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P541. Measuring Dopamine Synthesis Capacity: A Comparison of Compartment Modelling and Graphical Methods

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Background: The human dopamine synthesis capacity can be analyzed in vivo using 18F-FDOPA PET. This tracer kinetic model is known to be complex due to the presence of multiple compartments, as well as prominent metabolites that also enter the brain. Several methods have been proposed to analyze the data, but there is no general consensus of an optimal method.

Classical compartment modeling is complicated by the need for sequential arterial samples that may be difficult to obtain in a clinical setting. Replacing the arterial input function (AIF) with a population based input function has been suggested to avoid the need for invasive samples. Graphical analysis on the other hand is computationally simpler, and can be performed with or, in a modified version, without arterial input.

In the present study we compare non-linear compartment modeling to two graphical methods, using both simulated and real data. We further evaluate different strategies for estimating and scaling a population based arterial input function.

Methods: We collected experimental data in a study of 62 subjects (first episode psychosis and controls (ref 1), using 18F-FDOPA and arterial blood sampling with estimation of peripheral metabolites. Values of the clearance parameters (K1,k2,k3,k4) of the compartment model were estimated using non-linear fitting (in-house software, Matlab v. 2020a). We used either the true plasma input function, or population based AIF, estimated by scaling the averaged AIF of all other subjects.

Further, values of the influx parameter (Ki) were estimated from striatal regions using Gjedde-Patlak graphical analysis with input functions based either on arterial plasma or a cerebellar reference region. The true value of Ki depends on the underlying clearance parameters of the compartment model (K1,k2,k3,k4). For reference region method they also depend on the values of (K1,k2) in the reference region.

Finally, simulated data were constructed using known values of the variables and analyzed using the same methods.

Results: Simulated data revealed that the graphical analysis estimate of Ki is highly informative of K1 with plasma input values ($r > 0.8$), but only modestly so ($r < 0.46$) when using cerebellum reference. Graphical analysis was not informative of any other kinetic parameters.

For real data Ki values of the graphical method correlated significantly ($r = 0.64$, $p < 0.001$) with K1 based on the compartment model, but only with the plasma input function.

Results based on populations based AIF's were generally only weakly correlated with those based on the true plasma AIF. A maximum correlation of 0.54 ($p < 0.001$) was found between Ki based on a population based AIF scaled with injected dose, and Ki based on the true AIF.

Conclusions: With real data from an arterially derived input function, the graphical tissue reference method outcome parameter Ki is suitable as a proxy for the vascular clearance rate K1, although with a rather low correlation. The method is not informative of the remaining underlying kinetic parameters. Population based AIFs were not suitable to replace plasma samples with sufficient accuracy. The current data therefore suggest that an individual arterial input function may not be left out without significant loss of precision. Furthermore, studies to elucidate the relationship between the graphical reference tissue method and the underlying tracer kinetic parameters seems warranted.

Reference:

1. Sigvard AK, et al. Dopaminergic Activity in Antipsychotic-Naïve Patients Assessed With Positron Emission Tomography Before and After Partial Dopamine D2 Receptor Agonist Treatment: Association With Psychotic Symptoms and Treatment Response. Biol Psychiat. 2022;91(2):236–45.

Keywords: Dopaminergic System, Psychosis, Imaging

Disclosure: Nothing to disclose.

P542. Toward an Understanding of the Role of the Cholinergic System in Schizophrenia: A Pilot Study Using 18F-VAT PET

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Background: The cholinergic system has been of longstanding interest in schizophrenia (SCZ) and its potential treatments. However, to date the lack of imaging tools has limited in vivo investigation of the cholinergic system in SCZ, particularly as it relates to labeling presynaptic targets. Filling this void, an in vivo PET imaging probe of cholinergic tone ([18 F]VAT), which targets the vesicular cholinergic transporter (VAChT) with high affinity and selectivity, was recently validated for use in human research. As VAChT regulates the trafficking of acetylcholine into synaptic vesicles, it sets how much acetylcholine is available for activity, and [18 F]VAT binding (PET signal) can serve as a proxy for cholinergic synaptic integrity and capacity for transmission. Cholinergic system alteration in SCZ has been implicated across multiple domains: from higher prevalence of cigarette smoking among patients, to postmortem and genetic studies suggesting deficits in the cholinergic nicotinic system and models of auditory sensory processing suggesting disrupted cholinergic modulation may underly abnormal gamma rhythms in SCZ. In light of this, as well as the essential role of the cholinergic system for cognition and modulation of dopamine signaling, which is also abnormal in SCZ, we conducted a proof-of-concept neuroimaging study using [18 F]VAT PET to measure cholinergic integrity across the brain in patients with SCZ.

Methods: Eighteen patients with SCZ and 14 demographically-matched healthy controls (HC), aged 18-60, underwent clinical assessment, neuropsychological testing, and a PET/MR scan with [18 F]VAT. All participants were assessed and rated for psychosis-related signs and symptoms using the Positive and Negative Syndrome Scale (PANSS) and completed a computerized test battery (PennCNP) which consisted of 13 neurocognitive tasks, including the letter n-back (0-, 1-, and 2-back); short visual object learning tasks (sVOLT); and the abstraction, inhibition and working memory task (AIM). Participants were scanned on a combined PET/MR scanner, a Siemens Biograph 3 T mMR (Knoxville TN, USA), at Stony Brook University Hospital, equipped with a 12-channel head-and-neck coil. After a brief transmission scan, participants received an intravenous bolus injection (over 30 s) of ≤5 mCi (total mass ≤1.18 μg) of radiotracer [18 F]VAT while undergoing 150-min PET/MR scan with arterial blood sampling. There were no adverse reactions to the radioactive drug or study procedures. Time-activity curves were formed as the mean activity in each region of interest (ROI) in each PET frame. The main PET outcome measure of VAChT availability was derived for each ROI in each subject by calculation of the total distribution volume (VT) for [18 F]VAT using pharmacokinetic modeling of the PET data with the arterial plasma input function.

Simultaneous MR acquisitions included ultra-short echo time image for PET attenuation correction, followed by T1-weighted (T1w) and T2-weighted images with 1 mm isotropic voxels and whole-brain coverage for ROI delineation. ROIs of multiple cortical and subcortical brain regions were manually drawn on each subject's T1w MRI (1 mm isotropic voxels) and transferred to the coregistered PET images.

For each ROI, we performed two-sample t-tests of VT between groups. PANSS items scores were summed for total, positive, negative, and general items subscale scores. Scores on the PennCNP were assessed as total correct score or d' (for tasks having a true/false positive/negative structure). Pearson correlations were conducted between the VT for each ROI and PANSS subscale scores and each of the PennCNP outcome scores.

Results: [18 F]VAT VT did not significantly differ between patients with SCZ compared to HC in any of the striatal or extrastriatal regions. Similarly, regional [18 F]VAT VT did not differ significantly when patients were subgrouped based on whether they were currently taking antipsychotic medication ($n = 7$) or not ($n = 11$).

Despite non-significant group differences, we observed relationships between [18 F]VAT VT and key clinical and neuropsychological variables within the patient group. Most notably, patients' psychosis symptoms, measured by the PANSS Positive subscale score, were positively correlated with [18 F]VAT VT in multiple ROIs (strongest effects in the thalamus, cerebellum, and several cortical regions). Also, within patients with SCZ, performance on the 2-back task was negatively associated with VAChT availability in several cortical regions: the higher the VT, the worse the performance (e.g. strong negative association between performance on the 2-back task and [18 F]VAT VT in the dlPFC in SCZ: $R = -0.63$). These correlations should be considered preliminary, in that these were exploratory analyses in a small pilot study, but suggest the need for further investigations of the cholinergic system in SCZ.

Conclusions: These findings suggest the cholinergic system may be mediating a relationship between working memory deficits and psychosis severity in individuals with SCZ. An improved understanding of these modulatory systems could lead to a better mechanistic understanding of the disease.

Keywords: PET, Cholinergic System, Cognitive Impairment Associated With Schizophrenia, Psychosis and Memory

Disclosure: Nothing to disclose.

P543. Exploring Sex Differences (and Lack Thereof) in Schizophrenia: From Cognitive Control Brain Activity to Clinical Phenotypes

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Background: Sex differences in schizophrenia represent an important aspect of schizophrenia in need of further investigation. Male sex has previously been associated with earlier age of onset, higher rates of substance abuse, and in some studies a higher incidence of the disorder. Several studies have also identified differences in symptom profiles, with female patients presenting with lower levels of negative symptoms and higher depressive symptoms. However, the literature is largely mixed in terms of sex effects on cognition and brain function in patients with schizophrenia. Several studies have identified disproportionately worse performance in male patients on measures of verbal learning and memory and inhibition/switching tasks, while others suggest worse performance in female patients in visualspatial attention and spatial memory. Notably, the modest number of functional magnetic resonance imaging (fMRI) studies suggest altered activity and connectivity in male versus female patients on tasks of mental rotation and emotional memory that involve the frontal-parietal and limbic networks. The current study seeks to further expand these findings by examining a large sample of patients with schizophrenia and healthy controls who performed a cognitive control task during fMRI and completed thorough symptom and functioning evaluations.

Methods: A total of 148 (116 male, 32 female) individuals with schizophrenia-spectrum diagnoses were recruited, assessed using the Structured Clinical Interview for DSM-IV, Global Social/Role

Functioning Scales (GFS: R/S), Scale for the Assessment of Positive Symptoms (SAPS), Scale for the Assessment of Negative Symptoms (SANS), and Brief Psychiatric Rating Scale (BPRS). A sample of 132 control participants (78 male, 54 female) were also recruited from the community. All participants performed the AX-CPT while undergoing fMRI on a 1.5 Tesla General Electric or 3 Tesla Siemens Trio scanner. fMRI data were processed using SPM8, including slice timing correction, realignment, normalization to the MNI EPI template, and smoothing. General linear models (GLM) were used to test for the effect of sex, group and sex by group interactions. Protocol type (i.e. 1.5 T or 3 T scanner) and age were included as covariates. T-tests and Mann-Whitney U tests were used for assessing sex effects within the patient group on symptom and functioning measures.

