forced abstinence from heroin. Behavior during heroin self-administration or a cue-induced relapse session were measured to determine the impact of psilocybin or ketanserin on heroin intake or seeking. Rats were euthanized immediately after the relapse test for gene expression analysis of inflammatory cytokine and chemokines using a focused qPCR array. To determine the transcriptional consequences of psilocybin or ketanserin on gene expression in the prefrontal cortex (PFC), a region critical for the brain's reward circuitry, adult male drug-naïve rats were injected with 1.0 or 3.0 mg/kg psilocybin alone, or in combination with ketanserin. RNA-sequencing of mRNA expression patterns was performed on brain tissue collected 24 hours later and data was analyzed using DeSeq2 to identify differentially expressed genes, as well as rank-rank hypergeometric analysis (RRHO).

Results: Administration of 0.1-3.0 mg/kg psilocybin 4 hours prior to heroin self-administration did not alter heroin intake. Pretreatment with 3.0 mg/kg ketanserin 30 minutes prior to heroin self-administration increased heroin infusions, while lower doses had no impact. 3.0 mg/kg psilocybin administered either 4 or 24 hours prior to a relapse test significantly reduced cue-induced heroin seeking following forced abstinence. Conversely, 3.0 mg/kg ketanserin significantly increased heroin seeking behavior following forced abstinence. 0.1 or 1.0 mg/kg ketanserin or psilocybin did not impact heroin seeking during the relapse test. In drug-naïve animals, 3.0 mg/kg psilocybin induced regulation of 314 genes in the PFC, while 1.0 mg/kg psilocybin regulated the expression of 177 genes and a subset of genes were overlapping the two treatments. RRHO analysis determined that >90% of the effects of 3.0 mg/kg psilocybin on PFC gene expression were blocked by co-administration of 3.0 mg/kg ketanserin. One of the most significant KEGG pathways enriched by 24-hour exposure to 3.0 mg/kg psilocybin included genes involved in cytokine signaling. A focused qPCR array of PFC and nucleus accumbens tissue from heroin self-administration rats that displayed psilocybin-mediated inhibition of relapse revealed significant dysregulation of distinct cytokine or chemokine molecules in the two brain regions.

Conclusions: Pharmacological manipulation of 5-HT2AR signaling modulates heroin seeking and a single administration of psilocybin prior to a cue-induced relapse test is sufficient to reduce heroin seeking behavior. Ketanserin is adequate to block psilocybin-induced regulation of gene expression in the PFC. The highest doses of psilocybin and ketanserin examined in this study produced opposing effects on both heroin relapse behavior, as well as PFC gene expression. Psilocybin-mediated inhibition of heroin seeking is accompanied by regulation of inflammatory cytokine and chemokine genes in the brain.

Keywords: Opioid Craving, Psychedelics, RNA-Sequencing, Heroin Self-Administration

Disclosure: Nothing to disclose.

P712. THC Improved Learning in HIV-1 Transgenic Rats Without Affecting Wildtype Littermates, While They Did Not Differ in THC-Induced Physiological Responses

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Background: People with HIV-1 (PWH) use cannabis at high rates and the impact of such drug intake on HIV-associated neurocognitive impairment is not clear but is likely function-dependent. Given the difficulties in determining directionality of effects in humans, the HIV-1 transgenic (TG) rat provides a reliable model of neuroHIV in the current age of combination antiretroviral therapy, where viral replication is suppressed, and chronic low-level infection persists. Here, we tested the impact of acute and chronic delta-9-tetrahydrocannabinol (THC) on an operant battery of cognitive tasks in HIV-1 TG rats and their wildtype (WT) littermates (Experiment 1). We also examined possible genotypic differences in physiological responding to THC using the cannabinoid tetrad test (Experiment 2).

Methods: Experiment 1: Male HIV-1TG and WT ($n = 17$ and 21 respectively) rats were baseline tested in the Iowa Gambling Task (IGT; to measure risky decision-making), the Probabilistic Reversal-Learning Task (PRLT; to measure learning and cognitive flexibility), and the Progressive-Ratio Breakpoint Task (PRBT; to measure motivation). Rats were retested following acute and chronic THC (0, 0.3, or 3 mg/kg) exposure ($n = 6$-7/group). Experiment 2: Male and female TG and WT (62% male; TG $n = 39$ and WT $n = 45$), rats were administered THC (0, 0.3, or 3 mg/kg), used in cognitive testing and body temperature (rectal), nociception (tail flick response) and locomotor/exploratory (behavioral pattern monitor; BPM) behavior were assessed ($n = 3$-11/group). Data were statistically analyzed using ANOVAs. Rats were bred, raised, and maintained in a dedicated animal facility approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All procedures were approved by the University of California San Diego Animal Care and Use Committee and

adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: Experiment. 1: In the IGT at baseline, HIV-1TG rats exhibited more premature responding relative to WT rats [$F(1,36) = 5.54$, $p < 0.05$], indicative of heightened motoric impulsivity. During both acute and chronic IGT tests genotype * dose interactions [acute: $F(2,38) = 5.6$, $p < 0.01$; chronic: $F(2,30) = 3.4, p < 0.05$], revealed high dose THC reduced total trials in WT, but not TG rats. No effect of genotype, dose, or interaction was observed for risky decision-making at acute or chronic timepoints. In the PRLT acute THC had no effect on the number of reversals. Chronic THC in the PRLT tended to reduce the number of reversals irrespective of genotype [$F(2,34) = 2.9, p < 0.075$]. A trend genotype * dose interaction revealed that low dose THC improved initial learning in TG rats relative to vehicle-treated rats at both acute [$F(2,21) = 2.5, p = 0.1$] and chronic [$F(2,27) = 3.1, p < 0.06$] timepoints. In the PRBT, acute high dose THC reduced motivation across genotypes [$F(2,32) = 12.2, p < 0.001$], with this effect disappearing during chronic testing, suggestive of tolerance to THC-induced motivational deficits.

Experiment 2: As expected, THC produced dose-related decreases in nociception across sex and genotype [$F(1,64) = 16.376$, $p < 0.001$]. A sex * drug * dose interaction [$F(2,56) = 6.8$, $p<0.002$] revealed high-, but not low-dose THC, reduced body temperature in female, but not male rats. In the BPM, high dose THC significantly decreased activity [as measured by a dose effect on rears $F(2,72) = 3.4$, $p < 0.05$] and increased linear movements [as measured by spatial d; $F(2,72) = 6.0$, $p = 0.01$]. WT animals also exhibited more circumscribed movements (higher spatial d) compared to TG rats [$F(1,72) = 6.3, p < 0.05$], while no other genotypic differences were observed.

Conclusions: HIV-1TG rats exhibited elevated rates of motoric impulsivity relative to WT littermates, but exhibited few other differences at baseline. Importantly, THC selectively improved the learning rate of TG rats, an effect not seen in the WT controls. In other measures (e.g., reversal learning), THC exerted consistent deleterious effects in both TG and WT rats. The impact of acute THC also differed from chronic effects, wherein the amotivational impact seen after acute THC was lessened after chronic THC. These results support the premise that the cognitive effects of THC on cognition in PWH may be task-dependent, and that chronic treatment may be required to reveal beneficial effects. Finally, these genotypic cognitive differences were evident despite cannabinoid tetrad testing indicating similar physiological responses to the THC doses used in cognitive testing across genotype. Altogether, these findings implicate that THC differentially affects cognitive performance in HIV-1TG vs. WT rats in a way that is not accounted for by differences in physiological responsiveness to the drug.

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Keywords: THC, HIV, Cognition, Decision-Making, Reinforcement Learning

Disclosure: Nothing to disclose.

P713. PDGFRβ Selectively Mediates the Expression of Fentanyl Reward

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Background: Despite the current opioid epidemic, opioids remain the gold standard for the treatment of chronic severe pain. Identification of novel pathways that selectively mediate side-effects downstream of the μ-opioid receptor (MOR, target of clinically used opioids) are needed to develop treatments to increase opioid safety and prevent opioid use disorders (OUD, i.e., addiction). Opioid-activated MORs have been known to recruit receptor tyrosine kinase (RTK) signaling in vitro and in vivo. We provided physiological relevance to this phenomenon by discovering that inhibition of RTKs, such as the platelet-derived growth factor receptor beta (PDGFRβ), in pain processing neurons in the spinal cord, could selectively block opioid analgesic tolerance (side-effect causing reduced analgesia overtime). These findings showed that PDGFRβ signaling could selectively mediate opioid side-effects downstream of MOR. In the current pilot study, we aimed to determine if PDGFRβ could also be targeted to block opioid reward, a side-effect that promotes the development of OUD.

Methods: For these studies, 8–12-week-old female and male C57BL6/J mice (Jackson laboratories) were used. In previous work, we showed that opioid-activated MORs can phosphorylate PDGFRβ via the release of the platelet-derived growth factor type B (PDGF-B) ligand. Therefore, we performed a neuroanatomical study, to determine if PDGF-B, PDGFRβ could be co-expressed with MOR in brain structures that are essential in opioid reward, such as the ventral tegmental area (VTA) and nucleus accumbens (NAc). Mice brains ($N = 2$/sex) were collected and processed for in situ hybridization (RNAscope, ACDBio) using probes targeting Pdgfb, Pdgfrβ, and Oprm1 mRNAs. QuPath software was used for cellular segmentation to analyze images acquired with a wide-field fluorescence microscope (BZ-8000, Keyence).

Next, to test if PDGFRβ signaling could be involved in opioid reward, we used the mouse conditioning place preference (CPP) assay, which consists in conditioning mice to receive a reinforcing substance in a specific environment and test the rewarding effect of that substance by scoring time spent in the paired environment in the absence of the drug. In these pilot experiments, mice were conditioned to receive saline or fentanyl in a paired environment ($N = 4$ mice per sex). Imatinib (PDGFRβ inhibitor) was either co-administered with fentanyl during conditioning or only administered on the last day before testing. CPP behavioral data was analyzed using one-way ANOVA (GraphPad Prism software), $p < 0,05$ was considered significant.

Results: RNAscope studies showed that Pdgfrβ and Pdgfb mRNAs are abundantly expressed in VTA and NAc. Interestingly, only the Pdgfb ligand was co-expressed in cells expressing Oprm1, while Pdgfrβ was mostly only present in cells in the vicinity of Oprm1 cells.

In the CPP assay, when co-administered with fentanyl during conditioning, imatinib did not impact fentanyl place preference, showing that PDGFRβ inhibition did not have an impact on the development of fentanyl reward. However, when imatinib was only administered after the conditioning period, right before testing, it completely blocked fentanyl place preference. This sticking result showed that PDGFRβ inhibition was able to block the expression of fentanyl reward. Importantly, analysis of sex as biological variable did not show any sex effect. Also important, imatinib did not induce conditioning when administered by itself.

Conclusions: Our results show that PDGF-B and PDGFRβ are expressed in brain structures that express MOR and that are involved in opioid reward. Co-expression of PDGF-B with MOR suggests that opioid-activated MORs could recruit PDGFRβ signaling in a paracrine manner via the release of PDGF-B. We also discovered that imatinib, a PDGFRβ inhibitor, can effectively block the expression of fentanyl reward.

Together with our previous findings showing that PDGFRβ inhibition can block opioid tolerance without altering analgesia, these exciting preliminary findings, suggest that PDGFRβ could be targeted to prevent multiple opioid side-effects, and therefore increase opioid safety, and prevent OUD.

Future studies are underway to understand the precise brain circuits underlying PDGFRβ-mediated expression of reward, and test the potential for PDGFRβ inhibitors to prevent/treat OUD.

Keywords: Mu Opioid Receptor, Platelet Derived Growth Factor, Opioid Side-Effects, Opioid Reward

Disclosure: Nothing to disclose.

P714. Microdosing Partial Agonists Inactivates Kappa Opioid Receptors - An Alternative Strategy for Human Trials

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Background: Preclinical studies have established the role of endogenous dynorphins in mediating the anxiogenic, dysphoric, cognitive-disrupting, and proaddictive effects of stress. Kappa opioid receptor (KOR) antagonists able to block endogenous dynorphins' effects have therapeutic potential and selective KOR antagonists show promise in human clinical trials. Two pharmacological types of selective KOR antagonists have been described – reversible competitive (e.g. Aticaprant and NMRA-140) and KOR-inactivating antagonists (e.g. norBNI and JDTic); however, which type would be more effective is not yet clear. NorBNI produces its long-lasting KOR inactivating effects by modifying G-protein binding to KOR through a previously described cJun kinase (JNK)-PRDX6-peroxide cascade. In this study, we report that daily treatment with low doses of nalfurafine (a G-biased KOR agonist) or nalmefene (a nonselective mu opioid antagonist/KOR partial agonist) also inactivates KOR through the JNK mechanism. Because both nalfurafine and nalmefene have long-established safety profiles for human use (as antipruritic and mu-opioid antagonist medications, respectively), their potential utility as KOR-inactivating antagonists for human therapeutics seems plausible.

Methods: Adult male and female C57BL6 mice were injected daily for 7-days with low doses of vehicle, norBNI (0.1-30 mg/kg), nalmefene (0.1 ug-10 mg/kg), or nalfurafine (10 ng-1mg/kg), and the KOR-mediated analgesic, aversive, prolactin-releasing, diuresis, and antipruritic responses were determined ($n = 8$-12 for all doses). Anti-nociception was measured using tail-withdrawal from 52.5 o C water. Stress-induced aversion was measured using odorant-paired conditioned avoidance. Prolactin release was measured by ELISA in sera collected 60 min after 100 ug/kg nalfurafine. Diuresis was measured by weighing blotter paper 30 min after nalfurafine injection. Itch was measured following injection of 30 ug/kg 5'-GNTI. KOR activation of JNK was measured using the novel peroxide sensor oROS (AAV1-FLEX-oROS-Gr) expressed in the VTA of freely moving male or female KOR-Cre mice by fiber photometry or by 2-Photon imaging of midbrain slice. One- and two-way ANOVA used.

Results: Daily injection of norBNI (0.33 mg/kg and higher), nalmefene (10 ug/kg and higher), or nalfurafine (100 ng/kg and higher) significantly blocked KOR agonist-induced analgesia, KOR-stimulated release of prolactin, and odorant aversion. Consistent with the long-duration of norBNI action, recovery of KOR analgesic responses following nalfurafine or nalmefene treatment required 2+ weeks. No block of KOR-induced diuresis or itch inhibition was observed. Acute nalmefene or nalfurafine dose-dependently stimulated oROS-Gr fluorescence in the VTA, and this response was blocked by the selective KOR antagonist Aticaprant (in vivo), naloxone (in vitro). In female mice, KOR activation of oROS was only evident in low estrogen states (diestrus) as determined by vaginal cell morphology. Progesterone injection enabled nalfurafine-induced ROS generation.

Conclusions: Low, repeated doses of nalmefene and nalfurafine inactivated KOR by the established JNK mechanism. We show that ROS generation via KOR agonism with nalfurafine was dependent on estrus cycle stage, with high estradiol during proestrus (like the estrogen spike before ovulation in human females) blocking generation of ROS. This presents a unique problem when examining long-lasting inactivation in women. It has been shown that repeated dosing at 10-100 fold lower doses can overcome this effect in female mice. Compared to unbiased KOR agonists, nalfurafine and nalmefene do not efficiently activate the GRK3/arrestin-dependent cascade that otherwise blocks the phosphorylation of JNK. The inactivation using nalmefene and nalfurafine was dose dependent with a U-shaped curve. The low end of the doses for each drug are likely not causing enough ROS generation to inactivate a significant portion of the KORs. At high doses, we can assume that the GRK3/arrestin pathway is being activated enough to cause the JNK pathway to be blocked. The window of ideal doses for long-lasting block KOR signaling is low compared to the doses that have been shown to be safe in humans and could realistically be even lower given longer periods of administration (14-28 days instead of 7). These results suggest that chronic treatment with low doses of either nalfurafine or nalmefene, previously shown to be safe in humans, may inactivate KOR to promote stress resilience in men and women (on progesterone birth control). Slowly accumulating KOR inactivation by this mechanism could produce a titratable drug effect that would have a more stable drug response than a short-acting KOR antagonist. The low doses of nalmefene or nalfurafine required for KOR inactivation could theoretically produce fewer off-target adverse effects than produced by the saturating doses required for competitive KOR antagonism. The tissue-specificity of KOR inactivation observed (no block of diuretic or antipruritic effects) also has potential pharmacological selectivity advantages. The results obtained suggest that a direct comparison between competitive antagonists and KOR-inactivating medications in human trials is warranted.

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Keywords: Nalmefene, Nalfurafine, Stress-Resilience, Kappa Opioid Receptor, Substance Use Disorder

Disclosure: Nothing to disclose.

P715. FAAH-Regulated Endocannabinoid Signaling Controls Alcohol-Induced Hyperalgesic Priming

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Background: Chronic pain is a prevalent condition observed in individuals with alcohol use disorder (AUD), and may contribute to excessive drinking and relapse. Understanding the molecular mechanism through which alcohol intake causes persistent pain is essential for the development of disease-modifying therapies. Emerging evidence suggests that the endocannabinoid system could play an important role in this condition.

Methods: We subjected animals to a short-term, low-dose (5% v/v) alcohol drinking paradigm to investigate whether such exposure would cause hyperalgesic priming (HP). The latter is a form of nociceptor plasticity that heightens sensitivity to a normally subthreshold dose of noxious stimuli.

Results: Our findings demonstrated that intraplantar injection of the inflammatory mediator prostaglandin E2 (PGE2) induced a lasting hypersensitive state in approximately 63% (12 out of 19) of alcohol-exposed male mice. This effect appeared within 60 minutes and persisted for at least 14 days, irrespective of the amount

of alcohol intake. Moreover, all the alcohol-experienced female mice (19 out of 19) exhibited prolonged hypersensitivity to PGE2. Additionally, we observed that the acute injection of URB597, a potent inhibitor of the endocannabinoid-hydrolyzing enzyme fatty acid amide hydrolase (FAAH), failed to alleviate alcohol-induced HP. Yet, the chronic administration of the compound reversed HP in mice of both sexes. Importantly, this effect was phenocopied in transgenic animals lacking the Faah gene, as they were resistant to alcohol-induced HP. These findings suggest that alcohol drinking alters the endocannabinoid signaling in the nociceptive circuit.

Conclusions: Our results highlight FAAH-regulated endocannabinoid signaling as a potential control point in alcohol-induced HP and its related complications. Understanding and targeting this pathway could offer promising avenues for managing alcohol-induced pain syndromes.

Keywords: Alcohol, Chronic Pain, Endocannabinoids, Nociception

Disclosure: Nothing to disclose.

P716. Sex Differences of Locomotor Effects of Metformin in Cocaine-Conditioned Place Preference

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Background: Cocaine use disorder (CUD) remains a significant problem as cocaine-associated deaths in the U.S. continue to rise annually. Most importantly, there is no current FDA-approved treatment for CUD leading to high rates of treatment attrition, relapse, and overdose. Our lab has previously shown evidence supporting metformin, a Type II Diabetes drug, as a potential treatment for CUD by reducing cocaine-seeking behaviors. We hypothesize that metformin will diminish rewarding effects of cocaine in a conditioned place preference (CPP) model. The current experiments further elucidate the effect of metformin in motivated behavior and provide supporting evidence for future repurposing as treatment for CUD.

Methods: Cocaine conditioned place preference (CPP) was performed in adult (8-10 weeks) male and female Sprague Dawley rats in a 2-chamber box (MedAssociates). Rats were conditioned for 10 days in daily 30-minute sessions, alternating control and treatment sessions, and using an unbiased research design. Rats displaying >80% preference for either chamber during a baseline pre-test were excluded from the analysis. Pretreatment of metformin (175 mg/kg) or saline occurred 30 minutes before conditioning with cocaine (10 mg/kg, 20 mg/kg) or vehicle (saline). All injections were given intraperitoneal. After conditioning, time spent in each chamber was assessed during 15 minutes of free exploration with no drug exposure. Data were analyzed by two-way repeated measures analysis of variance (ANOVA) followed by Sidak's multiple comparisons.

Results: Sex differences were observed in the conditioned rewarding effects of cocaine. Female rats ($n = 8$; $p = 0.05$ for time spent in drug-paired chamber in pre-test vs. test) but not male rats ($n = 8$; $p = 0.93$ for time spent in drug-paired chamber in pre-test vs. test) acquired CPP for a 20 mg/kg dose of cocaine. Furthermore, female rats showed no preference for a cocaine-paired side when receiving a pretreatment of metformin ($n = 7$, $p = 0.84$ for time spent in drug-paired chamber in pre-test vs. test). Despite no CPP effects, metformin reduced locomotor stimulating effects of 20 mg/kg cocaine in male rats across multiple days of testing, and prevented locomotor sensitization with a 10 mg/kg dose of cocaine. Metformin had no locomotor effect in female rats.

Conclusions: Sex differences have been observed in the effects of cocaine and metformin as measured by performance on CPP, as well as locomotor activity. Metformin prevents cocaine CPP in female rats supporting our hypothesis that metformin has promise as a potential intervention for CUD. Despite no direct effects in CPP with male rats, pretreatment with metformin reduced enhanced locomotion induced by a high dose of cocaine over multiple days. Our work suggests increased sensitivity to the effects of cocaine and metformin in female rats, although the motor effect in male animals suggests unique pharmacological sex differences. Continuation of this study will inform the potential development of metformin as a pharmacotherapy for cocaine use disorder, while informing important therapeutic sex differences in the physiological response to metformin that may be applied broadly.

Keywords: Cocaine Sex Differences, Conditioned Place Preference, Locomotor Activity

Disclosure: Nothing to disclose.

P717. Organic Cation Transporter 3 (OCT3) is Crucial for the Reinforcing Effects of Amphetamine-Type Stimulants in Mice

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Background: Amphetamine-type stimulants (ATS) are one of the most commonly misused classes of drugs around the world, second only to cannabis. In the United States, overdose deaths involving ATS have risen sharply over the past 5 years. Despite the high incidence of ATS misuse, that there are currently no FDA-approved pharmacotherapies for stimulant use disorders highlights the dire need to identify novel targets to reduce the abuse-related and toxic effects of ATS. Organic Cation Transporter 3 (OCT3) is an uptake-2 (low affinity, high capacity), bidirectional monoamine transporter, and emerging evidence suggests that it plays important roles in the neurochemical and behavioral effects of ATS. Thus, the goal of this study was to determine the degree to which OCT3 is involved in the reinforcing effects of amphetamine in wild-type (WT) and constitutive OCT3 knockout (KO) mice.

Methods: Male and female WT and OCT3 KO mice were first trained to respond under a fixed ratio 1 (FR1) schedule of reinforcement for presentation of chocolate flavored Ensure®. Once acquired, mice were surgically prepared with an indwelling catheter in the left femoral vein to allow for intravenous drug infusion. Subsequently, mice were allowed to respond under an FR1 for 0.32 mg/kg/inf of cocaine. A dose-response curve (DRC) for amphetamine (0.0032-0.32 mg/kg/inf) was generated by dose-substitution from a cocaine baseline. A separate cohort of male and female WT and OCT3 KO mice were used to evaluate the effects of an uptake-2 transporter inhibitor, decynium-22 (D22), on responding maintained by 0.32 mg/kg/inf cocaine, or 0.032 mg/kg/inf amphetamine.

Results: WT and OCT3 KO OCT3-/- mice acquired responding for food and cocaine at comparable rates and levels. However, when amphetamine was substituted for cocaine, OCT3 KO mice earned significantly fewer infusions of amphetamine, resulting in their DRC being shifted downward and rightward relative to the amphetamine DRC in WT mice. Pretreatment with D22 inhibited amphetamine self-administration in WT but not OCT3 KO mice. D22 failed to alter cocaine self-administration in either genotype.

Conclusions: These studies provide convergent genetic and pharmacological data indicating that OCT3 plays a crucial role in mediating the reinforcing effects of ATS in both male and female mice. Together with the results of neurochemical studies, these finding suggest that OCT3 represents an exciting and novel target for the development of pharmacotherapies to help those suffering from an ATS use disorder.

Keywords: Organic Cation Transporters, Amphetamine, Self-Administration

Disclosure: Nothing to disclose.

P718. Single Vs. Multiple Cycles of Fentanyl Withdrawal in Mice

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Background: Opioid withdrawal is a significant obstacle to those wishing to enter abstinence. Recently it has been shown that microglia may play a role in the generation of opioid withdrawal symptoms, such as through activity of opioids at TLRs or through release of cytokines following cessation of opioid administration. Since Opioid Use Disorder demonstrates numerous cycles of abstinence and relapse, we investigated the physical and emotional symptoms of spontaneous opioid withdrawal in mice following a single withdrawal experience or a series of five, repeated cycles of fentanyl administration and withdrawal. Our hypothesis was that opioid withdrawal becomes more severe and/or more protracted after numerous cycles of tolerance and withdrawal. In addition, we are focusing on the role of striatal microglia in opioid withdrawal by investigating microglia morphology and gene expression in these animals based on early studies from our group finding surprising paradoxical changes in gene expression after morphine withdrawal.

Methods: Mice expressing microglia selective, HA-tagged ribosomes (Cx3cr1-CreERT2::floxed-RPL22HA Ribotag) were administered fentanyl or saline twice-daily by IP injection, with the fentanyl dose doubling each day, for five consecutive days. A separate cohort of animals received the same five-day fentanyl administration schedule repeated a total of five times, with four days of rest in the home cage between each period of opioid administration. Animals were then assessed for withdrawal symptoms at 16, 48 or 196 hours after their final IP injection. For some animals in both cohorts, we collected striatum to perform RNA sequencing of the microglia RNAs at an acute period of the withdrawal.

Results: In the behavioral analyses, we found that at 16 hours into withdrawal, male animals in both single withdrawal and multiple withdrawal groups showed reduced consumption of sucrose in the sucrose preference test. However, both male and female animals in the five-cycle withdrawal group showed reduced sucrose consumption at 48 hours, suggesting that anhedonia during withdrawal is exacerbated by repeated cycles of opioid administration and cessation. Both single and multiple withdrawal groups showed hyperalgesia to noxious heat at 16 and 48 hours in the tail flick test, regardless of sex. This hyperalgesia was not seen in animals at 196 hours. However, animals of both sex and in both withdrawal cohorts spent more time near the peripheral walls in the open field test at 196 hours into withdrawal, suggesting that anxiety induced by opioid withdrawal may be long lasting. We also performed fluorescent IHC to label microglia using antibodies against Iba1 for imaging by confocal microscopy. We found that microglia cell bodies become more compact and less ramified in the animals that experience five cycles of withdrawal regardless of sex, as revealed by morphological measurements of cells in the striatum. RNA sequencing analysis is underway and will be presented as well.

Conclusions: Together these experiments lead us to conclude that opioid withdrawal symptoms become significantly worse following repeated cycles of fentanyl exposure and withdrawal. We further postulate that microglia undergo physiological adaptations during multiple cycles of withdrawal, and we are currently investigating how functional changes in microglia activity either mitigate or contribute to the increasing severity of withdrawal over multiple cycles of withdrawal.

Keywords: Opioid, Microglia, Opioid Withdrawal, Striatum

Disclosure: Nothing to disclose.

P719. A Polypharmacy Approach Targeting Serotonin 2C and Dopamine D3 Receptor Provides Broad-Spectrum Efficacy to Reduce Drug-Taking Behavior in Rats

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Background: Substance use disorders (SUDs) continue to pose a significant threat to public health. Moreover, the continued and exponential rise in deaths related to acute drug overdose highlights the dire need to develop novel approaches to helping those suffering from an SUD. In preclinical studies, dopamine D3 (DAD3) and serotonin 2C (5-HT2C) receptor ligands have both been shown to reduce stimulant and opioid self-administrator, though clinical studies with buspirone (DAD3) and lorcaserin (5-HT2C) have been less promising. Two strategies to further the development of drugs targeting DAD3 and 5-HT2C receptors as pharmacotherapies for SUD are to improve receptor selectivity and/or develop polypharmacy approaches targeting both DAD3 and 5-HT2C receptors.

Methods: Male and female Sprague-Dawley rats ($n = 7$ per sex, per condition) were implanted with an indwelling venous catheter and allowed to self-administer cocaine (0.32 mg/kg/inf), methamphetamine (0.056 mg/kg/inf), fentanyl (0.0032 mg/kg/inf), or sucrose (1 sucrose pellet) under a progressive ratio (PR) schedule of reinforcement. Prior to evaluating mixtures of DAD3 and 5-HT2C receptor ligands, inhibition functions were generated for a DAD3 receptor-selective antagonist (VK4-116,1-32 mg/kg; IP), a DAD3 receptor-selective partial agonist (VK4-40, 1-32 mg/kg; IP), and a 5-HT2C receptor-selective agonist (CP809,101, 0.32-10 mg/kg; IP) in rats responding for cocaine, methamphetamine, fentanyl, or sucrose. Subsequently, inhibition functions were generated for binary mixtures of VK4-116 + CP809,101 and VK4-40 + CP809,101 at fixed dose ratios of 3:1, 1:1, and 1:3, relative to each drug's ED50 to reduce drug self-administration.

Results: When tested alone, VK4-116, VK4-40, and CP809,101 were all equipotent at reducing responding maintained by cocaine, methamphetamine, and fentanyl, though CP809,101 was more potent and effective than either VK4-116 or VK4-40; there were no sex-related differences. When combined, mixtures of VK4-116 + CP809,101 and VK4-40 + CP809,101 resulted in a near complete inhibition of responding maintained by cocaine, methamphetamine, and fentanyl, and significantly lesser effects on responding maintained by sucrose. Dose-addition analyses indicated that interactions between the DAD3 and 5-HT2C receptor ligands exhibited effects were largely additive, though supra-additive interactions were also observed.

Conclusions: Together these studies provide evidence that the selectivity and effectiveness of monotherapies targeting 5-HT2C and DAD3 receptors to reduce drug-taking can be improved

through the use of a polypharmacy approach targeting both DAD3 and 5-HT2C receptors. Ultimately, further developments of such an approach may provide a novel, and broad-spectrum strategy to help individuals suffering from (poly)SUDs.

Keywords: Dopamine D3 Receptors, Serotonin 5-HT2C Receptor, Stimulants, Opioids, Self-Administration

Disclosure: Nothing to disclose.

P720. GM-3009 is a Novel Noribogaine Analog That Disrupts Opioid Self-Administration in Rats Via Agonism of Kappa Opioid Receptors Without Pro-Arrhythmic Effects on Human Cardiomyocytes

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Background: Opioid use disorder (OUD) is a massive public health crisis, with over 3 million people in the United States suffering from the disorder and over 100,000 reported overdose deaths in 2022. Current treatments for OUD, such as methadone, buprenorphine and alpha-2 adrenergic agonists are useful for addressing some facets of OUD, but they are not effective in everyone. Furthermore, they lack rapid-acting and persistent effects that are crucial for a health emergency of this magnitude. Ibogaine, a psychoactive alkaloid, shows potential in interrupting opioid use, but is associated with well-documented cardiac safety risks. A new class of benzofuryl iboga analogs bearing kappa opioid agonist pharmacology are being investigated as safer alternatives.

Methods: The in vitro profile of GM-3009 racemate was determined using competition binding at human kappa opioid receptors (KOR) expressed in RBL cells ([3H]-U69593 ligand) and HEK293 cells ([3H]-diprenorphine ligand) and a cAMP assay of Gi activation in cAMP Hunter™ CHO-K1 cells. Cardiotoxicity was assessed by measuring pro-arrhythmic effects of noribogaine and GM-3009 at 4 concentrations (0.1-10 μM) in fresh adult human primary ventricular cardiomyocytes (n = 7). The antinociceptive effects of GM-3009 (1-30 mg/kg, sc) were evaluated in C57BL/6 mice (n = 8/group) using the tail-flick and hot plate assays as in vivo pharmacodynamic measures of KOR target engagement. All results were interpreted in the context of pharmacokinetic exposure.

The efficacy of GM-3009 (3-30 mg/kg) was tested compared to the active ibogaine metabolite (noribogaine) in a rat oxycodone self-administration model of opioid use disorder. Adult male Long-Evans rats were trained to self-administer 0.03 mg/kg/infusion of oxycodone under a fixed ratio 5 schedule of reinforcement. Rats (n = 7/group) were dosed with GM-3009, noribogaine or vehicle and self-administration of oxycodone measured for 2 h.

In addition to these key studies on the racemic lead compound, enantiomers of GM-3009 were individually evaluated in select in vitro (KOR binding) and in vivo assays (oxycodone self-administration, n = 8/group).

Results: At human KOR receptors, GM-3009 bound with high affinity (Ki 0.9 nM and 87.3 nM, for [3H]-U69593 and [3H]-diprenorphine, respectively).

In a KOR cAMP assay, GM-3009 also proved to be a potent agonist at KOR, demonstrating an EC50 of 0.8 nM.

Following administration in rats, GM-3009 (1 mg/kg, iv) achieved a C0 of 151.6 ng/mL in plasma with a T1/2 of ~4 h. In mice administration of GM-3009 (1 mg/kg, iv) resulted in a C0 of 155.3 mg/mL in plasma with a T1/2 of ~2.8 h.

GM-3009 had robust antinociceptive effects in the mouse tail flick and hot plate assays (1-30 mg/kg, sc), demonstrating efficacy with ED50 values of 4.7 and 11.2 mg/kg, respectively.

In fresh adult human primary ventricular cardiomyocytes noribogaine exhibited a concentration dependent increased frequency of aftercontractions and contraction failures at concentrations of 1 μM and higher, but GM-3009 showed no pro-arrhythmic potential at any of the tested concentrations.

GM-3009 (3-30 mg/kg, ip) produced a dose-dependent reduction of oxycodone self-administration in rats (ED50 = 7.35 mg/kg).

The resolved enantiomers "E1" and "E2" (named according to chiral chromatographic elution order) were found to bind to KOR with Ki values of >100 nM and 0.6 nM respectively indicating that GM-3009_E2 was the more active enantiomer.

The enantiomers were also evaluated in the rat oxycodone self-administration assay. GM-3009_E2 similarly attenuated oxycodone intake, with ED50 values of 6.04 mg/kg (ip) and 1.82 mg/kg (iv). GM-3009_E1 produced vehicle-like effects when tested at the same dose producing maximal suppression by GM-3009_E2.