Results: Analyses of demographic data revealed a higher proportion of males in the patient group ($p < 0.001$) along with lower education in the patient group compared to controls ($p < 0.001$). In terms of global functioning, male patients showed significantly lower social functioning ($p = 0.002$) and a trend for lower role functioning ($p = 0.062$). Analyses of symptom syndrome scores revealed significantly lower poverty symptoms in female patients ($p = 0.018$) while reality distortion and disorganization syndrome scores did not differ between the sexes. Male patients were also significantly more likely to endorse a history of cannabis use compared to females ($p = 0.027$, phi = 0.159). In terms of cognitive control, healthy controls showed higher d'-context performance compared to patients ($p < 0.001$), but no sex or group by sex interaction emerged (all $p > 0.05$). Similarly, on a measure of attention lapsing (i.e., average of AX and BY error rates), patients showed higher rates of lapsing compared to controls ($p < 0.001$) in the absence of a significant sex or sex by group interaction (both $p > 0.05$). Bilateral regions of interest in the DLPFC and superior parietal cortex were evaluated on the CueB-CueA contrast. Both regions revealed significantly increased activation in controls compared to patients ($p = 0.002$ in DLPFC and $p < 0.001$ in parietal) with no significant effect of sex. Interestingly, within the patient group, females showed significantly higher activity in the right DLPFC ($p = 0.042$) and a similar but nonsignificant pattern of activity in the right superior parietal cortex ($p = 0.243$).

Conclusions: These data suggest that sex differences in patients with schizophrenia are relatively circumscribed. In agreement with other published findings, female patients show a relatively more favorable clinical and functional profile, with lower negative symptoms and higher social functioning being the most pronounced in this sample. The higher proportion of males in the schizophrenia sample is also consistent with the literature. Notably, one of the few functional differences that was identified was increased prefrontal activity in female versus male schizophrenia patients. This difference is counterpointed by a lack of evidence for sex differences in terms of cognition (d'-context, attention lapsing). It is not readily apparent why sex differences in brain activity emerged without concomitant behavioral differences, although differences in laterality between the sexes might underlie this effect. Given some evidence of the impact of illness chronicity on sex effects, future analyses will also focus on age of onset and illness duration.

Keywords: Functional MRI (fMRI), Schizophrenia (SCZ), Gender Differences, Sex Differences, Cognition

Disclosure: Nothing to disclose.

P544. Sleep and Wake Markers of Thalamocortical Circuit Functioning are Abnormal in Early-Course Psychotic Disorders

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Background: Recent advances in psychiatric genetics and studies in rodent models reveal that abnormalities in thalamocortical circuitry contribute to the pathogenesis of psychotic disorders. Sleep spindles, rapid bursts of 12-15 Hz EEG oscillations characteristic of Stage 2 non-rapid eye movement (NREM) sleep, are initiated by the thalamic reticular nucleus (TRN) and propagated to the cortex and coordinated with other NREM oscillations by thalamocortical circuitry. Spindles mediate memory consolidation, and are associated with impaired sleep-dependent memory consolidation and symptom severity in psychotic disorders. During wakefulness, TRN gates information flow from the thalamus to the cortex to attenuate the transmission of redundant and irrelevant sensory stimuli. The goal of the present study was to investigate thalamocortical circuit functioning in early course, minimally treated patients with psychotic disorders, their young, first-degree relatives (familial high risk: FHR) and matched controls. We utilized resting-state functional connectivity MRI to determine if thalamocortical circuitry is abnormal in psychosis and FHR, and sleep and wake EEG to evaluate the functional implications of thalamocortical circuit dysfunction. This unique sample allows for the identification of trait-related alterations in thalamocortical circuitry.

Methods: Seventy-one participants (13-35 yrs, n-psychosis=19, n-controls=28, n-FHR = 24) completed an overnight sleep study, a sensory gating event-related-potentials (ERP) experiment, resting-state fMRI scans and clinical interviews. Sleep was monitored with a 64 channel high-density EEG device and NREM sleep oscillations were identified using validated automated detectors. Sensory gating was calculated as the suppression of the auditory P50 component for the second of a pair of identical clicks. The thalamus was defined using the FSL Oxford atlas and thalamocortical functional connectivity was quantified using a seed-based approach.

Results: For thalamocortical connectivity, the main effect of Group (psychosis, FHR or control) was significant in a cluster in the right Heschl's gyrus ($F(2,64)MIN = 7.7$, 131 voxels, MNI peak: [40,-20,12], BA41). This reflected that compared to controls, both the psychosis ($p < 0.001$) and FHR ($p < 0.001$) groups exhibited hyperconnectivity of the thalamus with the primary auditory cortex. When directly compared with controls, individuals with psychosis also exhibited hyperconnectivity of the thalamus with the right motor cortex ([34,4,54], BA6) and hypoconnectivity with the left cerebellum ([-22,-80,-50], Crus II). Psychosis patients (but not FHR) showed significant reductions in spindle density (all electrodes, Fsum=356.8, p-corrected = 0.002) and spindle amplitude (37 electrodes, Fsum=207.4, p-corrected = 0.005), altered temporal coordination between spindles and slow oscillations (43 electrodes, Fsum=207.6, p-corrected = 0.009), and abnormal P50 sensory gating ($F(2,54)=4.2$, $p = 0.02$). Spindles and P50 gating were correlated ($r = -0.29$, $p = 0.03$) reflecting that individuals with fewer spindles also failed to gate repetitive auditory information. In both psychosis and FHR groups, intensity of psychotic-like experiences correlated with decreased sleep spindle activity ($r = -.47$, $p = 0.003$) and abnormal sensory gating ($r = 0.31$, $p = 0.08$).

Conclusions: In young early-course patients with psychotic disorders and their first-degree relatives, we observed abnormally increased connectivity of the thalamus with the primary auditory cortex, which extended to motor and premotor regions only in patients. The function of this circuitry was impaired in patients, as evidenced by a reduction in spindle activity during sleep and abnormal gating of auditory sensory information during wakefulness. First-degree relatives of patients did not exhibit significant deficits in sleep spindles or sensory gating, perhaps due to heterogeneity of the sample, but these markers predicted

psychotic-like experiences in both groups suggesting that altered thalamocortical circuit functioning contributes to the emergence of psychotic symptoms.

Keywords: Sleep Spindles, Sensory Gating, Thalamo-Cortical Connectivity, Psychotic Disorders, Psychosis-Risk

Disclosure: Nothing to disclose.

P545. Associations Between Structural Covariance Network and Antipsychotic Treatment Response in Schizophrenia

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Background: Approximately 30% of patients with schizophrenia do not respond to antipsychotics and are termed treatment-resistant schizophrenia (TRS). Several studies showed that patients with TRS have widespread strong reductions in cortical thickness compared to healthy controls (HC) as well as patients with non-TRS. Recently, to evaluate these structural changes in terms of brain networks, structural covariance has been used to assess the associations of structural measures between two regions. Structural covariance increases when similar structural changes occur in two independent regions, suggesting that those regions share a common pathophysiology. A previous study reported that structural covariance between the two regions with strongly reduced cortical thickness increased in patients with schizophrenia. In this study, we examined the association between treatment resistance and structural covariance changes in this disorder.

Methods: We used international multi-site cross-sectional neuroimaging datasets comprising 102 patients with TRS, 77 patients with non-TRS, and 79 HC. Eighty-nine, seventy, and ninety-nine participants were enrolled at Komagino Hospital (Tokyo, Japan), Shimofusa Hospital (Chiba, Japan), and the Centre for Addiction and Mental Health (Toronto, Canada). Antipsychotic treatment resistance was defined by the modified Treatment Response and Resistance in Psychosis Working Group Consensus criteria.

T1-weighted structural images were preprocessed using the bpipe pipelines, then cortical thickness was calculated using CIVET for each of 68 regions of the Desikan-Killiany-Tourville atlas. We used a CovBat harmonization method to control for the site differences in the cortical parameters while considering disease status, age, and sex as biological variables. We calculated the residuals of cortical thickness controlling for age and sex. Analysis of variance and post-hoc pairwise t tests were used to examine group differences in residuals of cortical thickness for the 62 cortical regions. Structural connectivity was quantified with partial correlation coefficient between all pairs of regions, controling for age and sex. This approach yielded a separate connectivity matrix of dimension 62×62 for each group. Based on the Fisher method for comparing correlation coefficients, thickness correlations were r-to-z transformed. From the difference in the resulting r-to-z values between the 2 groups, we estimated a z-score that tested the null hypothesis of equality in thickness correlations between the 2 groups. Calculated z-score in each edge was normalized using the standardized deviation of z-scores in 1,000 bootstrap samples. The network-based statistic with 50,000 permutations was used to obtain the structural covariance network with significant difference, controlling the 1,891 multiple comparisons. A primary z-score threshold of 3 was used, with a family-wise error rate threshold of 5%. We estimated the degree centrality for each node of the obtained structural covariance network. This study was conducted after obtaining approval from the respective ethics review committees.

Results: Group differences in residuals of cortical thickness were found in 58 out of 62 cortical regions. A total of 59 and 31 regions were found to show cortical thickness reductions in TRS and non-TRS compared with HC, respectively. Also, cortical thickness in 26 regions was reduced in the TRS group than in the non-TRS group. Both In the TRS and non-TRS groups, regions with cortical thinning were located mainly in the frontal and temporal lobes and the cingulate cortex. No significant difference was found in the variances in residuals of cortical thickness among the three groups. Structural covariances across 1,891 pairs of cortical regions were higher in the non-TRS group compared with the HC and TRS groups. The null hypothesis of equality in structural covariance between the non-TRS and HC groups was rejected using network-based statistics. We found a single network comprising connections with elevated structural covariance in patients with non-TRS compared with HC. The inferior temporal gyrus and insula had high degree centrality in the structural covariance network. The null hypothesis could not be rejected for structural covariance between the TRS group and the nonTRS or HC groups.