Conclusions: The novel ibogaine analog GM-3009 was shown to bind and activate kappa receptors in vitro. The highly potent kappa agonist activity of GM-3009 was attributable to the E2 enantiomer. In vivo, GM-3009 produced antinociceptive effects in both rats and mice. In the oxycodone self-administration model, consistent with relative binding affinity, activity was shown to be derived from the (-)-enantiomer (GM-3009_E2), which bears the same stereochemical configuration as natural ibogaine. GM-3009 demonstrated therapeutic efficacy but lacked the cardiotoxic effects associated with ibogaine supporting an improved safety profile. Overall, GM-3009, and in particular the E2 enantiomer, represents a novel OUD medication with important therapeutic potential.

Keywords: Ibogaine, Psychedelic Medicine, Kappa Agonist, Alcohol and Substance Use Disorders

Disclosure: Gilgamesh Pharmaceuticals: Founder (Self).

P721. Alcohol Withdrawal-Induced Activation of Orexin System in VTA-Extended Amygdala Circuitry in Alcohol Dependent Rats

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Background: Humans with alcohol use disorder (AUD) often experience negative affect during withdrawal (WD), and depressed mood and anxiety are positively correlated with relapse during abstinence. The neural adaptations that occur during the transition to dependence are not entirely understood, but may include a gradual recruitment of brain stress circuitry by mesolimbic reward circuitry that is activated during early stages of alcohol use. We have previously demonstrated that chronic alcohol increases the activity of a ventral tegmental area (VTA) to central amygdala (CeA) circuit. The VTA and CeA are important for mediating acute alcohol reinforcement and alcohol withdrawal-associated behaviors, respectively, raising the possibility that activation of this circuit mediates increases in anxiety-like behavior during alcohol WD. The mechanism by which the VTA-CeA circuit becomes activated is unknown, but this may occur via orexin-mediated disinhibition. Here, we explored 1) expression of orexin- or orexin 1 receptors in the VTA and extended amygdala subregions following chronic alcohol exposure during both adolescence and adulthood, 2) the role of the VTA-CeA circuit in mediating anxiety-like behavior in alcohol-dependent and naïve adult rats, and 3) the effects of VTA orexin signaling on anxiety-like behavior in adult rats. We hypothesized that orexin- or orexin 1 receptor-expressing cells in the VTA and extended amygdala

would be activated during alcohol WD; that both VTA-CeA circuit activity and the VTA orexin system contribute to increased anxiety-like behavior during alcohol WD; and that VTA-CeA circuit activation during alcohol WD occurs via an orexin-mediated mechanism.

Methods: Male and female Wistar and Long Evans rats were used in all experiments. To evaluate the role of VTA-CeA circuitry in behavioral assays, we used a dual virus approach to isolate CeA-projecting VTA neurons with an intra-CeA injection of a retro-cre virus (pENN.AAV.hSyn.HI.eGFP-Cre.WPRE.SV40) and an intra-VTA injection of either a cre-dependent inhibitory DREADD (pAAV-hSyn-DIO-hM4D(Gi)-mCherry), or inactive control (pAAV-hSyn-DIO-mCherry). To characterize the role of intra-VTA orexin on behavior, orexin A (50 nM), the orexin 1 receptor antagonist SB334867 (10 µg), or vehicle was site-specifically administered in the VTA prior to anxiety-like behavioral assays.

Alcohol dependence was induced using a chronic ethanol vapor exposure model in adult and adolescent rats, and rats were tested during acute WD. Anxiety-like behavior was assessed using open field, light/dark box, and elevated plus maze behavioral assays. In situ hybridization was performed to probe Hcrt (orexin), Hcrtr1 (orexin 1 receptor), Drd1 (dopamine 1 receptor), or Th (tyrosine hydroxylase) expression in neurons in the VTA and extended amygdala subregions.

Results: Preliminary data suggest a role for the VTA-CeA circuit, as well as the VTA orexin system, in mediating anxiety-like behavior in adult rats. Using a chemogenetic approach to inhibit the VTA-CeA circuit in alcohol dependent rats, we demonstrate a potential rescue of increased anxiety-like behavior during alcohol WD. Ongoing experiments are replicating these findings, as well as utilizing pharmacological strategies to investigate the mechanism underlying activation of CeA-projecting VTA neurons. We demonstrate that intra-VTA orexin A administration is sufficient to produce an anxiety-like phenotype in otherwise experimentally naïve rats ($p = 0.024$; two-tailed t-test), and preliminary data suggest that intra-VTA SB334867 administration may rescue increased anxiety-like behavior during alcohol WD, similar to inhibition of VTA-CeA neurons.

In situ hybridization data demonstrates a significantly higher percentage of Hcrtr1+ neurons in the VTA of alcohol dependent adult rats compared to naïve controls ($p = 0.011$; two-tailed t-test), and Hcrtr1 expression is significantly negatively correlated with time spent in the open arm of an elevated plus maze ($p = 0.035$; $R2 = 0.35$), suggesting a relationship between VTA orexin signaling and anxiety-like behavior during alcohol WD. Additionally, a significantly greater percentage of Hcrtr1-expressing Th+ neurons were found in the VTA of alcohol-dependent adult ($p = 0.034$; two-tailed t-test) and adolescent ($p = 0.012$; two-tailed t-test) rats, compared to naïve controls. No significant difference in Drd1, Hcrtr1, or Hcrt expression was found in the BNST, CeA, NAc, or LH when comparing alcohol dependent and naïve rats.

Collectively, these data indicate a relationship between both VTA-CeA circuitry, as well as the VTA orexin system, and anxiety-like behavior, particularly during alcohol dependence. Ongoing efforts are replicating these findings and utilizing more sophisticated techniques to more closely examine orexin modulation of CeA-projecting neurons specifically.

Conclusions: Crosstalk between brain reward and stress systems plays a critical role in behavioral dysregulation induced by alcohol dependence. These studies expand on our published findings demonstrating increased activity of the VTA-CeA circuit in alcohol-dependent, withdrawn rats by exploring the possibility that this circuit mediates some aspects of behavioral dysregulation associated with alcohol dependence. Results of these studies have the potential to expand our understanding of circuitry involved in anxiety-like behavior, as well as informing therapeutic strategies for individuals with AUD.

Keywords: Alcohol, Orexin, Ventral Tegmental Area, Central Amygdala

Disclosure: Nothing to disclose.

P722. Effects of Repeated Nicotine Vapor Exposure and Cessation on Intracranial Self-Stimulation Brain Reward Thresholds in Adult Male and Female Rats

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Background: In recent years, electronic cigarette use has increased substantially, raising concern about the effect of vaping on public health. Importantly, studies have found that women use two-fold higher concentrations of nicotine in e-cigarettes and display greater symptoms of nicotine dependence than men. Research has begun to identify the distinct effects of repeated nicotine vapor exposure on the brain and behavior; however, its effects on brain reward function remain unclear. Thus, this study aimed to assess, in a sex-dependent manner, the effects of chronic nicotine vapor exposure and cessation on brain reward function, via intracranial self-stimulation (ICSS) of the mesolimbic brain reward circuitry.

Methods: Adult male ($n = 12$) and female ($n = 18$) Sprague-Dawley rats were trained in the ICSS task until responding was stable (less than 10% variability across 3 consecutive days). Rats were then tested in ICSS for 14 days immediately following 90 minutes of passive exposure to 0 mg/mL vehicle vapor (50/50 propylene glycol/vegetable glycerin, PG/VG) or 24 mg/mL nicotine vapor. After 14 days of vapor exposure with ICSS testing, rats continued to test in ICSS for an additional 14 days, post-vapor exposure. Whole brains were collected following post-vapor ICSS testing, for electrode placement verification. To assess nicotine vapor's effects on brain reward function in males and females, percent change from baseline of preferred stimulation thresholds in ICSS were compared between treatment groups using mixed model ANOVA, with subsequent t-tests analysis comparing treatment groups on each day of the vapor exposure or post-vapor exposure periods. Family of t-tests were conducted for each week of treatment or withdrawal, and critical values were adjusted to account for these multiple comparisons. Male and female rats were tested in two separate experiments; therefore, statistical analysis directly comparing the sexes' preferred ICSS reward thresholds were not conducted.

Results: Mixed-model ANOVA comparing male rats in the vehicle and 24 mg/mL nicotine vapor exposure groups across the 14 consecutive days of vapor exposure resulted in no main effect or interaction for day or treatment group. Conversely, this same analysis revealed a significant interaction of exposure day and treatment group during week 2 of vapor exposure in female rats [$F(6,36) = 3.08$; $p = 0.008$], such that thresholds decreased relative to baseline in the nicotine vapor group, across exposure days 8-14. Analyses comparing ICSS threshold percent change between vapor treatment groups on each day of week 2 exposure revealed no significant differences when using the adjusted critical value of 0.007, but did reveal a significant difference between groups on exposure day 14 with the unadjusted critical value of 0.05 ($p = .044$). A mixed-model ANOVA comparing nicotine vapor exposure groups across all 14 days following cessation of nicotine vapor exposure revealed a main effect of treatment group in both males [$F(1,10) = 11.01$; $p = 0.008$] and females [$F(1,16) = 7.01$; $p = 0.018$], such that thresholds increased relative to baseline in the nicotine vapor group, across all exposure days. No main effects or interactions were observed for day in male and female

rats across the 14 days following vapor exposure. Analyses compared ICSS threshold percent change between vapor treatment groups on each day following cessation of nicotine vapor exposure. In male rats analysis revealed significant differences between treatment groups on day 1 of withdrawal when the critical value was adjusted to 0.007 for the family of t-test ($p = 0.005$), and on days 3, 4, 8, 9, and 13 of withdrawal when using an unadjusted critical value of 0.05 ($p = 0.013$, $p = 0.031$, $p = 0.011$, $p = 0.019$, and $p = 0.010$, respectively). In female rats post-hoc analyses revealed no significant differences on any given day when using the adjusted critical value of 0.007, but did reveal significant differences between treatment groups on days 2, 6, 7, 8, 13, and 14 of withdrawal when using an unadjusted critical value of 0.05 ($p = 0.052$, $p = 0.028$, $p = 0.054$, $p = 0.044$, $p = 0.030$, and $p = 0.044$, respectively).

Conclusions: These data suggest that nicotine vapor exposure decreases ICSS brain reward thresholds in female, but not male rats, after 1 week of exposure. Findings also reveal that cessation from repeated nicotine vapor exposure causes increases in brain reward thresholds in both male and female rats, similar to that seen in other drugs of abuse. Studies are currently underway in our laboratory to identify the neurobiological mechanisms underlying sex-differences in ICSS, as well as the factors driving nicotine vapor intake patterns and dependence. The inclusion of females in subsequent studies assessing nicotine use disorder is essential for the development of sex-specific treatment strategies aim at reducing nicotine cravings and intake behavior.

Keywords: Nicotine, Intracranial Self-Stimulation, Electronic Cigarette (E-Cigarette), Rat

Disclosure: Nothing to disclose.

P723. Liraglutide, a Glucagon-Like peptide-1 (GLP-1) Receptor Agonist, Does Not Affect the Expression of Methamphetamine Preference in Adolescent Male and Female Rats

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Background: Adolescent methamphetamine (METH) use is especially harmful because early initiation leads to poorer treatment outcomes. There are currently no FDA-approved stimulant use disorder treatments. Liraglutide, an FDA-approved drug that activates glucagon-like peptide-1 (GLP-1) receptors, has been shown in preclinical studies to reduce the rewarding effects of cocaine, heroin, and oxycodone. However, the role of GLP-1 receptor activation in METH reward has not been studied, nor has it been studied in adolescent rats. Thus, the current study used the conditioned place preference (CPP) paradigm, a validated animal model of drug reward, to see if stimulating GLP-1 receptors with Liraglutide reduces METH-seeking behavior in adolescent rats.

Methods: A 10-day CPP procedure was performed on male and female Sprague-Dawley rats. Rats were tested for initial side preference in a two-chamber-sided box on day one. Conditioning days were held over the next eight days, with rats receiving alternating drug and saline treatments. On their respective days, rats were given METH (0.0, 0.3, or 1.0 mg/kg) or saline and immediately confined to one side of the CPP box for 30 min. On day 10, the rats were pretreated with Liraglutide (0.1 mg/kg) or saline (n = 14-16) 60 min before being placed in the CPP apparatus with free access to both sides for 15 min. The time spent in the drug-paired side during testing was subtracted from the time spent in the same compartment during baseline to calculate a preference score. Changes in time spent in the METH-paired side between baseline and testing sessions for each group were also investigated.

Results: Overall, male rats showed a strong preference for the METH-paired side during testing ($p < 0.05$), which was not reduced by Liraglutide pretreatment. Female rats did not prefer the METH-paired side over the control side. However, between baseline and testing, there were within-group preferences for the METH-paired side ($p < 0.05$). Nonetheless, Liraglutide, like male rats, had no effect on female rats' preferences.

Conclusions: Additional studies examining higher doses of Liraglutide are required to determine whether adolescent rats are less sensitive to the effects of Liraglutide and to investigate the effects of Liraglutide on the acquisition of METH-induced CPP. When the effects of Liraglutide on METH-seeking behavior in adolescent male and female rats are considered together, developmental differences in the effects of Liraglutide are apparent.

Keywords: Adolescent, Developmental, Addiction, Psychostimulant

Disclosure: Nothing to disclose.

P724. Advancing Development of Monoclonal Antibodies to Treat and Reverse Fentanyl Overdose

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Background: The incidence of fatal drug overdoses has increased dramatically due to the widespread availability of fentanyl and its potent analogs, surpassing 100,000 death per year since 2020. Monoclonal antibodies (mAb) offer a novel strategy to treat overdose from fentanyl and its analogs. Our previous studies showed that mAbs can reversal fentanyl toxicity post-exposure in rats. Here, efficacy of a lead anti-fentanyl mAb in reversing fentanyl effects was assessed in large animal models, including a porcine model of fentanyl-induced apnea and non-human primates (NHP).

Methods: In lightly anesthetized Hanford mini-pigs (n = 3/group), animals were infused with fentanyl to produce stable apnea, and fentanyl effects were reversed with anti-fentanyl mAb or naloxone control. In NHP (n = 5), fentanyl dose-responses were first determined to establish individual fentanyl doses that produced respiratory depression. Then, subjects were treated with fentanyl, and 15 minutes later anti-fentanyl mAb was administered intravenously to reverse fentanyl effects. On subsequent days, subjects were challenged with fentanyl to determine whether mAb prevents re-exposure to fentanyl, and duration of mAb efficacy was determined at several weeks post-exposure.

Results: Following fentanyl infusion in mini-pigs, control saline-treated subjects returned to spontaneous breathing in approximately 7-8 min after cessation of fentanyl exposure, whereas mAb-treated subjects recovered within 2 min of mAb injection. Treatment with mAb increased serum fentanyl 10-20X within the first minute after mAb infusion. Similarly, in NHP the mAb was effective in both pre- and post-exposure scenarios.

Conclusions: These studies further support advancement of anti-fentanyl mAb towards clinical evaluation.

Keywords: Antibody, Opioid Addiction, Opioid Overdose, Opioid Treatment

Disclosure: Nothing to disclose.

P725. The Parabrachial Nucleus Modulates Anxiety in Alcohol Abstinence and Following Repeated Stress

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Background: Chronic intermittent ethanol vapor exposure (CIE) and two-bottle alcohol choice (2BC) paradigms produce neuronal adaptations in the bed nucleus of the stria terminalis (BNST), a region critical for affective behavior. The parabrachial nucleus (PBN), a sensory alarm, sends calcitonin gene related protein (CGRP) and pituitary adenylate cyclase activating peptide (PACAP) projections to the BNST that remain relatively unexplored in abstinence.

Methods: Calca-CRE (gene name for CGRP) male mice (n = 5-10/sex/virus group) received bilateral injections of hM4D(Gi) DREADDs or control virus in the PBN. Anxiety was measured in early-withdrawal from CIE (4-6 hr) with the elevated plus maze (EPM) paired with the hM4D(Gi) ligand, CNO (3 mg/kg, IP). PACAP-CRE female mice (n = 4-9/sex/genotype/stress group) received bilateral injections of hM4D(Gi) DREADDs in the PBN and were exposed to 2BC. Anxiety was measured in prolonged-withdrawal (2 wks) from 2BC with the novelty suppressed feeding task (NSFT) followed by repeated restraint stress (RRS) paired with CNO (3 mg/kg, IP). Post-RRS anxiety was measured with NSFT.

Results: PBN(CGRP) inhibition increased EPM time immobile in CIE-withdrawal (2-way ANOVA vapor $p = 0.05$; treatment $p = 0.01$). PBN(PACAP) inhibition induced a trend for decreased latency to feed in NSFT with no changes on RRS behavior in 2BC prolonged withdrawal. A history of RRS paired with PBN(PACAP) inhibition blunted RSS-induced increase in NSFT latency (2-way ANOVA stress $p = 0.004$, genotype $p = 0.0481$).

Conclusions: These data demonstrate PBN(CGRP) and PBN(PACAP) neurons modulate anxiety-like behavior in alcohol-abstinence. Given the prominent role of the BNST in abstinence-induced anxiety current studies investigate changes in PBN to BNST circuit activity. Given pharmaceutical treatments for migraines targeting CGRP and PACAP inhibition are bioavailable, these studies inform a potential role for treatments in alcohol-abstinence.

Keywords: Anxiety and Stress, Parabrachial Nucleus, CGRP, PACAP, Alcohol Abstinence

Disclosure: Nothing to disclose.