Conclusions: While patients with TRS showed stronger cortical thinning in the frontal and temporal lobes compared to HC and patients with non-TRS, there was no structural covariance network with significant difference between the TRS group and the HC or non-TRS groups. On the other hand, the non-TRS group had a brain network with increased structural covariance compared to the HC group. Our findings suggest that coordinated cortical thinning in the brain network is related to treatment response in schizophrenia while TRS may have greater heterogeneity in the pathophysiology of cortical thinning than non-TRS, resulting in lower structural covariance. Another possibility is that cortical thinning was severe or had reached the plateau long ago in patients with TRS such that the elevation of structural covariance may not be detected. Further longitudinal study is warranted to confirm the link between pathophysiology of structural alteration and treatment resistance in schizophrenia.

Keywords: Schizophrenia (SCZ), Treatment-Response, Structural Covariance Analyses

Disclosure: Nothing to disclose.

P546. Salience Detection Abnormalities in First Episode Psychosis: An MEG, Whole Brain 1H-MRS and Midbrain Neuromelanin Study

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Background: In psychotic disorders, the processing of irrelevant stimuli as important may instill percepts and thoughts with abnormal salience, leading to inappropriate associations and causal attributions. In an auditory oddball paradigm, both Sz and BP-I (the best exemplars of psychotic disorders) have reduced P300a (novelty) and P300b (target) amplitudes compared to healthy volunteers (HVs). Furthermore, subjects at-risk for psychosis also have reductions in both P300 subcomponents. However, in the largest study of at-risk subjects, only the P300b reductions predicted subsequent conversion to psychosis. This suggests that a specific salience detection deficit may be critical to the development of psychosis. However, the underlying neurobiology of salience detection deficits in psychotic disorders remains unclear. In this pilot study we used MEG, 3 dimensional 1H-MRS and midbrain neuromelanin MR, to provide unprecedented spatial resolution and localization of evoked potentials and

their underlying tissue characteristics (glutamate and N-acetylaspartate), as well as their relationship to dopamine function in the substantia nigra (SN).

Methods: We studied 13 first episode psychosis (FEP) subjects and 12 HV with MEG, 3D-1H-MRS and MR neuromelanin. During MEG, subjects performed an auditory oddball evoked response task, pressing a button to infrequent target stimuli (10%). Also frequent standard (80%) and infrequent novel (10 %) stimuli were presented but the subject was instructed to ignore these. Magnetic fields were recorded using a Neuromag 306-channel whole-head system. Continuous data was collected at a digitization rate of 1000 Hz with filters set at 0.03–130 Hz. Epochs for each stimulus type were extracted from 200 ms pre-stimulus to 1500 ms post-stimulus and analyzed with BRAINSTORM. MRS data was acquired with Siemens PRISMA at 3 T using EPSI: TR/TE = 1551/17.6 msec; spatial array of 50 ×50 x 18, FOV of 280 ×280 x 180 mm3 (corresponding to a nominal voxel size of 5.6 ×5.6 ×10 mm3). Spectral data was processed with MIDAS. After source localization MEG regions that showed reduced amplitudes to the target in FEP ($p < 0.05$, non-corrected FWE) were examined for MRS group differences with MINT. MINT allows the selection of all spectral voxels in a particular region and integrates the data to generate one better quality spectrum that is fitted for metabolite quantification (eg: glutamate and N-acetylaspartate). Finally, neuromelanin MR was acquired at 3 T from a midbrain slab containing the SN: TR/TE = 444/4.11 msec; spatial array of 50 ×50 x 18, FOV 220 mm (corresponding to a nominal voxel size of 0.4 ×0.4 ×1.5 mm3).

Results: FEP had reduced amplitudes to the target minus standard stimuli at the 250 msec interval over the left hemisphere cortical area involving the posterior superior temporal gyrus (STG) and middle temporal gyrus (MTG; $p < 0.05$). FEP also had reduced target minus standard amplitudes at 100 msec over the left and right posterior STG and MTG ($p < 0.05$). HV and FEP had similar low error in performance though reaction times to targets were slower in FEP. Tissue neurochemistry group contrasts over the left and right STG plus MTG will be presented. Also the relationships between target evoked amplitudes, glutamate concentrations and SN neuromelanin will be examined.

Conclusions: Reduced amplitudes to targets at 250 msec in left temporal cortex in patients early in the illness, suggest specific abnormalities in the processing of relevant stimuli in psychosis. Speculations about the relationships between salience detection, glutamate and dopaminergic functions are abundant in the field of psychosis, but convergent data is limited. In rats, cue-reward learning is proportional to the strength of glutamatergic synapses onto dopamine midbrain neurons. Also in rats, NMDA hypofunction induced by acute systemic ketamine increases extracellular prefrontal glutamate and dopamine release. In humans, one study used PET and single-voxel 1H-MRS to measure striatal dopamine synthesis-capacity and medial frontal glutamate respectively, in first-episode psychosis. Glutamate and dopamine were inversely correlated in psychotic but not in healthy volunteers, supporting the importance of measuring both systems in this population. Ours is the first investigation to examine the relationships between glutamate and dopamine with electrophysiological measures of salience detection. Results from this study will have two significant specific implications. First, identifying the location of glutamate and P300 deficits may inform probe placement for future neuromodulation studies, aimed to improve cognitive deficits and persistent psychotic and negative symptoms. Second, these studies may also inform anatomical regional selection for future postmortem studies of Sz and BP-I aimed at examining the molecular underpinnings of the illness.

Keywords: P300, Disorders of Glutamate, Transition to Broad Psychosis Spectrum Psychopathology, MEG, Proton Magnetic Resonance Spectroscopy

Disclosure: UpToDate, Other Financial or Material Support(Self)

P547. Brain Functional Segregation, Psychotic Symptoms, and Treatment Response

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Background: Functional integration and segregation are the two guiding principles in human brain functional mapping. These two principles reflect the integrated and distributed nature of neuronal processing and brain organization. In brain imaging studies with fMRI, functional integration and segregation are indicated by the correlation and anticorrelation of time course of spontaneous blood oxygen level-dependent (BOLD) signal, respectively. To date, most research has focused on functional integration, with less attention paid to the role of functional segregation. It is unclear whether functional segregation is impaired only in some specifical brain regions such as between default mode networks (DMN) and the executive network, or if it is a global impairment. It is also unclear whether various psychotic symptoms have different neuropathological substrates that are related to functional segregation impairments in different circuits. Moreover, there is considerable biological and clinical heterogeneity across psychosis syndromes and in the longitudinal course of psychotic disorders. Conventional approaches investigating treatment outcomes based on clinical categorization and diagnoses are difficult to capture fundamental underlying neurobiological mechanisms of dysfunction and can be recall or responder biased. Thus, an objective neuroimaging biomarker for classifying patients and tracking the changes of psychotic symptoms would be crucial.

Methods: To address the above issues, we introduce the Negative Degree Centrality (NDC) method to quantify the degree of functional segregation between a specific region and all the other parts of the brain. A whole-brain data-driven analysis was applied for a comprehensive evaluation of impaired functional segregation in psychosis. We examined the difference of NDC among early-phase patients with schizophrenia (SZ, $N = 123$), bipolar disorder (BD, $N = 156$), and healthy controls (HC, $N = 139$); and monitored NDC changes in a subset of patients (31 SZ and 56 BD) with disease progression one year later. We adopted an unsupervised machine learning classifier to subdivide patients based on distinct NDC patterns of neuroimaging data at baseline and one-year follow-up and investigated whether these subgroups of patients would have different behavioral and treatment outcomes. We hypothesized that: 1) various psychotic symptoms will be associated with functional segregation impairments in different neural circuits; 2) the combination of functional segregation and a machine learning method will outperform traditional diagnostic categories in tracking psychotic symptom changes and predicting treatment outcomes.

Results: Compared to HC, both SZ and BD subjects showed significantly decreased NDC at baseline. Positive, negative, and general psychotic symptoms were correlated with impaired NDC in the salience, frontal-parietal, and DMN, respectively. Using a machine learning approach, we identified two transdiagnostic patient subgroups with distinct recovery trajectories: one subgroup with more NDC impairments at baseline showed significantly improved NDC and psychotic symptoms, however, the other subgroup with less NDC impairments at baseline showed only slightly improved NDC and symptom severity at one-year follow-up.

Conclusions: Our study indicated that various psychotic symptoms were linked to impaired functional segregation in different brain circuits. NDC combined with machine learning might provide a helpful biomarker in identifying the neural

underpinnings of outcome heterogeneity, and for psychotic symptom severity and treatment response prediction, which could not have been revealed by categorizing them based on conventional clinical diagnoses. Our approach may help to develop precision medicine and personalized interventions in psychiatry.

Keywords: Psychotic Disorders, Functional Segregation, Functional Magnetic Resonance Imaging, Machine Learning, Brain Circuit

Disclosure: Nothing to disclose.

P548. Nanoscale Probing of Synaptic Architecture in Human Prefrontal Cortex With Expansion Microscopy

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Background: Schizophrenia is associated with alterations in excitatory neurotransmission in the prefrontal cortex (PFC). The strength of this signaling depends, in part, on the spatial organization of synaptic proteins. For example, presynaptic regulators of vesicle release (e.g., Rab3-interacting molecule [RIM]), postsynaptic scaffolding proteins (e.g., PSD95) and AMPA receptors organize into nanoscale clusters that align with each other trans-synaptically. The alignment of these clusters is thought to facilitate efficient neurotransmitter movement from presynaptic terminals to AMPA receptors. Visualizing these nanoclusters requires imaging techniques with resolution higher than the light diffraction limit. Expansion microscopy physically expands biological specimens isotopically, providing nanoscale-resolution imaging of synaptic molecules with conventional confocal microscopy. Here, we explored the utility of expansion microscopy for assessing spatial organization of synaptic proteins in human PFC.