P726. Ketamine Metabolites as Next-Generation Pharmacotherapies for Treating Opioid Addiction

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Background: Prolonged abstinence from opioid use is associated with a negative affective state characterized by social avoidance and anhedonia, which act as motivational triggers for relapse. While substitution pharmacotherapies are currently used to maintain opioid abstinence, these drugs have limited efficacy in treating the negative affect of protracted opioid abstinence and preventing relapse. Evidence suggests that racemic ketamine might prolong abstinence and reduce relapse rates in heroin addicts; however, its side effects and abuse potential due to NMDAR inhibition limit its widespread use.

Methods: In this study, we utilized a classic mouse model of opioid dependence (i.e., 6-day, twice daily, escalating-dose morphine; 20-100 mg/kg, i.p.) and allowed the mice to undergo a 10-day withdrawal period. We also employed the conditioned-place preference paradigm with 5 mg/kg morphine conditioning to assess the efficacy of these metabolites in preventing stress-induced opioid-seeking behaviors following extinction.

Results: Administering select ketamine metabolites reversed and prevented decreased sociability, sucrose preference deficits, and reduced nest-building behavior induced by opioid withdrawal. Furthermore, in the conditioned place preference paradigm, these metabolites effectively prevented stress-induced reinstatement of morphine-seeking behavior after extinction.

Conclusions: Our findings highlight that ketamine's metabolites, which do not exhibit abuse liability, could serve as promising next-generation pharmacotherapies for the treatment of opioid addiction.

Keywords: Opioid Addiction, (R,S)-Ketamine, Racemic Ketamine and Metabolites

Disclosure: Nothing to disclose.

P727. The Lower Availability of the Cannabinoid Receptor Type 1 (CB1R) in Opioid Use Disorder (OUD): A Positron Emission Tomography (PET) Study (Preliminary Results)

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Background: There is an urgent need to develop novel targets in opioid use disorder (OUD) treatment. Despite all the nation-wide preventive and therapeutic efforts, drug overdose rates have surged by a factor of four in the last two decades, with opioids implicated in over 68% of cases. The current state-of-the-art treatment for OUD includes the use of medication assisted therapies, which mainly target the endogenous opioid system, especially opioid mu receptors. However, their effectiveness is limited by the high rates of treatment discontinuation and relapse.

The endogenous cannabinoid (endocannabinoid or eCB) system closely interacts with the endogenous opioid system. Both CB1R and MOR are Gi/o-coupled receptors, coexisting in various brain regions. A growing body of evidence suggests that the eCB system could present a novel target in treatment of OUD. Despite the current evidence from animal studies demonstrating that prolonged exposure to opioids alters the eCB function and CB1R levels, there is limited human studies on the endocannabinoid system in OUD. To the best of our knowledge, this is the first in vivo study to measure CB1R availability in individuals with OUD, using PET imaging and [11C]OMAR.

Methods: We enrolled otherwise healthy individuals with DSM5 diagnosis of OUD on stable agonist therapy (Methadone Maintenance) and healthy controls without drug use. Positron emission tomography (PET) and [11C]OMAR were used to measure the CB1R availability (the volume of distribution) composite (mean regional values) in different brain regions. The study protocol has been approved by the Yale School of Medicine Institutional review board.

Results: We present the preliminary results of 5 individuals with OUD compared to 15 age- and gender-matched healthy controls. Mean age was 45.3 (SD: 8.1) and 52 (SD: 17.2) in healthy and OUD group, respectively, with no significant differences. Mean dose of methadone was 87mg/day in the OUD group. Composite (whole brain) CB1R availability (VT) was significantly lower in individuals with OUD (1.22, SD: 0.11) compared to healthy controls (1.44, SD: 0.19).

Conclusions: Preliminary results suggest that the mean CB1R availability is lower in individuals with OUD compared to healthy controls. Given the essential role of the endocannabinoid system in stress regulation, the lower signaling of the endocannabinoid system in individuals with OUD could have important clinical implications in stress hyperactivity in individuals with OUD.

Keywords: Endocannabinoids, Opioid Use Disorder, Cannabinoid Receptor Type 1

Disclosure: Synendos Therapeutics: Advisory Board (Self).

P728. The Brain Dopamine System in Individuals Taking Medications for Opioid Use Disorder

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Background: Chronic use of drugs, including opioids, is associated with significant changes to the brain dopamine system. Indeed, using positron emission tomography (PET) we and others reported that actively using heroin users had lower striatal dopamine D2/3 receptor availability and stimulant-induced dopamine release than controls. However, whether these deficits resolve when individuals are treated with medications for opioid use disorder (MOUD), including methadone and buprenorphine or have achieved recovery without MOUD has not been investigated. This is important to address because MOUD are underutilized, in part due to stigma that they are just substituting one opioid drug for another. Further, little is known about dopamine D1-like receptors, which are crucial for drug reward, in opioid use disorder (OUD).

Methods: We completed studies in 55 individuals with OUD that were treated with methadone (subgroup 1: $n = 28$), buprenorphine (subgroup 2: $n = 17$) or had achieved recovery for 3+ months without MOUD nor illicit opioid use (subgroup 3 $n = 10$), and in 38 age/sex/BMI-matched healthy controls. Each participant underwent 3 PET scans; one to measure D1 receptors ([11C]NNC119) and two to measure D2 receptors ([11C]raclopride) with and without a methylphenidate challenge (60 mg oral), which was used to assess dopamine release. Scan were completed over a 2-to-3-day period (blinded, order counterbalanced).

Results: The OUD participants treated with methadone or buprenorphine, but not the OUD participants that had achieved recovery, showed significantly lower D2/3 receptor availability than controls in putamen ($F > 4.50$, $p < .05$), but not in caudate nor accumbens. The difference in D2/3 receptor availability was driven by individuals who were still occasionally using illicit opioids. All three OUD subgroups showed higher striatal D1 receptor availability than controls. There were no differences in methylphenidate induced increases in striatal dopamine between controls and OUD participants with or without MOUD. The samples were insufficient to assess sex differences or differences between buprenorphine and methadone.

Conclusions: This study provides preliminary evidence that individuals with OUD treated with methadone (full opioid agonist) or buprenorphine (partial opioid agonist) do not show deficits in striatal dopamine release (as measured with a methylphenidate challenge) and that the deficits in D2/3 receptor availability appear less severe than those previously reported in untreated patients and most notable for patients who achieved abstinence from illicit opioids. The elevated D1R in treated and untreated OUD participants might reflect persistent changes and/or predisposing characteristics that increased their risk for OUD. Our findings document evidence of recovery on D2R signaling and dopamine release but not D1R with MOUD, indicative of their beneficial effects to brain recovery and distinguishing them from continued misuse of opioids.

Keywords: Dopamine Receptors, Positron Emission Tomography Imaging, Substance Use Disorder

Disclosure: Nothing to disclose.

P729. Abstinence From Binge Alcohol Consumption Potentiates Neuronal Activity in the Lateral Habenula via Sex-Specific Mechanisms to Dysregulate Social and Arousal Behaviors

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Background: Binge alcohol consumption induces discrete social and arousal disturbances in human populations, which are thought to promote increased drinking. However, whether models of binge drinking in rodents can induce these same long-term negative behavioral symptoms is unknown. Serotonin receptors have been implicated in the pathophysiology of both mood disorders and alcohol use disorder, but the neuronal circuits and receptor subtypes controlling discrete aspects of pathological behaviors are unclear.

Methods: Male and female adult mice ($n = 8$/group) performed voluntary alcohol drinking (20% w/v) or water drinking in their home cages for three weeks using the Drinking in the Dark (DiD) paradigm. One week following DiD, mice were tested in the 3-chamber sociability test, open field test, and acoustic startle test. We next used fiber photometry to characterize the calcium and serotonin responses of lateral habenula neurons containing the serotonin 5HT2c receptor (LHb5HT2c) during startle and social behaviors ($n = 7$-21/group). We performed these recordings before and after DiD to ascertain how alcohol shapes the responses of LHb5HT2c neurons to rewarding and aversive stimuli. We next used patch-clamp electrophysiology to measure the effects of DiD on LHb5HT2c neuronal excitability, spontaneous activity, and spontaneous EPSCs ($n = 5$-6 mice, 2-4 cells/group). We also used qPCR to determine how DiD affects the expression of serotonin receptors and ion channels co-expressed on LHb5HT2c ($n = 8$-10/group). Finally, we used chemogenetics to inhibit the activity of LHb5HT2c during behavior in animals that went through the DiD procedure ($n = 6$-9/group).

Results: DiD increased acoustic startle in males ($p < 0.05$) and impaired social recognition in females ($p < 0.01$) (Two-way ANOVA). LHb5HT2c were activated by startle and social interaction but inhibited by voluntary alcohol or water drinking (t-tests, $p < 0.05$). DiD increased LHb5HT2c calcium activity in response to startle stimuli in males ($p < 0.01$) and marginally increased 5HT release onto LHb5HT2c in response to social stimuli in females ($p = 0.07$) (t-tests). In slice, DiD increased LHb5HT2c excitability selectively in males ($p < 0.05$) (Two-way repeated measures ANOVA). However, DiD increased spontaneous firing rates of LHb5HT2c in both sexes ($p > 0.05$) (t-tests). In addition, spontaneous EPSC amplitude was larger in DiD females compared to water females ($p < 0.05$) (t-test). These physiological changes were accompanied by sex-specific changes in a variety of 5HT receptors co-expressed on LHb5HT2c. DiD females displayed reduced expression of 5HT1a, 5HT1b, and 5HT3a, while DiD males displayed increased expression of 5HT2c. Interestingly, various K + channels were increased in the LHb of DiD mice of both sexes. Chemogenetic inhibition of LHb5HT2c following DiD normalized

social recognition deficits in females ($p < 0.01$) as well as startle behavior in males ($p < 0.01$) (Two-way ANOVA).

Conclusions: Binge alcohol consumption induces distinct changes in social and emotional behavior in males and females. Critically, these distinct behavioral changes are commonly mediated by alcohol-induced hyperactivity of a sub-population of LHb neurons (LHb5HT2c). The cellular mechanisms driving this hyperactivity are potentially related to sex-specific alterations in 5HT receptor and ion channel expression.

Keywords: Serotonin 5-HT2C Receptor, Alcohol, Lateral Habenula

Disclosure: Nothing to disclose.

P730. Organization and Function of Substance P Innervation to the Central Amygdala and Nucleus Accumbens

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Background: The neuropeptide Substance P (SP) serves as the preferred endogenous ligand of the neurokinin-1 receptor (NK1R). The SP/NK1R system plays a significant role in complex behaviors such as anxiety, depression, stress responses, and drug/alcohol seeking. For alcohol-related behaviors specifically, the NK1R mediates escalated alcohol consumption and stress-elicited alcohol seeking, but does not impact baseline intake or cue-induced alcohol seeking. We have previously found that these alcohol-related behaviors are mediated by NK1Rs in two primary regions, the nucleus accumbens (NAC) shell, and the central nucleus of the amygdala (CeA). For example, NK1Rs in the NAC shell mediate escalated alcohol consumption that is induced by exposure to chronic social defeat stress, and relapse-like behavior that is triggered by stress. Also, NK1Rs in the CeA influence escalated alcohol seeking in genetically selected, alcohol preferring rats.

Methods: In the experiments described here, we infused a retroAAV-hSyn-DIO-mCherry virus into the NAC shell or CeA of Tac1-cre mice. Tac1 is the gene that produces the propeptide for SP, and this approach allows us to identify SP neurons that innervate the regions into which the retrovirus is infused. After viral infusion, we allowed 3 weeks for expression and then perfused mice and extracted brains for analysis. Brains were sectioned on a cryostat, stained for mCherry immunoreactivity, and imaged on a fluorescent microscope.

Results: In mice infused in the NAC shell, we found mCherry positive cells in the paraventricular nucleus of the thalamus (PVT), basomedial nucleus of the amygdala (BMA), and the olfactory bulbs. For CeA infusion, mCherry positive cells were located in the PVT, BMA, and dorsal raphe (DR). To assess the function of these SP circuits in the brain, we infused a retroAAV-hSyn-DIO-h3D(Gq) -mCherry virus into the NAC shell of Tac1-Cre mice, and delivered CNO to activate the virally expressed excitatory Gq DREADD in NAC-innervating SP neurons. We found that this stimulation increased voluntary alcohol consumption.

Conclusions: The findings presented here outline the SP circuitry to the extended amygdala and provide evidence that they are involved in activating alcohol consumption. Future experiments will use similar DREADD-based strategies to assess CeA activation. We will also determine if SP+ projections from the PVT that innervate the CeA and NAC shell are distinct cell populations, or if individual neurons send collaterals to both regions.

Keywords: Neuropeptides, Alcohol, Neurokinin, Amygdala, Nucelus Accumbens

Disclosure: Nothing to disclose.

P731. Sexually Divergent Effects of Activity in the Ventral Hippocampus to Nucleus Accumbens Projection on Reward Learning in Rats

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Background: Cues in the environment can gain intrinsic motivational value when paired with rewarding substances. This relationship can become maladaptive when these cues are associated with harmful and addictive substances. The model of Pavlovian conditioned approach training is used to assess individual variation in attributing incentive value to reward cues, which is associated with a proclivity towards addiction and relapse. When the presentation of a lever precedes a food reward, sign-tracking rats will approach and manipulate lever itself, while goal-tracking rats treat the lever merely as a predictor and approach the location of impending food reward. The sign-trackers are attributing incentive salience to the lever itself, imbuing the cue with rewarding value. Sign-trackers are more prone to cue-induced reinstatement of drug-seeking behavior, and appear less able to use contextual information to modify their responses to cues. Neuronal activity in the nucleus accumbens (NAc) – a critical region of the motive circuit – seems to be necessary for the development of sign-tracking behavior and the attribution of incentive salience. However, little is known about the sources of afferent glutamatergic neurons that regulate NAc activity and impart individual variation to the associative learning process. The NAc is densely innervated by the ventral hippocampus (vHPC), and previous work in our lab showed that lesions of the vHPC attenuate the acquisition of sign-tracking. However, whether this effect is exerted via direct modulation of the NAc is unclear. We explored the hypothesis that the vHPC-NAc projection plays a functional role in determining sign- and goal-tracking behavior.

Methods: We used an in vivo dual-vector approach to bilaterally inject viruses expressing Cre recombinase into the NAc and either a virus expressing a Cre-dependent, inhibitory Gi-coupled or an excitatory Gq-coupled designer receptor exclusively activated by designer drugs (DREADD) into the vHPC to selectively target the vHPC-NAc projection. These viral injections were performed via stereotaxic surgery on male and female Sprague Dawley rats (7-8 weeks old). Five weeks after surgery, all rats underwent six daily sessions of a Pavlovian conditioning approach procedure with clozapine-N-oxide (CNO; 3mg/kg, i.p) or vehicle (6% DMSO) on board, followed by a crossover treatment test session. Each session consisted of 25 presentations of a retractable lever (conditioned stimulus) that extended into the chamber for 10 seconds, and 25 deliveries of a sucrose-free banana pellet (unconditioned stimulus) into the pellet magazine. Conditioned responses were classified based on whether they were preferentially directed toward the lever-cue (sign-tracking) or toward the food-cup (goal-tracking).

Results: Among males in the inhibition ($n = 18$), excitation ($n = 9$), and vehicle ($n = 22$) groups, we found group-by-session interaction effect for lever presses trending towards significance ($F(10,220) = 1.858$, $p = 0.0524$). Specifically, there was a significant difference between inhibition and excitation groups, where inhibition increased lever pressing while excitation decreased lever pressing ($p = 0.033$, 95% CI = [-99.32 to −3.570]). There was also a significant group-by-day interaction for food cup entries in males ($F(10,220) = 2.923$). Specifically, there was a significant difference between inhibition and excitation groups, where

excitation increased food cup entries while inhibition decreased food cup entries ($p = 0.33$, 95% CI = [3.137 to 72.09]). Despite these findings in males, we found no significant differences in lever press or food-cup entry number, latency or probability between vehicle treated (n = 12) vs CNO inhibition (n = 6) or excitation (n = 8) rats across all six training sessions among females suggesting that vHPC-NAc projection does not affect the acquisition of sign- and goal-tracking behavior in female rats.

Conclusions: Our data suggest that the vHPC-NAc projection may not be necessary for the acquisition of sign- and goal-tracking behavior in females. However, it may have a more complex and selective role in the performance of sign-tracking behavior in males, as chronic vHPC-NAc inhibition increased while excitation decreased the expression/performance of sign-tracking in male rats. More experiments need to be performed to further dissect this role and shed light on the neurocircuitry responsible for biasing sign- and goal-tracking behavior. Ultimately, this will lead to a better understanding of increased susceptibility to cue-driven psychopathologies such as addiction.

Keywords: Reward Learning, Addiction Circuitry, Individual Differences

Disclosure: Nothing to disclose.

P732. Endocannabinoid Tuning of Behavioral Engagement Via an Anterior Paraventricular Thalamus - Nucleus Accumbens Circuit

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Background: A key clinical feature of addiction is compulsive engagement with rewarding stimuli, such as drugs of abuse, despite negative outcomes. However, we lack a detailed understanding of the neural mechanisms that regulate compulsive behavioral engagement. The Nucleus Accumbens (NAc) represents an integral functional component of the mesocorticolimbic pathway, canonically known as the "reward" pathway. The NAc receives dense projections from the Paraventricular Thalamus (PVT), and these projections have been shown to regulate the behavioral effects of opiate withdrawal, sucrose seeking/consumption, and behavioral responses to painful stimuli. The PVT is a highly heterogenous structure, and recent studies examining the PVT-NAc circuit have generated contradictory results, partially driven by a lack of genetic and anatomical targeting within the PVT. In this study, we interrogate the physiological and functional role of aPVT projections to the NAc in regulating behavioral engagement.