Methods: Human PFC sections were incubated with methacrolein and a gelling solution to anchor the endogenous proteins to hydrogel matrix, and then incubated with a denaturant-rich solution to homogenize the contents of hydrogel while retaining endogenous proteins. Gels were then stained with antibodies for RIM1/2, PSD95 and Pan-GluA. Stained hydrogels were expanded in water and imaged with a confocal spinning-disk microscope under 40x lens. Peak-to-peak analysis was performed to quantify the axial distance between the clusters formed by RIM, PSD95 and GluA. The relative size of these clusters was assessed by auto-correlation analysis. Finally, the spatial alignment between pre- and postsynaptic clusters across synaptic cleft was assessed by enrichment analysis.

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Results: At 9-fold expansion, multiple clusters formed by RIM, PSD95 and GluA within individual synapses were visualized in human PFC. The axial distance between PSD95 and GluA, GluA and RIM, or PSD95 and RIM clusters was 27.5 nm, 51.8 nm or 64.8 nm, respectively. Auto-correlation analysis showed that PSD95, GluA and RIM proteins are organized in local clusters with an average radius of 80 nm. Finally, enrichment analysis showed that the expression level of each of these proteins is enriched within 40 nm radius from the center of clusters formed by the protein localized at the opposite side of synaptic terminals.

Conclusions: Our findings demonstrate an optical resolution for the spatial organization of key synaptic proteins that regulate the strength of excitatory neurotransmission in human PFC by expansion microscopy. This approach may provide a novel strategy to identify the role of synaptic nanoarchitecture alterations underlying PFC circuitry dysfunction in schizophrenia.

Keywords: Dorsolateral Prefrontal Cortex (DLPFC), Expansion Microscopy, Synaptic Organization

Disclosure: Nothing to disclose.

P549. Meta-Analytic Evidence of Elevated Choline, Reduced NAA and Normal Creatine in Schizophrenia and Their Moderation by Measurement Quality, Field Strength, TE, and Medication Status

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Background: Magnetic resonance spectroscopy (MRS) provides insights into the pathology of schizophrenia via measurement of molecules involved in neuronal mitochondrial function (n-acetylaspartate, NAA), phospholipid metabolism (choline), and cellular energy metabolism (creatine). Decreased NAA is consistently found in MRS studies of schizophrenia. However, recent investigations suggest that T2 relaxation effects may significantly moderate these findings. The field lacks consensus regarding choline and/or creatine abnormalities in schizophrenia. To address these knowledge gaps, we performed a new meta-analysis of NAA, choline, and creatine in patients across all phases of schizophrenia, compared to healthy control subjects. In addition, recent work has demonstrated a strong influence of data measurement quality on estimates of glutamate levels with MRS. We assessed potential moderating effects of measurement quality on the three singlet peaks – NAA, choline, and creatine. Other technical and clinical moderators were also examined.

Methods: All studies cited in three recent MRS meta-analyses were identified. A search of the PubMed database from December 1, 2019, and March 12, 2021 for MRS studies published after the most recent meta-analysis was conducted to identify newer studies for this updated meta-analysis. A total of 116 studies met our inclusion criteria. Pooled effect sizes (Hedges' g) of patient-control differences were calculated whenever ten or more studies reported data on a metabolite from a specific brain area. Medial prefrontal cortex (MPFC), dorsolateral prefrontal cortex, frontal white matter, hippocampus, thalamus, and basal ganglia met this threshold and were included in the meta-analysis.

To examine moderating effects of data measurement quality on results, we performed moving sub-meta-analyses akin to a moving average from the lowest to highest quality studies based on the coefficient of variation of metabolite values (standard deviation / mean). A best-fitting, 4-parameter, logistic function was fit to the pooled effect sizes and the resulting equation used to identify the inflection point. This inflection point was then used to test for a moderating effect of quality on meta-analytical results. The potential moderating effects of field strength, echo time, creatine versus water normalization, medication status, phase of illness, patient age and patient sex were also examined.

Results: Choline was significantly elevated in medial and dorsolateral prefrontal cortices and basal ganglia in schizophrenia ($p = 0.008$, $p = 0.007$, $p < 0.00006$, respectively). Elevation in MPFC was significantly stronger in studies with better measurement quality and tended to be stronger in studies with more medicated patients. The hippocampal choline effect size was significantly moderated by field strength, with greater elevation in studies conducted at ≥ 3 Tesla.

NAA was reduced in schizophrenia in medial and dorsolateral prefrontal cortices, frontal white matter, hippocampus, and thalamus, but not in basal ganglia ($p = 0.00007$, $p = 0.0059$, $p = 0.0015$, $p = 0.0088$, $p = 0.313$, respectively). The reduction in MPFC was significantly stronger in studies with higher signal-to-noise ratios and tended to be stronger in studies with more

medicated patients. Hippocampal NAA was significantly more reduced in studies with more medicated patients. When outlier studies were excluded, hippocampal NAA was more reduced in studies using longer echo times. This effect was only robust in studies reporting water-normalized NAA values. No effect of echo time was seen in other regions or in creatine-normalized hippocampal NAA datasets.

There was no meta-analytic evidence that creatine levels differed between patients and control subjects in any brain region investigated. Creatine effect sizes were not moderated by measurement quality or any other technical or clinical factors examined.

Conclusions: Elevated choline in prefrontal cortex and basal ganglia may reflect greater membrane turnover in these brain regions or a disturbance involving the role of choline-containing compounds in lipid metabolism, lipid signaling, or DNA methylation. Our finding of reduction in NAA is consistent with prior meta-analyses. This may reflect a diminished synthesis of NAA by neuronal mitochondria or an abnormality involving NAA catabolism by oligodendrocytes in schizophrenia. Prior evidence that increased dopaminergic tone can stimulate NAA synthesis in the basal ganglia may account for the selective absence of reduced NAA in this region. Although prior studies have reported more reduced NAA at longer echo times, this was not seen at the meta-analytic level except for water-normalized hippocampal studies. The positive association between antipsychotic medication use and both reduced NAA and elevated choline may have clinical significance and merits further investigation. The absence of evidence for abnormal creatine levels suggests that creatine normalization may be a valid option in 1H-MRS studies of schizophrenia patients. Moderation of higher choline and lower NAA by measurement quality may account for elevated choline not being consistently observed in prior meta-analyses and indicates a need for more stringent quality measurement thresholds to improve validity of, and consensus among, future studies.

Keywords: Schizophrenia (SCZ), Magnetic Resonance Spectroscopy, Meta-Analysis, Choline, N-acetylaspartate

Disclosure: Nothing to disclose.

P550. Neurochemical Alterations in Occipital and Prefrontal Cortex Measured With 7 T MRS as Part of the Psychosis Human Connectome Project

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Background: Psychosis spectrum disorders such as schizophrenia involve disrupted cognitive and perceptual functioning, but the neural basis of these deficits remains unclear. Neurochemical changes in people with psychotic psychopathology (PwPP) have been observed across a number of brain regions using methods including magnetic resonance spectroscopy (MRS). In particular, differences in glutamate and GABA have received attention, as an imbalance in excitatory and inhibitory neural processes has been hypothesized in PwPP. However, methodological issues including small sample sizes and difficulty isolating signals from low concentration metabolites have limited previous studies. We sought to address these limitations in the current study by acquiring 7 tesla MRS data as part of the Psychosis Human Connectome Project. We acquired data from healthy controls and PwPP as well as unaffected biological relatives, in order to explore the role of genetic liability for psychosis in brain chemistry. Our data will be made publicly available in order to facilitate further studies from other research groups.

Methods: We acquired 7 T MRS data using a STEAM sequence with an ultra short (8 ms) echo time in 44 healthy controls, 43 relatives, and 65 PwPP. We also obtained re-scan data from a subset of 44 participants (10 controls, 34 PwPP; median time between scans = 132 days). Data were acquired in two volumes of interest (VOIs) within the medial occipital and dorsomedial prefrontal cortex. Scan parameters were as follows: TR = 5 s, TE = 8 ms, TM = 32 ms, VOI size = 30 ×18 x 18 mm3 (occipital) or 30 ×30 x 15 mm3 (prefrontal), # data points = 2048, spectral bandwidth = 6000 Hz, VAPOR water suppression.

After frequency and phase correction, spectra were fit using LCModel to quantify concentrations for the following metabolites: ascorbic acid, aspartic acid, total choline, total creatine, GABA, glucose, glutamate, glutamine, glutathione, lactate, myo-inositol, N-acetyl aspartate (NAA), N-acetyl aspartylglutamate (NAAG), phosphorylethanolamine, scyllo-inositol, taurine, lipids, and macromolecules. Metabolite concentrations were scaled relative to the unsuppressed water signal, after correcting for relaxation time and differences in tissue composition within the VOIs across participants. We established quality metrics (linewidth of water, spectrum linewidth, and spectrum SNR) which we used to exclude low quality data. 92% of our data sets passed all quality checks.

Results: We first examined differences in 6 metabolites of interest (GABA, glutamate, glutamine, glutathione, NAA, and NAAG) which were identified a priori based on previously reported differences between PwPP and controls in occipital and prefrontal regions. Group differences for these 6 a priori metabolites were examined without corrections for multiple comparisons. We observed significantly lower NAA in occipital cortex among PwPP and relatives versus controls ($X2(2) = 7.03$, $p = 0.030$). In prefrontal cortex, NAAG was significantly lower among PwPP versus controls and relatives ($X2(2) = 7.66$, $p = 0.022$). Longitudinal stability for these metabolites was fair (ICC = 0.58 for prefrontal NAAG) to good (ICC = 0.82 for occipital NAA). None of the other metabolites examined a priori (including GABA and glutamate) showed significant group differences in either VOI.