Methods: 1. RNAscope: 4 mice total, 2 anterior and 2 posterior PVT slices per mouse were used for quantification of mRNA transcript numbers and colocalization using HALO software

2. Ex vivo electrophysiology: Acute coronal slices containing the NAc were prepared from NTS-Cre x D1-tdTomato mice injected with DIO-ChR2-eYFP. Minimum of 4 mice per group, 2 slices per mouse, and a maximum of 4 cells per group per slice.

3. GCaMP fiber photometry: 10 NTS-Cre mice were injected with DIO-GCaMP6s in the aPVT and implanted with a unilateral fiberoptic in the NAc. 6 weeks after surgery, mice were trained in Pavlovian reward and Pavlovian fear conditioning.

4. In vivo optogenetics: Activation: 12 NTS-Cre mice were injected with DIO-ChR2 in the aPVT and 11 were injected with DIO-eYFP control virus. Inhibition: 7 NTS-Cre mice were injected with DIO-PPO and 6 were injected with DIO-eYFP control virus. Both groups were implanted with bilateral fiberoptics above the NAc. Mice were trained in Pavlovian reward and Pavlovian fear conditioning.

5. GRABeCB fiber photometry: 7 WT were injected with hSyn-GRABeCB in the aPVT and implanted with a unilateral fiberoptic above the NAc. Mice were trained in Pavlovian reward and Pavlovian fear conditioning.

6. CRISPR/Cas9 deletion of the CB1 receptor w/ fiber photometry. NTS-Cre mice were injected with a 5:3 mixture of DIO-saCas9-sgCNR1 and DIO-GCaMP6s in the aPVT and were implanted with a unilateral fiberoptic above the NAc. Mice were trained in Pavlovian reward and Pavlovian fear conditioning.

7. 1 photon imaging of NAc PENK neurons. PENK-Cre mice were injected with a transsynaptic AAV1-EF1a-DIO-FLP in the aPVT and an fDIO-GCaMP6s in the NAc to capture NAc PENK neurons that receive direct input from the aPVT. This was followed by implantation with a GRIN lens in the NAc. Mice were trained in Pavlovian reward and Pavlovian fear conditioning.

Results: Here, we identify the neuropeptide neurotensin (NTS) as a novel marker for a select population of anterior PVT neurons. We further demonstrate that these neurons are highly colocalized with the cannabinoid 1 receptor (CB1), the primary neuronal receptor for endocannabinoids and a crucial regulator of presynaptic neurotransmitter release. We subsequently show that these NTS neurons send strong excitatory projections to the NAc, and that this input is biased toward Dopamine Receptor 1 (D1R) expressing medium-spiny neurons. This input bias is mediated through tonic endocannabinoid (eCB) suppression of excitatory input onto Dopamine Receptor 2 (D2R) expressing neurons. Using fiber photometry in mice expressing DIO-GCaMP in PVT NTS neurons, we observed that aPVT-NAc projections are inhibited during engagement in both sucrose seeking/consumption ($R2 = 0.2553$, $p = 0.0002$) and conditioned freezing behaviors ($R2 = 0.1300$, $p = 0.0068$), and activated upon behavioral disengagement ($R2 = 0.3299$, $p < 0.0001$). Ectopic activation and inhibition of this circuit using optogenetics was sufficient to attenuate ($p = 0.0413$) and prolong behavioral engagement ($p = 0.0389$), respectively. To assess the role of eCBs in modulating aPVT-NAc circuit activity and behavioral engagement, we used GRAB-eCB, a fluorescent sensor for eCB release, to demonstrate that eCBs are released in the NAc and bind to impinging aPVT terminals during engagement in reward-seeking behaviors ($p = 0.0009$). We augmented this approach by demonstrating that CRISPR/Cas9 deletion of the Cannabinoid 1 Receptor (CB1) from aPVT NTS neurons was sufficient to reduce engagement in reward-seeking behaviors ($p = 0.0481$) and concomitant aPVT-NAc terminal inhibition ($p = 0.0136$). Lastly, using an anterograde transsynaptic labeling technique combined with 1-photon miniscope imaging, we observed that D2R/PENK neural activity in the NAc precedes aPVT terminal inhibition, ostensibly linking NAc neural activity with resultant eCB release and retrograde action at aPVT terminals in the NAc.

Conclusions: Taken together, these results implicate a novel eCB mechanism for regulation of behavioral engagement through modulation of an aPVT-NAc circuit. Our data suggest that eCB inhibition of this excitatory circuit plays a critical role in regulating engagement with both rewarding and aversive stimuli. These data lay the groundwork for investigating how drugs of abuse elicit maladaptive changes in the innate function of this circuit to drive compulsive drug seeking behaviors.

Keywords: Endocannabinoids, Addiction Circuitry, Slice Electrophysiology, Paraventricular Thalamus, Nucleus Accumbens

Disclosure: Nothing to disclose.

P733. The Anterior Cingulate Cortex Controls Associative Fentanyl Analgesic Tolerance

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Background: Opioid analgesics are highly prescribed for pain management. However, opioids have considerable abuse liability. As users increase their opioid intake, they build tolerance against their therapeutic effects. Despite well-described molecular and cellular mechanisms, tolerance can be reversed when the drug is taken in another context, a phenomenon known as associative analgesic tolerance. Since associative analgesic tolerance can lead to escalation of use, it increases the risk of opioid use disorder (OUD). While associative tolerance and its reversal have been described in animal studies, the biological mechanisms underlying this process are poorly understood. We, therefore, determined how associative tolerance and its reversal change the activity of neurons and circuits throughout the brain, identifying the anterior cingulate cortex (ACC) as a critical orchestrator of associative tolerance in male mice.

Methods: In experiment 1, we established a training procedure that induces associative analgesic tolerance. Male and female C57BL/6J mice (n = 20/ group) received injections of either fentanyl (FEN) (25 ug/kg, s.c.) or saline (SAL) before being placed in a context with distinct multisensory features each day for 14 days. After 15 minutes in the context, basal nociception and FEN-induced analgesia were measured using the hotplate assay. After the training phase, associative tolerance was tested by administering FEN in the FEN-paired or SAL-paired context before the hotplate test. Experiment 2 identified neuronal populations engaged during associative tolerance across the brain using Arc-TRAP: Ai14 mice (n = 4/group), allowing for activity- and time-dependent tagging of neuronal populations with the fluorescent marker tdTomato. To tag tolerance-active neurons, mice received the TRAP activator 4-OH tamoxifen on the last day of tolerance training. Four weeks later, mice were injected with FEN and exposed to either the FEN- or SAL-paired context. Brains were extracted and cleared using SHIELD to preserve endogenous fluorescence. Images were acquired using a custom-built high-speed selective plane illumination microscope (SPIM), and active neurons were detected using an automatic cell-counting algorithm. Experiment 3 determined how context and FEN changed neuronal activity in brain areas implicated in contextual memory and pain processing in tolerant mice. Mice (n = 3-4/group) underwent tolerance training before exposure to one of the following conditions: SAL-context and SAL, SAL context and FEN, FEN-context and SAL, or FEN context and FEN. Brains were extracted and analyzed using immunohistochemistry against cFOS. In experiment 4, we used fiber photometry to measure neuronal activity in the ACC in vivo throughout associative tolerance training. Male mice (n = 4) were stereotaxically injected with a viral vector containing the fluorescent calcium sensor GCaMP6f in the ACC. Fluorescent signal was recorded during tolerance acquisition, expression, and reversal. Finally, in experiment 5, the ACC of male mice (n = 6-9/group) was targeted for stereotaxic injection of viral vectors containing inhibitory Designer Receptors Exclusively Activated by Designer Drugs (Gi-DREADDs) or fluorescent controls. Mice then underwent tolerance training before being injected with the DREADD activator CNO and FEN in the FEN context or SAL in the SAL context.

Results: Experiment 1: male and female mice that received FEN in the FEN-paired context showed tolerance, defined as a decrease in analgesic responses in the hotplate assay from the first FEN session (F1) to the seventh FEN session (F7) (male change = 11 seconds, female change = 11 seconds, F1, 33 = 7.985, $p < .0001$). However, tolerance was reversed in male, but not female, mice exposed to FEN in the SAL-paired context (F1, 33 = 12.89, $p < .01$). Experiment 2: FEN exposure in the FEN-paired context significantly increased activity in 8 brain regions which play roles in sensory, learning, pain, and reward-associated behaviors. FEN exposure in the SAL context significantly increased activity in areas that negatively regulate arousal, memory, and pain. Experiment 3: administration of FEN in the FEN context increased cFOS+ neurons specifically within the ACC (F1, 8 = 5.536 $p < .05$). Experiment 4: preliminary results suggest that ACC fluorescent signal (df/f) is suppressed in FEN-naïve mice during the hotplate test but normalizes by the 7th FEN presentation when compared to SAL treatment (mean A.U.C. of df/f = 174 for SAL, 17 for F1, and 171 for F7). ACC signal is suppressed in tolerant mice that received FEN in the SAL context (A.U.C. of df/f = 55). Experiment 5: chemogenetic silencing ACC in the FEN-paired context blocked analgesic tolerance but did not alter nociception when mice received SAL in the SAL-paired context (F3, 21 = 74.92, $p < .0001$).

Conclusions: These results establish a robust behavioral training procedure for studying associative opioid analgesic tolerance in mice. The findings also reveal how associative tolerance and its reversal change activity across the brain. Among the identified regions, the ACC appears to be a target for the modulation of associative analgesic tolerance.

Keywords: Opioid Tolerance, Context, Whole-Brain Rodent Imaging

Disclosure: Nothing to disclose.

P734. Identification of Novel Brain Regions Underlying Reinstatement to Oxycodone Seeking

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Background: The US is in the midst of an opioid abuse and overdose epidemic, which has been declared a public health emergency. Oxycodone is one of the most prescribed analgesics, is the first opioid many people experience, and has physiochemical properties that allow it to accumulate in the brain at rates higher than other opioids, perhaps explaining its considerable abuse potential. In the past two decades, a great deal of research using animal models drug addiction have focused on a small number of neurobiological systems, most notably the mesocorticolimbic dopamine system, the corticostriatal glutamate system, and the extended amygdala. Recent advances in tissue clearing and light-sheet microscopy technologies now enable high-throughput, unbiased examination of protein expression (e.g. c-Fos) or neurocircuitry throughout the entire brain. Using these techniques to study addictive-like behaviors reveals previously unstudied brain structures that are highly correlated with drug seeking, and may warrant further investigation. Here we present data on one such novel structure, the anterior pretectal nucleus (APtN).

Methods: We used the AdipoClear+ tissue clearing method and light-sheet microscopy to examine whole-brain c-Fos expression following experimenter-administered oxycodone, and following cue-induced reinstatement of oxycodone seeking. We trained Fos2A-iCreER (i.e. FosTRAP2) x ai14-tdTomato reporter mice to self-administer intravenous oxycodone, with yoked-saline and sucrose self-administering control groups (N = 9-14 per group). Following a final extinction or cue-induced reinstatement test, administered 4-OHT to induce Cre recombinase activity, 'tagging' cells activated during the preceding session. 3 days later, we extracted and cleared their brains to identify neuronal ensembles activated by cue-induced reinstatement of oxycodone, but not

sucrose seeking. We identified the APtN as a novel structure that showed a highly significant upregulation of Fos-dependent tdTomato+ cell counts following cued reinstatement of oxycodone seeking, respectively. We then injected a Cre-dependent inhibitory (M4/Gi) DREADD into the APtN of FosTRAP mice, and again administered 4-OHT immediately following cued reinstatement of oxycodone seeking, to 'tag' the activated ensemble with the inhibitory construct. One week later we administered CNO or vehicle to examine the effect of inhibition of this ensemble on subsequent reinstatement. To determine behavioral roles of the APtN in drug-naïve mice, we then employed optogenetics in C57 mice during real-time place preference tests. To anatomically characterize the APtN, we used retrograde and anterograde tracing viruses and brain clearing to identify whole-brain connectivity of the APtN. Furthermore we used single-nuclei RNA-sequencing (snRNA-Seq) to characterize the composition of cell types within the APtN, and future studies will examine cell-type specific contributions of the APtN to reward, aversion, and addictive-like behaviors.

Results: The APtN is robustly and specifically activated by cued reinstatement of oxycodone seeking, and the magnitude of its activation is correlated with drug seeking (i.e. lever-pressing). Chemogenetic inhibition of this reinstatement-activated ensemble effectively reduced subsequent cued reinstatement, providing the first evidence for the APtN's involvement in drug-seeking behavior. Preliminary data indicates that optogenetic stimulation of the APtN in drug-naïve mice is aversive, as mice spend less time on an LED-paired side of a chamber. The APtN receives dense inputs from the reticular formation nuclei of the mid- and hindbrain, the amygdalostriatal transition area, and the retrosplenial cortex. It sends diffuse projections throughout the thalamus, and dense projections to the rostroventral medulla, deep mesencephalic nuclei, zona incerta, and ventral mesencephalon. Analysis of single-nuclei sequencing data is ongoing but will be complete before this annual meeting.

Conclusions: These data identify the APtN as a highly novel regulator of cue-induced reinstatement. Optogenetic data indicate that stimulation of this region is aversive, leading to the hypothesis that opioids act on inhibitory receptors in the APtN to inhibit aversion, causing reward through negative reinforcement. The connectivity patterns of the APtN suggest it may be involved in mechanisms of arousal, sensory gating, and pain. Ongoing studies will determine the molecular composition of the APtN and cell-type specific contributions to reward, aversion, and addictive-like behaviors.

Keywords: Opioid Addiction, Substance Use Disorders, Light-Sheet Microscopy, Optogenetics, Single-Nuclei Sequencing

Disclosure: Nothing to disclose.

P735. A Thalamostriatal Behavioral Inhibition Circuit is Weakened by Opioid Use and its Restoration Prevents Relapse

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Background: A lack of behavioral inhibition is a hallmark of all addiction-related disorders. For example, in substance use disorders relapse results from the disinhibition of drug seeking during abstinence. Despite this knowledge, a vast majority of research has focused on neural mechanisms that facilitate, rather than disinhibit, drug seeking. Hence, whether relapse results from drug-induced dysregulation of the neuronal mechanisms that normally guide behavioral inhibition remains unclear. This study will highlight a key thalamostriatal behavioral inhibition circuit that is weakened in mouse models of opioid use, and that can be restored to prevent relapse.

Methods: Here we study behavioral inhibition circuit adaptations across drug use by developing deep brain two-photon calcium imaging in heroin and sucrose self-administering male and female mice (n > 8 mice per group, equal males and females). We record from neurons in the paraventricular thalamus (PVT) that project to the nucleus accumbens (PVT-NAc), a circuit that is key for behavioral inhibition. Furthermore, we use patch-clamp electrophysiology ex vivo to identify drug-induced synaptic adaptations at cell-type specific PVT-NAc synapses (n > 6 mice per group, equal males and females, > 12 neurons per group). Finally, we combine chemogenetics and optogenetics to determine the sufficiency and necessity of PVT-NAc neurons, and downstream NAc cell types for the inhibition of heroin seeking (n > 8 mice per group, equal males and females).

Results: We find select neuronal ensembles (n = 3) that become profoundly hypoactive across the development of heroin use and during reinstatement to heroin seeking (p-values < 0.001). Furthermore, we find select postsynaptic adaptations at PVT-NAc synapses, such that thalamostriatal input to NAc parvalbumin interneurons (PV-INs) are profoundly weakened following heroin use (p-values < 0.001). Finally, we show that while stimulation of PVT-NAc neurons is sufficient to prevent cue-induced reward seeking in sucrose-trained mice, the same manipulation has no effect in heroin-trained mice (p-values < 0.001). Behavioral disinhibition can be prevented by the knockout of PVT μ-opioid receptors or by combinatorial stimulation of PVT-NAc neurons with chemogenetic excitation of the downstream NAc PV-INs (p-values < 0.01). We quantified but did not observe sex differences in our calcium imaging, slice electrophysiology, optogenetic, or chemogenetic studies.

Conclusions: Our findings identify precise functional and physiological adaptations within a thalamostriatal behavioral control network, such that PVT-NAc activity and synaptic efficacy is weakened by opioid use. Furthermore, our data reveal that restoration of this system would prevent relapse to drug seeking. Our findings highlight the critical need for basic research examining behavioral suppression circuitry in the context substance use disorders.

Keywords: Behavioral Inhibition System, Disinhibition, Drug Self-Administration

Disclosure: Nothing to disclose.

P736. Social Isolation Recruits Amygdala-Cortical Circuitry to Escalate Alcohol Drinking

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Background: Stress can trigger the urge to drink alcohol, which become even more apparent with the recent COVID-19 pandemic. While alcohol drinking and sales went up during this time, many individuals reduced or abstained from drinking. This individual variability in responses to stress and alcohol drinking can be observed in humans as well as rodents. It remains unclear what underlies this individual variability in alcohol drinking and the brain circuits contributing to these differences. Identifying a biomarker for individual variability in alcohol drinking could lead

to the prevention of an alcohol use disorder in susceptible individuals.