We also performed an exploratory analysis of group differences in the remaining 11 metabolites (excluding lipids), which included corrections for multiple comparisons. Only glucose in prefrontal cortex showed a significant group difference in this exploratory analysis, and was higher among PwPP versus controls and relatives ($X2(2) = 12.3$, FDR-corrected $p = 0.024$). Interestingly, a similar trend was observed for glucose in occipital cortex, but did not survive correction for multiple comparisons ($X2(2) = 7.67$, uncorrected $p = 0.022$, FDR-corrected $p = 0.24$). We saw fair stability over time for glucose (ICC = 0.59 in occipital cortex, ICC = 0.54 in prefrontal cortex). Preliminary results from a hierarchical clustering analysis suggested that glucose levels in both occipital and prefrontal VOIs were associated with individual differences in measures of psychopathology (e.g., Brief Psychiatric Rating Scale, Schizotypal Personality Questionnaire) across all groups (Spearman's r-values = 0.14 to 0.28, uncorrected p-values = 0.12 to 5 ×10^-4).

Conclusions: People with psychosis showed lower NAA and NAAG (in occipital and prefrontal cortex, respectively), consistent with findings from previous studies. Lower occipital NAA levels were also observed among first-degree biological relatives compared to controls, suggesting a link to genetic liability for psychosis. To the best of our knowledge, our study is the first to observe higher brain glucose levels among PwPP, which may be associated with more severe psychopathology. Together, our findings indicate a unique profile of neurometabolic disruption across brain regions in PwPP, which may be associated with both genetic factors and individual differences in psychopathology.

Keywords: MR Spectroscopy, Psychosis, Schizophrenia (SCZ), Occipital Cortex, Prefrontal Cortex

Disclosure: Nothing to disclose.

P551. Metabolomic Signatures Associated With Antipsychotic -Induced Weight Gain and Psychosis Spectrum Diagnoses

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Background: Psychosis spectrum disorders (PSDs) are associated with intrinsic metabolic abnormalities. Beyond the intrinsic metabolic risk, antipsychotics (APs), the cornerstone of treatment for PSDs, incur additional metabolic adversities including weight gain. Currently, major gaps exist in understanding psychosis illness biomarkers, as well as risk factors and mechanisms for AP-induced weight gain. Metabolomic profiles may provide insight into biomarkers of PSDs and antipsychotic-induced weight gain.

Methods: In this 12-week prospective naturalistic study, we compared serum metabolomic profiles of AP-naïve cases to healthy controls at baseline to examine biomarkers of intrinsic metabolic dysfunction in PSDs. We then examined changes in serum metabolomic profiles over 12 weeks of antipsychotic treatment in AP-naïve cases to identify metabolites that may predict or associate with AP-induced weight gain. Statistical analyses of the metabolomic datasets were conducted using Metaboanalyst 5.0. T-tests were used to compare mean baseline metabolite concentrations between 1) AP-naïve cases and controls at baseline, and 2) AP-naïve cases who do and do not develop ≥5% body weight gain at 12 weeks. Changes from baseline to endpoint between AP-naïve cases who do and do not develop significant (i.e. ≥5%) body weight gain were compared with two-way repeated measures ANOVA testing. Pearson correlations were calculated between change in weight gain and change in metabolite concentrations from week 1 to week 12 for cases. To control for multiple comparisons, a false discovery rate (FDR) of the resulting post hoc P values was calculated.

Results: Twenty-five AP-naïve cases were enrolled, and 17 cases completed both baseline and 12-week follow up visits, while the remaining 8 cases had baseline visits only. Six healthy controls completed baseline visits. Following 12 weeks of AP exposure, cases experienced increases in body weight ($p < 0.001$), BMI ($p < 0.001$), waist circumference ($p = 0.010$), LDL cholesterol ($p = 0.026$), and total cholesterol ($p < 0.001$). Additionally, a subgroup of cases ($N = 11$) experienced clinically significant increases (≥5%) in body weight. Overall, 20 amino acids, 20 bile acids, 30 fatty acids, and 29 acylcarnitines were identified and quantified. AP-naïve cases were distinguished from controls by six fatty acids when compared at baseline (FDR of <0.05), namely reduced levels of palmitoleic acid, lauric acid, and heneicosylic acid and elevated levels of behenic acid, arachidonic acid, and myristoleic acid. Baseline levels of the fatty acid adrenic acid was increased in individuals who experienced a clinically significant body weight gain (≥5%) following 12 weeks of AP exposure as compared to those who did not (FDR = 0.0408). Among quantified metabolites, none met the threshold for significance when examining associations between changes in metabolite concentrations and body weight over 12 weeks.

Conclusions: Specific fatty acids may represent illness biomarkers of PSDs and early predictors of AP-induced weight gain. The findings hold important clinical implications for early identification of individuals who could benefit from prevention strategies to reduce future cardiometabolic risk, and may lead to targeted treatments to counteract metabolic dysfunction in PSDs.

Keywords: First Episode Psychosis, Metabolomics, Intrinsic Metabolic Risk, Antipsychotic-Induced Weight Gain

Disclosure: Alkermes: Consultant (Self).

P552. Proteomic Plasma Profiling of Patients With First-Episode Psychosis Identifies Inflammatory Markers Associated With Illness Severity

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Background: Over the last 20 years numerous studies depict that the immune system and inflammatory responses may play an important role in psychiatric disorders. Thus, enhanced secretion of inflammatory cytokines and chemokines have been found in patients with depression, schizophrenia and bipolar disorder. Increased levels of immune markers have also been found to correlate with impaired cognitive ability.

In the present study, Olink Proximitiy Extension Assay (PEA) technology was used to investigate immune activation in first-episode psychosis (FEP) patients and healthy controls (HC) and to further analyze if the immune profile differs between FEP patients later diagnosed with schizophrenia and those not receiving such diagnosis. Furthermore, since correlations of disease severity and neurocognitive decline has been shown previously, we aim to explore patterns of correlations with altered inflammation markers and psychopathology and cognitive scores.

Methods: Somatically HC ($n = 55$) with no prior drug addiction and FEP patients ($n = 73$) were recruited in the Karolinska Schizophrenia Project (KaSP) in Stockholm, Sweden. The existence of neurologic illnesses or severe somatic illness, a history of illegal substance addiction, and the presence of co-existing neurodevelopmental abnormalities were exclusion criteria. Global Assessment of Functioning (GAF, the Positive and Negative Syndrome Scale (PANSS), Clinical Global Impression (CGI) were performed to assess clinical characteristics of the patients. 46.6% of the patients ($n = 34$ of 73) were treated with antipsychotics at the time of serum sampling. Maximum number of days of antipsychotic treatment was 26 days, mean number of days was 9.6. Thirty-eight patients have not used any kind of antipsychotics prior, or at the time of serum sampling. Venous blood samples were collected using standard venipuncture techniques. 92 inflammatory proteins in the serum were analyzed by Olink Bioscience, Uppsala, Sweden using the multiplex proximity extension assay technology (PEA) inflammation panel.

Results: No significant differences were observed between HC and FEP patients regarding age, gender, or body mass index. Total PANSS score for patients was 71.9 ± 2.4. DUP was 8.5 ± 1.6 (mean ± s.e.m.) months for FEP patients. Differential expression analysis showed 12 proteins upregulated in FEP patients. AXIN1 was identified to be the most differentially abundant (logFC = 1.48, adj.p.val = 0.00002). The second most differentially abundant protein, with highest log fold change, was STAMBP (logFC = 1.01, adj.p.val = 0.00002). CASP-8 and SIRT2 are other top differentially abundant inflammation markers with high log fold changes found in patients. The chemokines CXCL1, CXCL5, CXCL6, IL7 and TNFSF14, playing important roles in ERK signaling pathways and neutrophil activation, were also found to be elevated. The levels of the anti-inflammatory cytokine, IL-10RA, was found to be decreased in patients. Differential expression analysis between HC and disease groups (SCZ and Non-SCZ) showed that 15 proteins are upregulated in patients diagnosed with schizophrenia compared to HC. Here, AXIN1 was identified to be the most differentially expressed with an elevated log fold change. (logFC = 1.87, adj.p.val = 4.54E-07). Ten out of 12 of the inflammation proteins (namely AXIN1, STAMBP, CASP-8, 4E-BP1, CXCL1, CXCL5, IL7, TNFSF14, CXCL6 and IL-10RA) showed a significant correlation with PANSS positive scores. The NPX values

for AXIN1 (rs = 0.36), STAMBP (rs = 0.38), IL-7 (rs = 0.50) and IL-10RA (rs = −0.7) showed a high correlation with PANSS positive score. Correlations between differentially expressed proteins and symptom ratings were also performed in the subgroup of patients diagnosed with SCZ. The NPX values for AXIN1 (rs = 0.45), STAMBP (rs = 0.4), CASP-8 (rs = 0.67), SIRT2 (rs = 0.53), CXCL5 (rs = 0.35), IL-7 (rs = 0.56), TNFSF14 (rs = 0.33), CXCL6 (rs = 0.43), CD40 (rs = 0.34) and MCP-2 (rs = 0.50) showed a high correlation with PANSS positive score. PANSS Negative was found to correlate to the NPX values for CXCL6 (rs = 0.33), CCL11 (rs = 0.36), CXCL11 (rs = 0.32), PANSS General was found to correlate to the NPX values for AXIN1 (rs = 0.35) and PANSS total scores was found to correlate to the NPX values for AXIN1 (rs = 0.34), STAMBP (rs = 0.32), CASP-8 (rs = 0.5), IL-7 (rs = 0.35), CXCL6 (rs = 0.39) and MCP-2 (rs = 0.34). Correlations were not significant after correction for multiple testing.