Methods: Using a combination of behavior, whole-cell patch clamp recordings, cellular resolution calcium imaging combined with optogenetic manipulations, and machine learning, we tested the hypothesis that social factors, including social rank and social isolation, may contribute to individual differences in alcohol drinking which may be mediated by the BLA-mPFC circuit.

Results: We found that social rank predicts alcohol drinking, where subordinates drink more than dominants (Pearson correlation, $r2 = 0.25$, $^{**}p < 0.01$, $N = 23$). Furthermore, social isolation escalates alcohol drinking, particularly impacting subordinates who display a greater increase in alcohol drinking compared to dominants (unpaired t-test, $^{**}p < 0.01$). Using cellular resolution calcium imaging, we show that the basolateral amygdala-medial prefrontal cortex (BLA-mPFC) circuit predicts alcohol drinking (Pearson correlation, $r2 = 0.59$, $^{**}p < 0.01$, $N = 10$) in a rank-dependent manner, unlike non-specific BLA activity (Pearson correlation, $r2 = 0.15$, $p = 0.26$). The BLA-mPFC circuit becomes hyperexcitable during social isolation (two-way ANOVA, $F(25, 1200) = 4.45$, $^{****}p < 0.0001$, $n = 21\text{-}29$ neurons from $N = 5\text{-}6$ mice/group), detecting social isolation states. Mimicking the observed increases in BLA-mPFC activity using optogenetics was sufficient to increase alcohol drinking (two-way ANOVA, $N = 6$ mice/group), suggesting the BLA-mPFC circuit may be a neural substrate for the negative impact of social isolation. To test the hypothesis that the BLA-mPFC circuit conveys a signal induced by social isolation to motivate alcohol consumption, we first determined if this circuit detects social information. Leveraging optogenetics in combination with calcium imaging and SLEAP automated pose tracking, we found that BLA-mPFC circuity governs social behavior and neural representation of social contact. We further show that BLA-mPFC stimulation mimics social isolation-induced mPFC encoding of sucrose and alcohol, and inhibition of the BLA-mPFC circuit decreases alcohol drinking following social isolation.

Conclusions: Collectively, these data suggest the amygdala-cortical circuit mirrors a neural encoding state similar to social isolation and underlies social isolation-associated alcohol drinking. Thus, the BLA-mPFC circuit emerged as a key mediator of alcohol drinking associated with social rank and social isolation advancing our understanding of the neural mechanism underlying the interplay between social factors and alcohol drinking that may increase susceptibility to developing an alcohol use disorder.

Keywords: Alcohol, Amygdala, Prefrontal Cortex, Social Isolation, Social Hierarchy

Disclosure: Nothing to disclose.

P737. Binge-Like Ethanol Drinking and Withdrawal Effects on Hyperalgesia and Ventrolateral Periaqueductal Gray (vlPAG) Neuronal Properties in Mice

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Background: People with acute and chronic pain report drinking ethanol to relieve pain. Clinical and animal studies have shown that chronic pain is associated with and may promote an increase in ethanol intake. However, chronic ethanol use is associated with hyperalgesia, an effect that is especially apparent during periods of ethanol withdrawal. Ethanol withdrawal hyperalgesia has been demonstrated using several rodent drinking models, for example, chronic intermittent exposure to ethanol vapor protocol and the two-bottle choice drinking paradigm in rats and mice. However, this phenomenon has not been reported in models of human binge drinking, such as the drinking in the dark (DID) paradigm in mice. Ethanol withdrawal reduces the activity and strength of inhibition from central amygdala (CeA) GABAergic inputs onto ventrolateral periaqueductal gray (vlPAG) neurons, a region important for pain modulation. This project aims to investigate whether voluntary binge-like ethanol consumption using the DID paradigm (1) leads to hyperalgesia and (2) alters the intrinsic properties of vlPAG neurons in male and female adult mice.

Methods: Adult male and female mice were chronically exposed to ethanol using DID paradigm for 4 weeks (16 sessions). The experimental group had their water bottles switched out for a 20% ethanol solution for 2 hours, three days per week, and for 4 hours, one day per week, followed by 3 days of abstinence. In the control group, water bottles were switched out for another bottle containing water during the same amount of time as the experimental group. To assess whether DID generates ethanol-induced hyperalgesia, nociceptive testing occurred during the first day of abstinence. Mechanical sensitivity was assessed using the von Frey test and thermal sensitivity was assessed using the hot plate test. To assess changes in the intrinsic properties of vlPAG neurons, a separate cohort of mice were subjected to the same DID paradigm and whole-cell patch-clamp slice electrophysiology recordings were performed after 3-6 days of ethanol abstinence.

Results: The DID paradigm engenders ethanol drinking resulting in high blood ethanol concentration levels, above intoxication levels (80 mg/dL). Adult male and female mice exposed to 20% ethanol using DID developed mechanical and thermal hypersensitivity after 3 weeks of DID compared to control group. We hypothesize that DID followed by acute abstinence will generate changes in intrinsic properties of the vlPAG (these experiments are ongoing).

Conclusions: DID is useful as a model to examine neurobiological mechanisms underlying alcohol-associated hyperalgesia, and may lead to the identification of potential pharmacologic targets to help treat individuals experiencing alcohol withdrawal and those at risk of developing AUD due to chronic pain.

Keywords: Pain Sensitivity, Alcohol Abstinence, Periaqueductal Grey (PAG)

Disclosure: Nothing to disclose.

P738. Effects of Dorsomedial or Dorsolateral Striatum Lesions on Punishment Resistance for Cocaine Seeking in Rats

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Background: Addiction is characterized by continued drug use despite negative consequences. In an animal model, a subset of rats continues to self-administer cocaine despite footshock consequences, showing punishment resistance. Recently, our lab found that punishment resistance was associated with the continued use of habitual responding, whereas punishment sensitivity was associated with increased goal-directed control. These findings indicate that punishment resistance is related to habits that have become inflexible and persist under conditions that should encourage a transition to goal-directed behavior. Here we wanted to investigate the effects of lesions of dorsomedial striatum (DMS) and dorsolateral striatum (DLS) on punishment of cocaine seeking. Given that DMS and DLS are necessary for goal-directed and habitual responding, respectively, we hypothesized that DMS lesions would increase punishment resistance, while DLS lesions would increase punishment sensitivity, as compared to sham lesions.

Methods: Male Sprague Dawley rats received pre-training NMDA lesions of bilateral DMS (n = 12) or DLS (n = 10), or received sham lesions (n = 22), and were then trained on a seeking-taking chained schedule of reinforcement for cocaine self-administration (2 h per day). At the final stage of training, the seeking lever required completion of a random interval (RI60) or random ratio (RR20) schedule, which led to the extension of the taking lever. Rats were then exposed to four days of punishment testing in which footshock (0.4 mA, 0.3 s) was delivered randomly on one-third of trials after the completion of seeking. Two-way ANOVAs were used to compare lesion groups across days.

Results: We found that all groups (DMS, DLS, or sham lesions) showed a significant reduction in cocaine trials by the fourth day of punishment, as compared to baseline self-administration ($p < 0.0001$). Rats with DLS lesions were significantly more sensitive to punishment and completed less trials, as compared to rats with sham lesions ($p < 0.05$). In contrast, rats with DMS lesions showed similar punishment sensitivity and were not different from rats with sham lesions.

Conclusions: These findings indicate that DLS plays a role in punishment resistance for cocaine, which may be related to the role of DLS in habitual behavior. However, despite the role of DMS in goal-directed behavior, lesions of DLS did not drive increased punishment resistance for cocaine. Ongoing work is determining the effects of DMS and DLS lesions in female rats.

Keywords: Cocaine Self-Administration, Punishment, Dorsal Striatum, Habit, Addiction

Disclosure: Nothing to disclose.

P739. The Dopamine Transporter and D2 Autoreceptor Drive Local Differences in Dopamine Release

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Background: The release of dopamine (DA) in different areas of the brain varies in terms of quality and quantity. For instance, in the dorsal striatum and lateral nucleus accumbens (NAcLat), spontaneous DA transients fluctuate rapidly, while in the ventromedial striatum and prefrontal cortex, they fluctuate at lower frequencies. Furthermore, during reward seeking, DA released in the NAcLat exhibits patterns reminiscent of those observed during the encoding of reward prediction error, whereas in the medial nucleus accumbens (NAcMed), it is released more gradually, ramping up to the moment of reward consumption.

Several mechanisms may explain these local differences in DA dynamics. First, variability may be due to basic firing properties of different subpopulations of midbrain DA neurons, which express different ion channels and receive inputs from varying brain structures. Second, it has been suggested that acetylcholine released by striatal interneurons can trigger the release of DA without relying on firing of the dopamine cell bodies. Finally, two membrane proteins expressed on DA neurons, the dopamine transporter (DAT) and the dopamine D2 autoreceptor (D2-AR), may contribute to local DA dynamics. The DAT pumps extracellular DA back into the neuron, while D2-ARs on DA neurons decrease neuronal activity and the release of DA. These proteins play a role in determining how long DA remains in the extracellular space and provide an autofeedback mechanism that inhibits the release of DA. Notably, DAT and D2-AR are implicated in various psychiatric conditions, such as depression, psychosis, and addiction. However, the extent to which these two proteins contribute to local differences in DA dynamics across the corticolimbic system is not yet understood.

Methods: First, we used RNAscope in situ hybridization in combination with retrograde tracing to quantify mRNA levels of DAT, D2-AR and tyrosine hydroxylase in five projection-defined DA subpopulations (projecting to the NAcMed, NAcLat, dorsal striatum, tail of the striatum, and medial prefrontal cortex). We also recorded spontaneous and task-evoked DA release in these five projection targets using dLight, and correlated mRNA levels to different spontaneous and task-evoked measures of DA release. We then used a viral CRISPR/Cas9 strategy to knock down DAT and/or D2-AR in midbrain DA neurons and recorded DA release in the NAcMed and NAcLat. Currently we are using Neuropixels probes to record the activity of projection-defined DA neurons before and after knockdown of DAT and/or D2-AR.

Results: In situ hybridization revealed different levels of DAT and D2-AR mRNA expression across different projection-defined DA subpopulations. Interestingly, projections with high levels of DAT and D2-AR mRNA expression showed more rapid spontaneous DA fluctuations, suggesting that DAT and D2-AR, at least in part, may explain local differences in DA release. CRISPR/Cas9-mediated knockdown of DAT and D2-AR in DA neurons projecting to the NAcLat, a projection with high expression levels of these proteins, significantly slowed spontaneous DA transients in the NAcLat. In contrast, knockdown in DA neurons projecting to the NAcMed, a projection with lower expression levels of these proteins, had little effect on NAcMed DA transients. Lastly, knockdown of DAT and D2-AR in NAcLat-projecting DA neurons significantly modulated NAcLat DA transients in response to a tail shock, such that the offset of the shock led to an exaggerated rebound in DA release.

Conclusions: We conclude that DAT and D2-AR can to a large extent explain local quantitative differences in spontaneous and task-evoked DA release. Additionally, knock down of DAT and D2-AR in striatal projection neurons rich in gene expression can lead to qualitative changes in DA release, such as rebound excitation after tail shock. Together, these findings provide a molecular mechanism that may explain functional differences in DA signaling across the corticolimbic system.

Keywords: Dopamine, Reward, Striatum

Disclosure: Nothing to disclose.

P740. Contexts Facilitate Dynamic Value Encoding in the Mesolimbic Dopamine System

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Background: Exploiting contextual information to update and guide our behavior is essential for survival. Dopamine neurons and dopamine release are essential for learning and utilizing the long-running value of learned associations to guide behavior. However, the value of cues can be rapidly altered dependent on the context they are encountered and the ability of the dopamine system to control this dynamic scaling of behavior is still unclear.

Methods: Here we make use of pharmacology (n = 24), in vivo electrophysiology (n = 3; n = 235 neurons), calcium imaging (n = 6), and optogenetics (n = 24) to determine how contexts allow for flexibility in the encoding and utilization of value to guide behavior. Male and female rats were trained to discriminate when a conditioned stimulus would be followed by sucrose reward by exploiting the prior and non-overlapping presentation of a separate discrete contextual cue, an occasion setter. Only

when the occasion setter's presentation preceded the conditioned stimulus did the conditioned stimulus predict sucrose delivery.

Results: Reversible inactivation or dopamine antagonism within the nucleus accumbens prevented rats from exploiting contextual cues to properly estimating when the conditioned stimulus would be rewarded. We recorded single neurons within the nucleus accumbens ($n = 295$) and observed that the magnitude of conditioned-stimulus evoked inhibitions were greater when the conditioned stimulus would be followed by reward ($n = 35$; $p < 0.05$), than when it would not. Moreover, a population of neurons in the nucleus accumbens dynamically altered their firing to the conditioned stimulus (significant effect of trial type $p < 0.05$; $n = 23$), being excited when this cue was not predictive and inhibited when it would predict reward. Dopamine release in the nucleus accumbent tracked the presentation of the occasion setter. Critically, dopamine release to the conditioned stimulus only occurred when the occasion setter informed that this cue was predictive of reward ($p < 0.05$ compared to each other trial). In agreement, selective optogenetic inhibition of midbrain dopamine neurons during the occasion setter prevented accurate behavioral responding to the conditioned stimulus when it would be predictive of reward receipt.

Conclusions: Together these results reveal a mechanism for mesolimbic dopamine release and stratal activity to dynamically control reward-seeking. This has implications for our understanding of the dopamine system in adaptive and maladaptive behavioral flexibility.

Keywords: Dopamine, Motivation, Context

Disclosure: Nothing to disclose.

P741. Adolescent Alcohol Exposure Impairs Response Inhibition and Alters OFC-Striatal Dynamics in Adult and Adolescent Rats

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Background: There is a strong relationship between adolescent alcohol use, and adult addiction severity. Additionally, development of alcohol use disorder (AUD) is associated with initiation of drinking during adolescence. Adolescent alcohol use impacts cortical-striatal networks and may impair behavioral flexibility in adulthood. A better understanding of the impact of alcohol exposure on the adolescent brain in the context of motivated behavior is fundamental for understanding the etiology and pathophysiology of AUD. Deficits in response inhibition are a critical feature of AUD and are a clinically relevant endophenotype of the disorder. Little is known about the functional consequences of adolescent alcohol drinking on response inhibition and related cognitive measures in adulthood. We therefore sought to understand how adolescent alcohol exposure alters response inhibition and corresponding cortical-striatal dynamics during adolescence and adulthood by performing in vivo electrophysiology recordings in the orbitofrontal cortex (OFC) and dorsomedial striatum (DMS) of adolescent and adult rats following adolescent alcohol exposure.

Methods: A drinking-in-the-dark model where male and female adolescent rats had access to 5% sucrose or 20% v/v ethanol (EtOH) for five days in their home cage was used. They were then trained in a Cued Response Inhibition Task (CRIT) while we simultaneously recorded single units and local field potentials (LFPs) from adolescent and adult rats in the OFC and DMS. Cell types in both regions were classified based on waveform characteristics and firing rates for events of interest were computed. Spectral analyses were performed to quantify LFP fluctuations at different frequencies. Neural synchrony between the OFC and DMS was measured by computing the phase locking index γ. We performed mixed design analysis of variance (ANOVA) for all dependent variables with group factors exposure group and age.

Results: Adolescent alcohol drinking was associated with increased premature responding and decreased correct responding in adolescence and adulthood. In adolescents, alcohol exposure decreased firing rate of OFC neurons but increased firing rate of DMS neurons during premature responding. In adults, adolescent alcohol exposure was associated with an attenuated response to the inhibitory cue but an augmented response to reward. Relative to sucrose controls, animals in the EtOH exposed group showed increased OFC-DMS synchrony in the theta frequency band in response to premature actions.

Conclusions: Our analysis reveals that adolescent alcohol exposure produces several dynamic changes to OFC-striatal ensembles beginning in adolescence and lasting through adulthood. These findings suggest that alcohol drinking in adolescence induces changes that begin in adolescence, alters development of OFC-DMS circuits, and results in alterations in adult brain function. Because dysfunction of cortico-striatal circuits is a critical feature of AUD, our results enhance our mechanistic understanding of brain changes that occur in these circuits as a consequence of adolescent alcohol exposure.

Keywords: Adolescent Alcohol Use, Orbitofrontal Cortex, Dorsal Striatum, Single-Unit Electrophysiology in Vivo

Disclosure: Nothing to disclose.

P742. Neuroinflammation and Diffusion MRI Relationship in Opioid Use

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Background: Neuroinflammation is increasingly recognized as an essential feature in mental health disorders, including substance use disorders (SUD): therefore, in vivo markers of neuroinflammation will be a valuable clinical tool to improve clinical tracking of mental health disorders. Neuroinflammation induces a myriad of cellular and molecular signals that, in turn, transiently changes the cytoarchitectonic organization of the brain where the process is active; hence, it will presumably alter the diffusion MRI signal. Using multicompartment models, such as Neurite Orientation Dispersion and Density Imaging (NODDI), we can potentially track this process in vivo and provide a marker of neuroinflammation. NODDI yields three distinct markers that can then be biologically interpreted and related to distinct stages in the neuroinflammatory process. Given MRI translational applicability, the results can easily be integrated into human studies of SUDs; however, a careful examination using animal models will provide the quantitative relationship between the timing of the neuroinflammatory response and observable changes in the MRI indices of NODDI.