Conclusions: The current study's findings demonstrate that peripheral immunological markers are significantly elevated in FEP patients. Interestingly, several of the markers had previously been proposed to be implicated in psychosis. We also discovered that the markers significantly associated to illness severity and cognition. This is consistent with our discovery that immunological activation was even stronger in a subset of patients eventually diagnosed with schizophrenia.

Keywords: First Episode Psychosis, Chemokines, Cytokines, Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P553. Associations of Longitudinal Inflammatory Biomarker Changes With Cognitive, Mental, and Physical Health in Schizophrenia: Using a Mixed Models Approach to Compare 4-Year Changes in a Cohort of People With and Without Schizophrenia

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Background: Schizophrenia has been linked to a chronic inflammatory state, with previous research demonstrating elevated plasma biomarkers reflective of aberrant immune functioning. Pro-inflammatory cytokines are thought to have a direct effect on neurotransmission and are associated with severity of psychopathology. People with schizophrenia (PwS) who have elevated inflammatory biomarker levels may benefit from anti-inflammatory treatments during the early stages of the illness. Postmortem brain samples from PwS have shown increased expression of pro-inflammatory proteins, indicating persistence of some aspects of inflammation throughout the course of the illness. However longitudinal data evaluating the change in immune dynamics over time among PwS remain sparse. The current study addresses the limitations of previous cross-sectional studies by examining the rate of change of key inflammatory biomarkers from baseline to subsequent follow up, as well as their association with measures of cognitive, mental, and physical health.

We hypothesized that 1) PwS would have greater increases in pro-inflammatory biomarkers over time compared to non-psychiatric comparison subjects (NCs), and 2) these increased rates of change will predict worsening cognition over time. We conducted further exploratory analyses using various measures of physical and mental functioning.

Methods: The cohort included 123 PwS and 108 NCs (mean age 49.1, SD 10.2, range 26 to 66 years, 49.4% female), with no significant difference in age or sex between diagnostic groups. Plasma biomarker levels were quantified using Meso Scale Discovery MULTI-SPOT® Assay System and analyzed on a SECTOR Imager 2400 instrument. Plasma high sensitivity C-reactive protein (hs-CRP) levels were measured by enzyme-linked immunosorbent assay at UCSD. Interferon (IFN)-gamma, interleukin (IL)-10, IL-6, IL-8, and tumor necrosis factor (TNF)-alpha were assayed at UCLA.

Executive functioning measures included a composite score of subtests from the Delis-Kaplan Executive Function System (D-KEFS): Trail Making, Color Word Inhibition, and the Letter Fluency task. Other health measures included waist-to-hip ratio, BMI, HgbA1c (assessed using a standard clinical assay), medical comorbidity (Cumulative Illness Rating Scale), depression (Patient Health Questionnaire-9 item), anxiety (Brief Symptom Inventory-Anxiety), and positive and negative symptoms (Scales for Assessments of Positive and Negative Symptoms).

Rates of change of inflammatory biomarker levels, cognition, and other health measures were calculated as the difference between level/score at the final visit with level/score at baseline and converted to a yearly rate. Spearman's correlation was used to examine the relationship between biomarker rates of change and cognitive measures. Mixed effect linear models analyzed the association of inflammatory biomarker rates of change with diagnostic group (PwS vs. NCs) as well as with cognitive/health outcomes, controlling for age, sex, baseline inflammation, and baseline cognition.

Results: Over the mean 4.4-year follow-up period, PwS showed increases in IL-8 (mean yearly rate 0.496, SE .104) and IL-6 (mean yearly rate .0694, SE .0680) levels, whereas these inflammatory biomarkers remained unchanged in NCs. Primary analyses indicated elevated rate of change for IL-8 in PwS as compared to NCs ($t(152.7) = -3.178$, $p = 0.002$). D-KEFS scores improved over time in both diagnostic groups, likely due to practice effects. While D-KEFS score was not associated with biomarkers in PwS, there was a positive correlation between change in IFN-gamma levels and Color Word Inhibition (rs = 0.295, p = 0.005), indicating worse performance with decreasing biomarker levels, and a negative correlation between change in IL-10 levels and Letter Fluency (rs = −.242, p = 0.024), indicating better performance with decreasing biomarker levels. In NCs, there was a negative correlation between rate of change in IL-6 and that of D-KEFS score (rs = −.264, p = 0.041), with no other significant correlations. General linear models controlling for sex, age, diagnostic group, baseline inflammation, and baseline cognition indicated increases in baseline IL-8 level ($Z = 0.249$, $SE = 0.081$, $p = 0.0024$) and rate of change of IL-8 levels ($Z = 0.517$, $SE = 0.184$, $p = 0.0056$) as predictors of improved D-KEFS score over time.

Secondary analyses demonstrated a greater number of significant correlations between rates of change of inflammatory biomarkers with physical functioning among PwS compared to NCs. This relationship was especially notable with significant positive correlations between changes in waist-to-hip ratio and changes in IFN-gamma, IL-10 and 8, and TNF-alpha (rs = 0.382, .250, .266, .400 respectively) only among PwS. Conversely, NCs showed stronger correlations than PwS between rates of change of inflammatory biomarkers with mood, as illustrated by a significant positive correlation between change in depression and hs-CRP (rs = 0.244), as well as negative correlations between change in depression with IL-10 and TNF-alpha (rs = −.475 and -.311 respectively).

Conclusions: Collectively, these results indicate distinct trajectories in plasma inflammatory biomarkers in PwS when compared to NCs. Notably, increased IL-8 levels were found to be predictive of improvements in executive function over time, regardless of diagnostic group. This study is ongoing and updated results will be presented at the meeting.

Keywords: Psychosis, Cytokines, Cognition

Disclosure: Nothing to disclose.

P554. Tumor Necrosis Factor is Associated With Altered Activation in Ventral Striatal and Anterior Insula in Response to Reward and Effort in Patients With Schizophrenia

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Background: Increasing data implicates inflammation as a driver of negative symptoms in patients with schizophrenia. Relative to the mechanism by which inflammation effects negative symptoms, inflammation has been shown to decrease VS activation in response to reward in healthy controls and depressed patients. Negative symptoms, specifically motivational deficits, have been associated with decreased activation of the ventral striatum in response to reward anticipation. Previous data demonstrated that administration of inflammatory stimuli is reliably associated with decreased activation in the ventral striatum in the context of reward anticipation, whereas inflammation increases activation in the anterior insula in association with punishment prediction errors. Thus, we hypothesized that inflammation would be associated with motivational deficits as well as altered signaling in reward-relevant regions.

Methods: 37 patients with schizophrenia, on medications, were recruited from Grady Hospital in Atlanta, Georgia. Patients were excluded if they had evidence of unstable medical conditions, inflammatory illness, use of anti-inflammatory medications, or active substance use. Negative symptoms were measured using the Brief Negative Symptom Scale (BNSS), with subscores for a motivated behavior factor as well as an expressivity factor. Functional connectivity analyses ($n = 30$) were used with an a priori seed placed in the nucleus accumbens. A subset of subjects ($n = 22$) performed the Monetary Incentive Delay Task (MID) and the Effort Based Decision Making Task (EBDM) in a 3 T fMRI scanner. A predefined nucleus accumbens mask was used given the a priori hypothesis regarding the ventral striatum in response to reward anticipation. A whole brain analysis was used for the EBDM task to look at the effect of increasing effort (using a parametric modulator). Linear regression models were tested to determine the relationship between inflammation and brain activation, controlling for age and sex.

Results: Increases in CRP, a non-specific marker of inflammation, were significantly correlated with decreases in the BNSS motivated behavior factor ($r = -0.340$, $p = 0.042$) and specifically the avolition subscale ($r = -0.438$, $p = 0.029$). No association was found for the BNSS expressivity factor ($p > 0.8$). These relationships remained significant after controlling for depression. Using resting state functional connectivity analyses, increasing CRP was associated with greater connectivity between a seed in the right nucleus accumbens and activation in a cluster that included the right insula (peak [42, -14, 2] $T = 3.41$, p(uncorrected)<0.001). Regarding more specific inflammatory mediators, tumor necrosis factor (TNF) at higher concentrations were associated with lower activation in the nucleus accumbens in response to reward anticipation in the MID task (Win > Neutral contrast; $= \beta = 0.462$, $p = 0.039$) [both the left (peak left [-14, 22, -8], $T = 3.48$, p (uncorrected) $= 0.001$) and right (peak right [4, 22, -6], $T = 2.73$, p (uncorrected) $= 0.003$)]. Higher concentrations of TNF were also associated with increased activation in the right anterior insula ($\boldsymbol{\beta} = 0.690$, $p < 0.001$) in response to increasing effort on the EBDM task (peak [38, 20, 6], $T = 4.35$, p (uncorrected) < 0.001).Of note, activation in the nucleus accumbens in respons to reward was significantly correlated with activation in the anterior insula in response to effort ($r = -0.512$, $p = 0.015$).

Conclusions: These data support a relationship between inflammation and negative symptoms, and specifically motivational deficits. Increased TNF may lead to negative symptoms through reduced ventral striatum activation in response to reward and increased anterior insula activation in response to increasing effort. The ventral striatum is involved in the regulation of reward and previous data has demonstrated that inflammation may target brain reward circuitry, including the striatum. The anterior insula is involved in interoceptive processing and punishment prediction errors. Previous data has shown that the anterior insula may be sensitive to inflammation in response to punishment prediction errors. The anterior insula may control motivational vigor and influence downstream dopaminergic signaling in the nucleus accumbens, suggesting a coordinated circuit between the insula and basal ganglia that may be sensitive to inflammation. These findings support the hypothesis that inflammation may target the ventral striatum and anterior insula to lead to reward and effort processing deficits that may underlie negative symptom severity.

Keywords: Schizophrenia (SCZ), Inflammation, TNF-Alpha, Effort Based Decision Making Task, Monetary Incentive Delay Task

Disclosure: Nothing to disclose.

P555. Peripheral Inflammation is Associated With Impairments of Inhibitory Behavioral Control and Visual Sensorimotor Function in Psychotic Disorders

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Background: Elevated markers of peripheral inflammation are common in psychosis spectrum disorders and have been associated with brain anatomy, and physiology as well as clinical outcomes. Preliminary evidence suggests a link between inflammatory cytokines and C-reactive protein (CRP) with generalized cognitive impairments in a subgroup of individuals with psychosis. Whether these patients with elevated peripheral inflammation demonstrate deficits in specific cognitive domains remains unclear.

Methods: Seventeen neuropsychological and sensorimotor tasks and thirteen peripheral inflammatory and microvascular markers were quantified in a subset of participants (129 psychosis, 55 healthy controls) recruited from the first wave of the Bipolar-Schizophrenia Network on Intermediate Phenotypes consortium (B-SNIP1). Principal component analysis was conducted across the inflammatory markers, resulting in five inflammation factors. Three discrete latent cognitive domains (Visual Sensorimotor, General Cognitive Ability, and Inhibitory Behavioral Control) were characterized based on the neurobehavioral battery and examined in association with inflammation factors. Hierarchical clustering analysis identified cognition-sensitive high/low inflammation subgroups.

Results: Among persons with psychotic disorders but not healthy controls, higher scores on an inflammation factor representing elevations of CRP, IL6, and C4a and reduction of IFNγ, IL8, IL10, and VEGFD had significant associations with impairments of Inhibitory Control ($R2 = 0.100$, p-value=2.69e-4, q-value=0.004) and suggestive associations with Visual Sensorimotor function ($R2 = 0.039$, p-value=0.024, q-value=0.180), but not with General Cognitive Ability ($R2 = 0.015$, p-value=0.162). Greater deficits in Inhibitory Control were observed in the high inflammation patient subgroup, which represented 30.2% of persons with psychotic disorders, as compared to the low inflammation psychosis subgroup. These relationships were not

significantly impacted by the use of psychotropic medications, cardiometabolic diagnoses, cardiometabolic medications, or non-steroidal anti-inflammatory drugs (NSAIDs).

Conclusions: These findings indicate that inflammation dysregulation may differentially impact specific neurobehavioral domains across psychotic disorders, particularly performance on tasks requiring ongoing behavioral monitoring and control. The present findings provide further supporting evidence that one important underlying mechanistic contributor to the disruption of cognition in persons with psychotic disorders is immune/inflammation dysregulation and that targeting this dysregulation may be an avenue for novel therapeutic interventions to improve cognitive outcomes in these patients.

Keywords: Cognition, Psychosis, Inflammation

Disclosure: OptumRx: Consultant (Self).

P556. Levels of Matrix Metalloproteinase 9 (MMP-9) are Elevated in Persons With Serious Mental Illness

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Background: Matrix metalloproteinases constitute a diverse set of calcium-dependent endopeptidases with a wide range of biological functions. While there are many human metalloproteinases, MMP-9, also known as gelatinase B, has been of particular interest since it modulates several arms of the immune system, alters blood-brain barrier functioning, and can affect synaptic functioning. Increased levels of MMP-9 have also been associated with increased rates of mortality caused by impaired cardiopulmonary functioning. Due to these CNS and systemic properties, there has been interest in defining the relationship between MMP-9 and serious mental illnesses and in developing methods for lowering MMP-9 levels.

Individual levels of MM9 are determined by both genetic and environmental factors. Environmental factors associated with elevated MMP-9 levels include tobacco smoking and obesity, which are common in individuals with serious mental illness and are potentially modifiable. We measured circulating MMP-9 levels in a cohort of individuals with serious mental illness and without a psychiatric disorder and determined the effects of diagnosis, tobacco smoking, obesity, and other factors on MMP-9 levels.

Methods: Participants were enrolled in the period Jan 1, 2008 - Sept 1, 2021 and assessed on demographic and clinical characteristics; diagnoses were verified by SCID interview. Participants included three sub-cohorts consisting of individuals with schizophrenia, bipolar disorder, or depressive disorder enrolled from Sheppard Pratt clinical programs. A group of comparison persons without any history of psychiatric disorder was recruited from posted announcements. Levels of MMP-9 were measured in blood samples by means of sensitive solid phase immunoassays.

Statistical methods: We performed separate analyses for the three psychiatric sub-cohorts, comparing them each with the common comparison group. In each analysis, the psychiatric vs. non-psychiatric condition was used as the dependent variable in logistic regression. In preliminary analyses we determined that log-transformed MMP-9 was appropriate as an independent variable for the analyses, consistent with its linear relationship with the logit of the dependent variables. In addition, age as a continuous variable, male vs female and White vs other race as dichotomous variables and the variables of tobacco smoking, and obesity (BMI ≥ 30 kg/m2) and the interaction between these two terms were included as covariates. We estimated the contributions of various independent variables to the variance of each psychiatric diagnosis vs the non-psychiatric comparison group as a continuous variable as the squared partial correlation. We employed a linear regression model with log (MMP-9) and demographic covariates as well as an additional model adding tobacco smoking, obesity and the interaction between these two. We noted the change in the partial r2 of log-MMP-9 in these sequential models. Additional models examined the effects of psychiatric medications in individuals with psychiatric diagnoses.

Results: A total of 1256 individuals were enrolled in the study: 440 with schizophrenia, 399 with bipolar disorder, 135 with major depressive disorder, and 282 without any current or past psychiatric disorder. The mean age of persons in the larger cohort was 35.3 (±13.0); a total of 667 (53%) were female, 700 (56%) White; 470 (37%) were current tobacco smokers and 490 (39%) were obese.

Logistic models which included current smoking, obesity and their interaction along with demographic variables indicated significant associations between MMP-9 levels (per 1 natural log-unit higher levels) and the diagnosis of schizophrenia (OR 4.71, 95% CI 2.41, 9.21, $p < 0.001$), bipolar disorder (OR 2.99, 95% CI 1.79, 5.01, $p < 0.001$) and major depressive disorder (OR 9.63, 95% CI 4.59, 20.19, $p < 0.001$).

We also examined the fractional effects of smoking, obesity and their interaction on reducing the partial correlation of MMP-9 levels for each psychiatric group. In the case of schizophrenia, 59.6% of the partial r2 attributable to MMP-9 with age, sex and race adjustment alone was lost when smoking, obesity and their interaction were added to the model, while the remaining 40.4% or the original partial r2 remained attributable to log(MMP-9) and thus not be explained by these factors. In the case of bipolar disorder, 39.9% of the age-sex-race adjusted explanatory fraction of MMP-9 was lost when explained by smoking and obesity and their interaction, while in the case of major depressive disorder only 19.3% of the original age-sex-race adjusted explanatory fraction of MMP-9 elevation could be explained by these factors.

Among persons in the psychiatric groups, levels of MMP-9 were not significantly associated with the receipt of specific anti-psychotic, anti-depressant, or mood-stabilizing medications suggesting that the differences in MMP-9 levels among the diagnostic groups are not explained by medications.

Conclusions: Levels of MMP-9 are significantly elevated in persons with schizophrenia, bipolar disorder, and major depressive disorder. Some of the increases are related to tobacco smoking and obesity, particularly in individuals with schizophrenia where these factors are highly prevalent. In light of the potential adverse effects of MMP-9, efforts should be directed at lowering MMP-9 levels by means of smoking cessation and weight loss interventions as well as specific pharmacological interventions.

Keywords: Immune Biomarkers, Schizophrenia (SCZ), Matrix Metalloproteinase-9 (MMP-9)

Disclosure: Nothing to disclose.

P557. A Disease-Informed, Network-Targeted Neuromodulation Intervention Affects Craving in Individuals With Schizophrenia and Nicotine-Dependence

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Background: Tobacco use is the top cause of early preventable mortality in schizophrenia, leading to a 25-year decreased life expectancy. The prevalence of nicotine use in schizophrenia is more than three times that of the general population. There is a significant gap in our understanding of nicotine dependence in

schizophrenia and how this diagnosis affects treatment. Treatments for nicotine dependence are significantly less effective for people with schizophrenia compared to controls. This may be because current interventions for nicotine dependence in this population do not target schizophrenia-specific pathophysiology. We therefore sought to identify and test a neuromodulation intervention on a schizophrenia-specific circuit of nicotine dependence.

Methods: This study consisted of 3 phases: Network Discovery, Network Validation, and Network-Targeted Intervention. In Network Discovery, we used a data-driven approach to identify a schizophrenia-specific circuit of nicotine dependence. We reanalyzed existing data from 35 smokers (18 schizophrenia, 17 controls) who underwent resting-state fMRI and clinical characterization. We conducted a transdiagnostic assessment to identify shared and diagnosis-specific circuits of nicotine use. A multivariate pattern analysis of whole-connectome data was used to identify the strongest links between daily cigarette consumption and functional connectivity. In Network Validation, 12 participants with schizophrenia and 12 controls participated in a randomized, controlled crossover study of nicotine patch with resting-state fMRI. Based on the results from Network Discovery, we calculated mean change in default mode network (DMN) connectivity (FCnicotine – FCplacebo) and correlated change in whole-network functional connectivity with nicotine dose. We then determined the effect of acute nicotine administration on this network. In Network-Targeted Intervention, 10 individuals with schizophrenia and nicotine dependence were enrolled in a randomized, sham-controlled crossover study of single session DMN-targeted repetitive transcranial magnetic stimulation (rTMS) with fMRI immediately before and after each rTMS session. Individuals underwent baseline resting state fMRI where the DMN was mapped. The point of maximal connectivity of the left parietal DMN node was selected as the TMS target for all 3 sessions. Individuals received 1 session of intermittent theta-burst stimulation (iTBS, 100% active motor threshold, AMT), 1 session of continuous theta-burst stimulation (cTBS, 80% AMT), and 1 session of sham rTMS (100% AMT, coil flipped 180 degrees away from participant). Craving was assessed before and after each rTMS session using a Visual Analog Scale. Each rTMS session was separated by at least 48 hours in order to minimize any carryover effect of the previous stimulation. Using a within-subjects design, we compared changes in craving between each type of rTMS using a mixed ANOVA.