Methods: In this study, twenty Sprague Dawley rats were trained to self-administer (SA) morphine 0.4 mg/kg/inf over twelve days. We acquired MR images and fecal samples at three time points: 1) baseline (before SA), 2) after the first two days of SA, and 3) after the last day of SA. MR images will be analyzed with NODDI and traditional tensor scalar metrics, and fecal samples will be analyzed for alpha and beta diversity using 10s-RNA sequencing to identify potential relationships between the gut and brain.

Ultimately, we perform spatial transcriptomics to determine region-specific differences in neuroinflammatory biomarkers.

Results: We have found that male rats self-administer higher doses of morphine than females, but both sexes administered at least more than 4 mg/kg in at least the last three consecutive days to be included in this study. We are in the process of analysis of the transcriptomic, MRI, and fecal samples.

Conclusions: With this study, we have established a first longitudinal study to determine temporal relationships between the gut, brain, and behavior within the subjects. We believe this will inform about the relationship between microstructural indices of brain structure derived from MRI and the neuroinflammatory process due to opioid use.

Keywords: Neuroinflammation, MRI, Opioid

Disclosure: Nothing to disclose.

P743. Resolving Neuronal Activity Dynamics and Noradrenergic Modulation of Prelimbic Cortex During Heroin Seeking

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Background: Cue-induced drug seeking requires activation of the prelimbic prefrontal cortex (PrL) that is dysregulated in substance use disorder. The heterogeneity in PrL cell types has made it difficult to unveil the precise PrL circuit dynamics which orchestrate drug seeking.

Methods: To address this, we developed a head-fixed heroin self-administration procedure to allow for longitudinal tracking of PrL neuronal activity in mice during behavior. To measure the activity of PrL projection neurons, we virally labeled these neurons for calcium imaging (AAVdj-CaMKIIa-GCaMP6s) and implanted a GRIN lens dorsal to PrL. Using in vivo two-photon microscopy, we measured the calcium activity dynamics of single PrL excitatory output neurons (n = 15-17 mice, mixed sex; n = 783-2592 cells) from the onset of intravenous heroin self-administration through extinction and reinstatement. We used PCA-based unsupervised spectral clustering analyses to reveal how discrete groups of PrL neurons differentially encode behavioral events during drug seeking. Using chemogenetic approaches, we determined the necessity for locus coeruleus (LC) noradrenergic inputs to PrL (hM4Di n = 8, eYFP = 7) for cue-induced reinstatement. Intra-PrL application of lofexidine (n = 6) was used to determine the necessity of PrL noradrenergic signaling for cue-induced reinstatement. Finally, combining chemogenetics with two-photon recordings, we examined the necessity of LC noradrenergic inputs for the observed PrL cluster activity dynamics and for cue-induced reinstatement of drug seeking (hM4Di n = 7, tdTomato control = 6).

Results: Subsequent two-photon recordings reveal both frequency and amplitude of calcium events in PrL neurons are reduced following acquisition of heroin seeking, effects which persist through extinction, but then resurge during cue-induced reinstatement. Spectral clustering analyses reveal 4 distinct neuronal clusters, exhibiting either excitatory (clusters 1-3) or inhibitory (cluster 4) activity, emerge in a manner time-locked to active lever presses during each session. As local noradrenergic signaling rapidly increases PrL activity and mediates drug-cue memory retrieval, we hypothesized that inputs from the locus coeruleus (LC) contribute to the rescue of PrL activity during reinstatement. We found that chemogenetic inhibition of LC-PrL axon terminals blocks cue-induced reinstatement, an effect which surprisingly persisted upon a subsequent cue test. The effects of LC terminal inhibition in PrL were reproduced by intra-PrL application of the alpha2-adrenergic receptor agonist lofexidine. Two-photon recordings reveal that LC inhibition suppresses cue-induced reinstatement in a manner coinciding with suppressed activity of a discrete PrL excitatory cell cluster which ordinarily decodes the drug-cue. Analyses are currently underway to dissect sex differences and changes in single-cell activity dynamics throughout heroin self-administration, extinction, and reinstatement.

Conclusions: These studies suggest LC-PrL axon activation and noradrenergic activity rescues excitatory activity in PrL projection neurons for cue-induced reinstatement of heroin seeking.

Keywords: Substance Use Disorder, In Vivo Calcium Imaging, Prefrontal Cortex

Disclosure: Nothing to disclose.

P744. Chronic Ethanol Exposure Remodels Perineuronal Nets and Inhibitory Synapses on Striatal Fast-Spiking Interneurons

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Background: Persistent drinking in the face of a negative consequence, or compulsive drinking, is a prominent feature of alcohol use disorder. Parvalbumin-expressing dorsolateral striatal fast-spiking interneurons are necessary for the expression of compulsive-like drinking in mice. How chronic ethanol exposure modulates the physiology of dorsal striatum fast-spiking interneurons is poorly understood.

Methods: Exploring this, we exposed adult mice of both sexes to chronic intermittent ethanol vapor (or air) and performed ex vivo slice electrophysiology combined with optogenetics to interrogate both glutamatergic and GABAergic synaptic transmission onto fast-spiking interneurons. Additionally, we assessed synaptic markers and perineuronal net integrity using both western blot and confocal microscopy approaches.

Results: We discovered that chronic ethanol exposure reduced GABAergic, but not glutamatergic, transmission onto dorsolateral striatum fast-spiking interneurons. This corresponded with a significant loss of GABAergic synapses predominantly on the soma and proximal processes of fast-spiking interneurons where extracellular matrix perineuronal nets were enriched. We found that chronic ethanol exposure degraded perineuronal nets, and that enzymatically degrading perineuronal nets similarly reduced GABAergic transmission onto fast-spiking interneurons.

Conclusions: Collectively these data suggest that chronic ethanol exposure degrades perineuronal nets to downregulate GABAergic synaptic transmission onto fast-spiking interneurons. Given that perineuronal net formation in closes critical periods of learning and that alcohol facilitates habit learning, these data suggest ethanol re-opens a critical period for habit learning through modulation of perineuronal nets surrounding fast-spiking interneurons.

Keywords: Basal Ganglia, Cerebral Cortex, Thalamus, Compulsivity, Brain Circuits

Disclosure: Nothing to disclose.

P745. Dynamic Endogenous Opioid Neuromodulation Shapes Goal-Directed Behavor

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Background: Goal-directed behavior is essential for survival and co-opted during multiple neuropsychiatric disorders, including substance use disorder (SUD). A prevailing theory suggests that drugs co-opt existing mechanisms encoding goal-directed behaviors for natural reward. The endogenous opioid dynorphin (dyn), signaling through the kappa opioid receptor (KOR) is implicated in the escalation and reinstatement of drug seeking; although how dyn regulates goal-directed behavior for natural reward is unknown. The dorsomedial striatum (DMS) is essential for goal-directed behavior and ~50% of DMS neurons express dyn. However, the role of dyn neurons, the impact of dyn signaling through the kappa-opioid receptor (KOR) on striatal circuitry, and its contributions to behavior are largely unknown.

Methods: All studies were conducted in equal numbers of male and female mice in accordance with NIH guidelines, approved by the IACUC at the University of Washington. No sex-dependent effects were observed. Below were the methods used:

1. In-vivo 2-photon imaging of DMS dyn neurons during head-fixed operant behavior (D1-tdTom, n = 4 mice).
2. In-vivo fiber photometry during operant behavior of dyn release in the DMS (KOR-cre, n = 6 mice).
3. Operant behavior with conditional dyn deletion (Ctrl or pdyn-cKO, n = 12 mice).
4. Time-locked optogenetic manipulations of DMS dyn neurons in-vivo during operant behavior for activation using ChR2 (Ctrl or pdyn-cre, n = 9 mice).
5. Time-locked optogenetic BLA stimulation and dyn release during operant behavior (KOR-cre, n = 4 mice).
6. Ex-vivo viral tracing and in situ hybridization of BLA projections to DMS (WT, n = 3 mice, 6 slices Ai14, n = 3 mice, 6 slices; vglut1-cre, n = 2 mice, 6 slices; KOR-cre, n = 2 mice, 6 slices).
7. Ex-vivo electrophysiology of DMS neurons during optogenetic activation of BLA terminals (D1-tdtmto, n = 5 mice, 16 cells); KOR activation via bath application (U69,593 – 1uM) at BLA terminals during optogenetic activation (D1-tdtmto, n = 4 mice, 9-16 cells).
8. In-vivo fiber photometry during operant behavior of BLA terminals in the DMS multiplexed with conditional dyn deletion (WT or pdyn-cKO, n = 8 mice) or dyn neuron stimulation (pdyn-cre, n = 4 mice).

Results: We used in vivo 2-photon microscopy through implanted microprisms, affording longitudinal tracking of >5000 dyn neurons across a large area of the DMS (n = 4 mice). We find that as animals learn head-fixed operant behavior (n = 4 mice, operant behavior, t test; $p < 0.05$, $t(3) = 3.4$), dyn neurons dynamically change their activity across multiple timescales, and significant clusters of DMS dyn neurons are active during anticipation of reward. To determine what dyn is doing, we developed a novel dyn biosensor for use in vivo using fiber photometry. Similar to dyn neuron activity, we observe dyn release across multiple timescales - dyn tone in the DMS increases as animals learn goal-directed behavior across days (n = 6 mice, z-score, paired t test; $p < 0.01$, $t(5) = 7.03$ – early vs. trained), and dyn is released dynamically at the timescale of seconds in anticipation of reward delivery (n = 6 mice, z-score, unpaired t test; $p < 0.0001$ – early vs. trained). To determine necessity of DMS dyn in goal-directed behavior, we conditionally deleted dyn from the DMS and observe a reduction in the learning of goal-directed behavior, without impacting innate preference for the reward (n = 12 mice, Linear Regression; $p < 0.005$, $R2 = 0.48$). Furthermore, to measure sufficiency we used optogenetics to elicit dyn release from the DMS, and find that optogenetic mimicry of dyn release during the anticipation of reward delivery enhances behavior (n = 8 mice, paired t test, $p < 0.005$, $t(7) = 10.55$ – early vs. trained). To ascertain the source of dyn activation, we multiplexed optogenetics with photometry in vivo, and find that stimulating KOR-expressing basolateral amygdala (BLA) terminals in the DMS elicits dyn release (n = 4 mice, unpaired t test $p < 0.001$ – stim vs. no stim). Prior studies have shown that DMS-projecting BLA neurons are involved in goal-directed behavior. We show that ~20% of BLA neurons project to the DMS and are glutamatergic (vglut1+). Additionally, BLA terminals preferentially activate DMS D1+/dyn neurons (n = 16 cells, 2 Way ANOVA; $p = 0.0014$ – D1(+) vs. D1(-), $F(1,203) = 10.50$) Hence, to determine whether dyn may be acting on KOR at BLA terminals, we measured the activity of BLA terminals in the DMS. Using in vivo fiber photometry, we show that BLA-DMS terminal activity is increased during action, and inhibited during outcome. Importantly, we find that DMS dyn, or BLA KOR deletion, significantly diminishes BLA-DMS activity dynamics to action and outcome (n = 7 mice, z-score, unpaired t test; $p < 0.0005$ – WT vs. dyncKO), and negatively impacted the learning and maintenance of goal-directed behavior (n = 7 mice, operant behavior, 2 Way ANOVA; $p < 0.0005$ – day x genotype, $F(4,40) = 8.99$ - WT vs. dyncKO). Conversely, optogenetic stimulation of DMS dyn neurons during reward anticipation enhanced BLA-DMS activity dynamics (n = 4 mice, z-score, t test; $p < 0.05$ – stim vs. no stim) and goal-directed behavior (n = 4 mice, operant behavior, t test; $p < 0.05$, $t(3) = 3.2$ – stim vs. no stim), without inherently being reinforcing.

Conclusions: Altogether, we reveal that retrograde dyn transmission from the DMS onto KOR at BLA terminals dynamically regulates BLA-DMS activity and shapes goal-directed behavior.

Keywords: Dynorphin, Kappa Opioid Receptor, Goal-Directed Behaviors, Substance Use Disorder

Disclosure: Nothing to disclose.

P746. Endocannabinoids Influence the Dopaminergic Substrates of Cognitive Flexibility

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Background: Mesolimbic dopamine is responsible for reward-based learning. Specifically, activation of dopamine neurons facilitates the association of cues with the value of related rewards and is responsible for flexibly updating cue-reward learning when conditions are altered. Endocannabinoids are key modulators of dopamine neuron activation and enable dynamic changes in reward-driven dopamine release. Therefore, endocannabinoids, such as 2-AG, may facilitate changes to cue-reward associations during reversal learning when conditions are altered.

Methods: Male and female mice (n = 40) were first trained to discriminate between two levers of differing reinforcement probabilities (80% vs. 20%); this was followed by a reversal phase in which the reinforcement probabilities were inversed across levers. During the reversal session, mice were pre-treated with a monoacylglycerol lipase (MAGL) inhibitor JZL-184 (0, 8, 18 mg/kg, ip) to increase synaptic levels of 2-AG. DA was recorded during early and late acquisition, and early, mid, and late reversal

sessions. Performance was analyzed to assess mean differences across various metrics on the first day of the reversal phase. Analysis of error probabilities (win-stay and lose-shift) were also conducted. Finally, dopamine release was compared between- and within-subjects across sessions to determine differences in phasic dopamine release following different trial outcomes.

Results: Results demonstrate significant impairment in behavioral flexibility and dopamine release following elevation of 2-AG. Errors (% correct choices) were dose-dependently increased by JZL-184 administration ($p < 0.05$ vs. vehicle), and dopaminergic encoding of trial outcomes was attenuated. These results suggest disruption of the endocannabinoid signaling hinders reversal learning performance and dysregulates associated mesolimbic dopamine release.

Conclusions: These findings give critical insight into the role of the endocannabinoid system in flexible reward-based learning and may have significant implications for the use of cannabinoids for recreational or therapeutic purposes.

Keywords: Dopamine, Cannabinoid, Behavioral Flexibility, Nucelus Accumbens

Disclosure: Nothing to disclose.

P747. CB1R Signaling Regulates Cocaine-Taking and -Seeking Behavior in Male and Female Rats with a History of Repeated Stress

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Background: Illicit substance use (e.g., cocaine use) is a major public health concern with no FDA-approved therapeutics making this a critical unmet need. Clinical literature investigating current cocaine users suggests that many dimensions of substance use are exacerbated by stress, including drug-taking and -seeking behavior. Stress is particularly problematic as stress is unavoidable in daily life. Therefore, it is critical to elucidate the neurobiological mechanisms that underlie the contribution of stress to SUD. We have previously shown that repeated daily stress at the time of cocaine self-administration (SA) induces a significant increase in cocaine intake in male rats. However, this stress-induced increase in SA is likely influenced by sex, as females show increased sensitivity/susceptibility to stress and many aspects of substance use disorder. These behavioral processes also likely involve systems that connect stress-responsive and reward systems in the brain, such as the endocannabinoid system (eCB). We hypothesize that repeated stress at the time of SA recruits cannabinoid receptor 1 (CB1R) signaling that results in increases in cocaine SA and increased susceptibility to later reinstatement and that these effects are influenced by sex.

Methods: Male and female SD rats were trained to SA cocaine (0.5 mg/kg/inf) on a FR 4 schedule in 4 X 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. We examined the involvement of the CB1R in stress-escalated cocaine intake by administration of a CB1R antagonist systemically. To explore the role of CB1R signaling in cocaine-evoked dopamine, the dopamine biosensor, dLight 1.3, was expressed and a fiber optic cannula was implanted in the nucleus accumbens (NAc shell) to measure dopamine via in vivo fiber photometry. Rats were pre-treated with a systemic CB1R antagonist followed by passive administration of iv cocaine (0, 1, 3 mg/kg). Additional rats were tested for changes in extinction training and increased susceptibility for reinstatement to various stimuli. Rats were also tested for the effects of the CB1R antagonist systemically or directly into the prelimbic cortex (PrL) on cocaine-primed reinstatement (10 mg/kg, i.p.) to examine the involvement of CB1R signaling in augmented reinstatement in rats with a history of repeated stress.

Results: Footshock stress administered daily at the time of self-administration induced a significant increase in cocaine self-administration in both male and female rats ($n = 16$-18/group). However, female rats show more front-loading behavior and increased time-out responding, suggesting more compulsive-like behavior. Systemic administration of the CB1R antagonist attenuates cocaine intake only in stress-escalated rats in males but attenuates cocaine intake in both no stress and stressed female rats though there is a greater sensitivity to the antagonist in the stress-escalated rats ($n = 8$-10/group). Female rats show a greater cocaine-evoked dopamine response than males ($n = 4$-6/group) and administration of rimonabant (1 mg/kg, i.p.) only attenuates cocaine-induced dopamine in females at the current dose. Rats are currently being tested with a higher dose of rimonabant. Rats are also being tested for the role of CB1R signaling in stress-evoked dopamine changes. Sex differences in extinction training and reinstatement behavior is currently being tested. In males, rats with a history of stress show augmented cocaine- and stress-induced reinstatement. Furthermore, the recruitment of eCB signaling to influence drug-related behavior is long-lasting as systemic or intra-PrL administration of the CB1R antagonist 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).

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 CB1R as CB1R effects predominantly emerge in rats with a past history of stress. All of these behaviors are also likely influenced by sex as females appear to be more sensitive to the role of CB1R signaling in cocaine self-administration and cocaine-evoked dopamine in the NAc shell. Understanding the unique mechanisms by which stress can drive drug intake and seeking has implications for identifying and treating patients with SUD in whom stress is a contributing factor.

Keywords: Acute and Chronic Stress, Endocannabinoid System, Cocaine Sex Differences, Medial Prefrontal Cortex, Cocaine Self-Administration

Disclosure: Nothing to disclose.

P748. Gating of Opioid Withdrawal Aversion by a Unique Class of Neurons in the Nucleus Accumbens

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Background: Lethal overdose from opioids have increased dramatically over the past decade, critically contributing to the "opioid crisis". The aversion of opioid withdrawal and its associated environmental cues act as potent drivers of relapse and increase the risk of future overdose. More detailed understandings of circuit mechanisms underlying opioid withdrawal aversion are needed to develop new therapeutic strategies to target opioid dependence and relapse. The nucleus accumbens (NAc) has been long known to be involved in drug reward and cued reinstatement. Here we uncover a new subpopulation of medium spiny neuron in the nucleus accumbens shell capable of mediating the aversive nature and learning associated with acute opioid withdrawal.

Methods: Here we have combined anatomical and molecular characterization of subtypes of medium spiny neurons of the nucleus accumbens shell, chemogenetic and optogenetic manipulations, cell type specific fiber photometry measurements using calcium sensor GCAMP and dopamine sensory GRABDA. Photometry recordings were taken from two non overlapping populations of D1 medium spiny neurons using transgenic mouse lines Pdyn-Cre and Tshz1-Flp during a baseline period, acute morphine intoxication and naloxone precipitated withdrawal (n = 8 each, both sexes). Fiber photometry data was z-scored and effects of different states of intoxication vs withdrawal were determined with signed-rank tests. Additionally the percentage of gcamp responses rising above a mean absolute threshold was calculated during periods of drug condition. Chemogenetic manipulations (inhibitory and excitatory) of both cell types were conducted during morphine conditioned placed preference and naloxone induced withdrawal conditioned placed aversion (n = 8-10 each, pre vs post test, student T-test). Dopamine concentrations using fiber photometry and the DA sensor GRABDA were measured in the nucleus accumbens during morphine intoxication and naloxone precipitated withdrawal (n = 6-8, both sexes). Cell type specific modulation of dopamine release in the NAc were conducted using optogenetic stimulation of Pdyn and Tshz1 cells while performing photometry recordings with GRABDA (n = 6-8 each, both sexes).

Results: We identified neurons in the NAc that express mu opioid receptor (MOR) and found two major populations expressing D1 dopamine receptors, one marked by Pdyn and another with Tshz1, a cell-type recently found to mediate aversion learning in the dorsal striatum. Photometry recordings with GCaMP6f revealed distinct activity dynamics, such that acute morphine intoxication reduced activity in both cell-types (percent GCAMP signal above mean absolute threshold baseline vs post morphine injection, t-test $p < 0.01$) yet naloxone precipitated withdrawal triggered a large rebound in activity selectively in Tshz1 cells ($p < 0.05$). Reducing activity of Tshz1 neurons with hM4Di disrupted morphine withdrawal conditioned place aversion (CPA) ($p < 0.009$), while inhibition of Pdyn neurons had no effect on withdrawal CPA but reduced morphine conditioned place (CPP) ($p < 0.003$). Acute stimulation of Tshz1 neurons with ChR2 led to avoidance of the stimulation-paired chamber in a real time place preference test, whereas stimulation of Pdyn neurons led to a preference ($p < 0.01$). We hypothesized that these behavioral effects are mediated through cell-type specific modulation of dopamine (DA) release in the NAc. To test this idea, we stimulated Tshz1 or Pdyn cells with ChRmine while performing photometry recordings with GRAB DA. Stimulation of Tshz1 neurons led to a rapid suppression of DA release in the NAc, whereas stimulation of Pdyn neurons increased DA release. DA levels rose substantially after morphine administration but were significantly suppressed below baseline after naloxone injection, indicating a hypodopaminergic state during withdrawal.

Conclusions: Ongoing studies are examining the discrete mechanism by which Tshz1 neurons modulate DA release in the NAc, whether Tshz1 neuron activity mediates withdrawal-induced decreases in DA, and the role of MOR in Tshz1 neurons in these effects. Together, our data demonstrate a unique population of NAc neurons that mediate the aversive nature of acute opioid withdrawal through strong modulation of mesolimbic DA release.

Keywords: Opioid Addiction, Opioid Dependence, Pharmacotherapy, Animal Model, Withdrawal, Nucleus Accumbens Shell, Dopamine, Medium Spiny Neuron

Disclosure: Nothing to disclose.

P749. Hippocampal Volume Loss in Individuals with a History of Non-Fatal Opioid Overdose

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Background: Incidence of opioid-related overdoses in the United States has increased dramatically over the past two decades. Despite public emphasis on overdose fatalities, most overdose cases are not fatal. Although there are case reports of amnestic syndromes and acute injury to the hippocampus following non-fatal opioid overdose, the effects of such overdoses on brain structure are poorly understood.

Methods: Here, we investigated the neuroanatomical correlates of non-fatal opioid overdoses by comparing hippocampal volume in opioid use disorder (OUD) patients who had experienced an opioid overdose (OD; $N = 17$) with those who had not (NOD; $N = 32$). Groups did not differ in demographics or clinical characteristics: age, sex, race, body mass index (BMI), years of education, IQ scores, Addiction severity index drug scores, years of opioid use, days since last opioid use. All participants completed a high-resolution, T1-weighted 3D magnetization-prepared gradient-echo structural MRI scan at 3 Tesla. Structural and voxel-based analyses of MRI data were performed using SPM12 (www.fil.ion.ucl.ac.uk/spm) and CAT12 (www.neuro.uni-jena.de/cat). A priori hippocampal regions of interest (ROIs) were anatomically defined based on the Neuromorphometrics atlas (www.neuromorphometrics.com).

Results: Using the a priori bilateral ROI, the OD group had a significantly lower hippocampal volume than the NOD group (mean ± SD = 0.48 ± 0.05 vs. 0.51 ± 0.06, $F(1,43) = 4.43$, $p = 0.041$). Exploratory post hoc analysis examining the left and right ROIs separately showed a significant group difference only for the left hippocampus (0.49 ± 0.05 vs. 0.51 ± 0.06, $F(1,43) = 5.42$, $p = 0.025$) but not the right hippocampus (0.48 ± 0.05 vs. 0.51 ± 0.06,$F(1,43) = 3.18$, $p = 0.082$). Voxel-wise analysis within the bilateral hippocampal ROI identified one cluster in the left hippocampus with significantly lower volume in the OD compared to the NOD cohort (cluster extent = 1401 mm^3, $Z = 3.47$, x/y/z = –24/–15/–16).

Conclusions: These findings strengthen the evidence that hippocampal injury is associated with non-fatal opioid overdose, which is hypothesized to underlie overdose-related amnestic syndrome.

Keywords: Opioid Use Disorder, Overdose, Hippocampus

Disclosure: Nothing to disclose.

P750. Increased Resistance for Footshock Punishment of Cocaine Seeking in Female Rats With a History of Footshock Exposure

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Background: One of the defining characteristics of addiction is compulsive drug use that continues despite negative consequences. In an animal model of compulsive drug seeking, a subset of animals will continue to seek cocaine despite footshock consequences, while another subset reduces cocaine seeking to avoid footshock. Recent work from our lab found that non-contingent footshock, unlike contingent footshock, did not reduce cocaine seeking in male or female rats. These findings indicate that the contingency of the footshock plays a key role in the

suppressive effects of footshock. Here, we wanted to investigate whether prior exposure to footshock affects subsequent sensitivity to contingent or noncontingent footshock during cocaine self-administration in male and female rats.

Methods: Male (n = 41) and female (n = 15) Sprague Dawley rats were trained to self-administer intravenous cocaine on a seeking-taking chained schedule of reinforcement during daily 2-h sessions. After ~3 weeks of self-administration, rats were assigned to contingent footshock (randomly 1/3 trials, delivered after completion of seeking) or noncontingent footshock (same parameters and average number of shocks, but independent of behavior). They received 4 days of testing with footshock maintained at a consistent intensity (0.4 mA, 0.3 s) and 5 days of ramped footshock (increasing and/or duration). Rats were then exposed to the opposite footshock condition (4 days at 0.4 mA) such that rats first exposed to contingent footshock were given noncontingent footshock and vice versa. Between each testing block rats received ≥ 3 days of non-footshock self-administration. Data were analyzed using two-way ANOVAs and Sidak's multiple comparison tests for post hoc analyses.

Results: We found that noncontingent footshock did not reduce cocaine self-administration in males and females. However, contingent footshock reduced self-administration as compared to noncontingent footshock in both males (main effect of group: $p < 0.0001$) and females (main effect of group: $p = 0.0004$). When males then received the opposite footshock condition, we again saw that the contingent group was sensitive to footshock and significantly different from the noncontingent group, which did not reduce self-administration (main effect of group: $p = 0.0005$). However, when females received the opposite footshock condition, the contingent group was more resistant to footshock and not significantly different from the noncontingent group. Therefore, a history of footshock exposure led to increased punishment resistance in females but not males.

Conclusions: These results show that a history of footshock exposure has lasting effects in female rats and affects subsequent sensitivity to contingent footshock. Further work is necessary to determine whether increased punishment resistance would be seen following a history of either contingent or noncontingent footshock in females.

Keywords: Cocaine Self-Administration, Punishment Learning, Cocaine Addiction

Disclosure: Nothing to disclose.

P751. Loss of Behavioral Inhibition in Alcohol Dependence: Role of the PVT to NAc Circuit

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Background: Alcohol use disorder (AUD) is a chronic, relapsing neuropsychiatric condition that is characterized by a loss of behavioral inhibition. Although our understanding of the circuitry involved in alcohol-motivated behaviors is substantial, recent studies on the paraventricular thalamus (PVT) to nucleus accumbens (NAc) circuit in regulating sucrose reward-seeking show its critical role in behavioral inhibition, and thus may prove to be a critical circuit in promoting behavioral disinhibition in alcohol dependence. PVT inputs onto parvalbumin interneurons (PV-INs) in the NAc may serve as a "brake" on reward-seeking and appear to be dysregulated in models of dependence on other substances, like heroin. Therefore, understanding how alcohol dependence might alter the PVT-NAc pathway is imperative for dissecting the etiology of AUD.

Methods: Here we used a multi-faceted approach employing slice electrophysiology, optogenetics and 2-photon microscopy in a head-fixed operant conditioning paradigm. In wildtype mice, we targeted the PVT-NAc circuit using AAVrg-Cre into the NAc, and either a Cre-dependent Halorhodopsin or Channelrhodopsin into the PVT to manipulate circuitry during alcohol self-administration and in the presence of behavioral suppressors. For 2-photon calcium imaging of PV-INs, we injected ChRimson into the PVT, and Cre-dependent GCaMP6m into the NAc with a gradient-refractive index (GRIN) lens above the site, of PV-Cre mice.

Results: Our current data suggests that optogenetic activation of the PVT-NAc pathway reduces alcohol-seeking behaviors, evidenced by decreased active lever presses and alcohol deliveries, in nondependent mice. Interestingly, after inducing alcohol dependence via chronic intermittent ethanol (CIE) exposure, activation of PVT-NAc circuit no longer reduces alcohol seeking, indicative of a functional uncoupling of this circuit. Conversely, optogenetic inhibition of the PVT-NAc releases this "brake" and promotes active lever pressing, even in the face of behavioral suppressors, like quinine. Electrophysiology data indicate that compared to controls, CIE significantly reduces optically evoked EPSCs as well as AMPA/NMDA ratios in PV-INs, suggesting a synaptic change in PVT-NAc PV-INs. Additionally, 2-photon calcium imaging in NAc PV-INs show shifts in calcium dynamics during the acquisition of operant responding for alcohol. Simultaneously, we examine changes in PVT inputs onto PV interneurons by optogenetic activation of PVT terminals within the NAc.

Conclusions: Here we show that alcohol dependence alters the functional synaptic connectivity of PVT inputs onto PV-INs in the NAc. Further characterization of how these PV-INs function within the PVT-NAc pathway through a series of 2-photon imaging experiments coupled with simultaneous optogenetics may provide insight into how these neurons function across the development of dependence.

Keywords: Alcohol Dependence, Paraventricular Nucleus of the Thalamus, Nucleus Accumbens, 2-Photon Techniques, Circuit Optogenetics

Disclosure: Nothing to disclose.

P752. Role of Dorsal Raphe - Basolateral Amygdala CRF Circuits in Cocaine-Memory Reconsolidation

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Background: Exposure to cocaine-associated environmental contexts elicits the recall of salient cocaine memories that can induce drug craving and relapse. Retrieval can also destabilize drug memories, requiring their reconsolidation into long-term memory stores for continued environmental stimulus control over drug-seeking behaviors. Thus, disruption of labile cocaine memories or interference with their reconsolidation may be a viable therapeutic approach to reduce cue-induced relapse propensity. This requires an increased understanding of the mechanisms of drug-memory reconsolidation. Corticotropin-releasing factor (CRF) signaling within the basolateral amygdala (BLA) is critical for memory reconsolidation, yet the source of CRF to the BLA is not known. Here, we investigated the contribution of a dorsal raphe (DRN) → BLA CRF circuit to cocaine-memory reconsolidation.

Methods: Male and female Sprague-Dawley rats were trained to lever press for cocaine infusions in a distinct environmental

context and then received extinction training in a different context. They were then briefly re-exposed to the cocaine-predictive environment in the absence of drug reinforcement in order to retrieve and destabilize cocaine memories and trigger their reconsolidation. In experiment 1, brain tissue was collected two hours after memory retrieval to capture activation in multiple BLA-projecting CRF-immunoreactive neuronal populations ~30 minutes into memory reconsolidation. In experiment 2, we examined the effects of chemogenetic CRF pathway inhibition on the strengths of extinction and cocaine memories that were assessed based on lever pressing in each context 24 hours and 72 hours after memory retrieval, respectively. Data were analyzed using analysis of variance with appropriate post-hoc tests. Alpha was set at 0.05.

Results: Cocaine-memory retrieval increased c-Fos expression, an index of neuronal activation, in BLA-projecting CRF-immunoreactive neurons within the DRN, anterior cingulate cortex, and prelimbic cortex during reconsolidation, relative to no-memory retrieval (i.e., tissue collected from home cage controls). Inhibition of the DRN → BLA CRF circuit during cocaine memory reconsolidation using Gi-coupled designer receptors exclusively activated by designer drugs (DREADDs) and the DREADD ligand, deschloroclozapine (DCZ; 0.1 mM, 0.5 uL/BLA/hemisphere), weakened cocaine-memory strength as indicated by a cocaine context-, memory reactivation-, DREADD-, and DCZ-dependent reduction in lever pressing in both males and females. Further analysis revealed that neurons that exhibited increased c-Fos immunoreactivity during memory reconsolidation co-expressed CRF with serotonin and/or glutamate markers.

Conclusions: In conclusion, BLA projecting DRN CRF neurons regulate cocaine-memory strength during reconsolidation. Future studies will need to determine the relative contributions of serotonin and glutamate co-release to this phenomenon.

Keywords: Cocaine Addiction, Memory Reconsolidation, Amygdala, Raphe, Relapse

Disclosure: Nothing to disclose.

P753. Genetic Profiles within Reward-Specific Neuronal Ensembles

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Background: Reward-based positive reinforcement is an evolutionary strategy shared across species. However, in drug addiction, reward seeking becomes maladaptive and endangers survival. While drug and natural rewards such as sucrose involve overlapping brain nuclei, we have recently shown that drugs of abuse and natural rewards are linked to different neuronal ensembles, defined as a discrete number of neurons synchronously activated. Mounting evidence indicates that drug exposure strongly and uniquely impacts gene expression transcriptome-wide. However, aside from having identified a small number of plasticity-related genes in neuronal ensembles linked to methamphetamine seeking in rodents, little is known about how drug exposure alters mRNA expression profiles, specifically in drug-related ensembles. To address this gap in knowledge, we aim to investigate gene expression within reward-specific ensembles and how it differs in neurons activated while seeking different types of reward. We hypothesize that a discrete number of key genes, likely activity-dependent ones, are differentially expressed within each reward-specific ensemble and in cells responding to all types of reward.

Methods: Using inducible cFosiCreERxAi14 reporter mice, we demonstrated we can fluorescently tag different reward-specific neuron ensembles in the nucleus accumbens core (NAcore), a hub of reward processing. To investigate gene expression in these different ensembles, we used this same approach to induce fluorescent tagging of 3 different groups: the cocaine-, sucrose- or overlapping ensembles. We then isolated the NAcore neurons from mouse brains, sorted the tagged ensembles using the FACSMelodyTM cell sorter, extracted RNA and performed RNA sequencing to compare gene expression within the 3 ensembles to the untagged, non-ensembles cells. Differences in gene expression profiles amongst the 4 cell groups were compared and used to identify cell types and to create cell clusters based on transcriptional profiles.

Results: We found sex-, reward-, and region-specific transcriptomic changes that refine clinically relevant genetic approaches to decrease cocaine-seeking behavior without altering sucrose-based positive reinforcement.

Conclusions: Establishing differential gene expression profiles exclusively linked to drug-seeking raises the possibility of therapeutically targeting maladaptive drug seeking without affecting essential and biologically adaptive seeking of natural rewards.

Keywords: Cocaine Addiction, Reward, c-Fos-Expressing Ensembles, Neuronal Ensembles

Disclosure: Nothing to disclose.