Results: In Network Discovery, the strongest ($p < 0.001$) correlate between functional connectivity and daily cigarette consumption was driven by individual variation in the topography of the DMN. This effect was entirely driven by participants with schizophrenia despite the fact that groups were matched for severity of nicotine dependence. Specifically, with greater severity of nicotine use in schizophrenia, the DMN was pathologically expanded into territory normally occupied by the dorsal attention network. This relationship between network organization and nicotine use was specific to schizophrenia and not observed in control smokers. In Network Validation, we tested the causality of nicotine-network interactions in an independent cohort. We we observed that acute nicotine administration reverses DMN hyperconnectivity in schizophrenia in a dose-dependent relationship ($R = -0.50$; 95% CI -0.75 to -0.12, $p < 0.05$). In Network-Targeted Intervention, we observed a significant treatment x time relationship ($p = 0.017$) where craving was significantly increased by iTBS but not by cTBS or sham.

Conclusions: We identified a schizophrenia-specific network of nicotine dependence, validated the effect of acute nicotine administration on that network, and then applied a neuromodulation intervention on that network. This disease-informed, network-targeted intervention significantly affected nicotine craving in schizophrenia. This is the first evidence for a circuit-based intervention for substance use in schizophrenia that was empirically derived, unique to schizophrenia, and affected clinical outcomes. Future experiments should test repeated sessions of rTMS in clinical trials.

Keywords: Schizophrenia (SCZ), Nicotine Dependence, Resting State Networks, Default Mode Network (DMN), Repetitive Transcranial Magnetic Stimulation (rTMS)

Disclosure: Nothing to disclose.

P558. Taking AIMS: Characterization of Abnormal Involuntary Movement Scale Utilization in the VA Healthcare System

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Background: Tardive dyskinesia (TD) is a medication-induced movement disorder that is classically precipitated by long-term exposure to dopamine receptor blocking agents. TD is often irreversible, is debilitating, and has been associated with a poorer quality of life and increased mortality. TD surveillance is typically carried out via routine documentation through the Abnormal Involuntary Movement Scale (AIMS), especially in those prescribed medications with elevated TD risk, including antipsychotics.

Methods: We reviewed electronic health records (EHRs) for 700,000 participants in Cooperative Studies Program (CSP) #572 and the Million Veteran Program, including ICD-9/10 billing codes, prescription records, and AIMS, and spanning more than 20 years. We used "phecodes", which are groupings of conceptually related ICD codes, to assign putative diagnoses of schizophrenia (295.1), bipolar disorder (296.1), psychosis NOS (295.3), depression, and extrapyramidal symptoms (EPS; 333*). We intersected these data to characterize the clinical correlates of antipsychotics-induced EPS assessed using the AIMS and corroborated by a diagnosis of TD.

Results: The AIMS was administered 126,766 times to 38,364 individuals (median = 12, mean=27.3, SD = 43). AIMS scores were documented in X% of all individuals who were taking antipsychotic medications. Of those with any AIMS data, $n = 18,591$ had AIMS score >0; most had no incapacitation ($n = 13,100$;70%), followed by minimal ($n = 3,3403$;18%), mild ($n = 1,390$;7%), moderate ($n = 533$;3%), and severe ($n = 129$,1%). Symptoms most commonly affected the jaw, and least commonly affected the trunk. Schizophrenia ($n = 9,033$;48.6%), bipolar disorder ($n = 4,775$;25.7%), psychosis NOS ($n = 1,123$;6%) and depression-related diagnoses ($n = 3,221$;17.3%) were the most common diagnoses in those with AIMS > 0. AIMS scores were greater for those with a recorded diagnosis of EPS or abnormal movements compared to those without (5.4,SD = 4.8 vs 3.2, SD = 3.2; t = 30.6, df=7120.2, $p < 10$^-193). Consistent with expectation, patients who received treatment with a first-generation antipsychotic medication (44.8%) had higher AIMS than those treated with second-generation agents alone (55.2%; unpaired t-test, t = 20.8, df=14585, $p <$ 10-93).

Conclusions: We have undertaken a preliminary survey of extrapyramidal side-effects of antipsychotic medications in the VHA, and describe the clinical correlates of EPS and TD with respect to medication classes, specific agents, lifetime psychiatric diagnoses. Available AIMS data revealed appreciable individual differences in risk and severity of EPS/TD among patients with comparable treatment histories, and support an AIMS score of 2 or more as a relevant clinical cutoff for TD. Ongoing analyses include more granular investigations of particular medications based on receptor affinity and with consideration to dosage, and genome-

wide association studies (GWAS) to identify novel TD/EPS susceptibility (or resilience) loci.

Keywords: Tardive Dyskinesia, Electronic Health Record (EHR), Extrapyramidal Symptoms, Abnormal Involuntary Movement Scale (AIMS)

Disclosure: Nothing to disclose.

P559. Where is the Reliable Signal in the Total and New Short Forms of the PANSS in an Outpatient RCT

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Background: The Positive and Negative Symptom Scale (PANSS) is a venerable interview for rating severity of psychotic symptoms, and it is the most common outcome measure in clinical trials with youths and adults. It has 30 items that are usually summed or averaged to produce a total severity score. However, factor analyses consistently indicate that the PANSS items do not reflect a single construct. Instead, five factor solutions appear most favored in prior work, usually with low correlations between them. These findings call into question the use of a total score as a main outcome, as well as invalidating Cronbach's alpha as a measure of reliability. If the group factors are sufficiently reliable and distinct from a general factor, that would support using them as distinct targets for intervention development or outcome. The goal of the present investigation is to use omega reliability estimates, which model the number of factors underlying the items to produce estimates of total variance in the scores related to all factors (omega total), versus related only to the general factor (total psychosis), or distinct to each factor subscale (supporting interpretation of the subscale).

We use a well-characterized pediatric samples, and we calculate the omega values for the 30 item, 20 and 10 item versions we developed, and also the 19 item and two 6 item short forms proposed by others.

Methods: Secondary analyses of the TEOSS study (Sikich et al., $N = 180$). Analyses were performed using the mirt, lavaan, BifactorIndicesCalculator, and psych packages in R. Omega estimates specified 5 factors (2 factors for the 6 item forms), and calculated alpha and omegaTotal, omegaGeneral, and omegaGroup. Alpha estimates reliability for a single underlying factor; omegaTotal combines reliability for the general (psychosis total) and 5 group factors; omegaGeneral estimates the variance attributable to the general factor alone (with values > 0.80 indicating a strong general factor), and OmegaHS is the variance in subscales attributable to the specific factors (with values > 0.40 indicating reliable specific scores, likely to support independent interpretation and replication)(Rodriguez, Reise, and Haviland, 2015; Revelle and Condon, 2019).

Results: Every analysis in both samples indicated that a multifactor model fit better than a unidimensional one across all item sets. OmegaTotal was always substantially larger than alpha. The OmegaGeneral was .45 (poor) for the 30 item TEOSS PANSS, and .17 to .39 for the short forms. In contrast, all of the subscales on the 10 item TEOSS form has OmegaHS ranging from .45 to .76, supporting their individual interpretation. The general factor only explained 16% to 26% of the variance in items, whereas subscales based on the factors were more reliable and interpretable, correlating with their respective factor scores .71 to .97 for the 10-item version.

Conclusions: Results strongly indicate that the PANSS is better considered a "composite" containing different factors, rather than treating it as a single score (Revelle and Condon, 2019). Existing datasets could be reanalyzed focusing on the subscale scores. New studies and clinical applications could use shortened versions, perhaps omitting scales (e.g., internalizing) that may be redundant with other parts of a battery (e.g., CDRS-R or HDRS).

Keywords: Clinical Assessment, Psychosis, Pediatric

Disclosures: Signant: Consultant (Self), Guilford Press, American Psychological Association: Royalties (Self).

P560. Content Validation of a Functional Co-Primary Measure for Clinical Trials Targeting Cognitive Impairment Associated With Schizophrenia (CIAS): An FDA-Funded Study

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Background: The FDA requires clinical trials for new drugs targeting CIAS to demonstrate the functional relevance of any cognitive improvements with a co-primary measure. The Virtual Reality Functional Capacity Assessment Tool (VRFCAT) is a performance-based measure that uses a realistic simulated environment to assess skills required for independent activities of daily living, including planning a meal, using transportation, using currency, and shopping. The FDA has accepted the VRFCAT into its Clinical Outcome Assessment (COA) Qualification Program for use as a co-primary measure in CIAS trials. For a full Qualification Package submission, the FDA COA Program requires data supporting content validity, including qualitative evidence that key stakeholders view the measure as relevant and important in the target population. We present findings from an FDA-funded study designed to obtain qualitative data on the VRFCAT.

Methods: First, we convened a panel of academic, industry, and regulatory experts to develop a semi-structured interview to elicit key stakeholders' views about (a) the definition of independence and associated behaviors, and (b) the functional relevance of the four domains assessed by the VRFCAT (meal planning, using transportation, using currency, shopping). Second, three stakeholder groups were interviewed: outpatients with schizophrenia ($n = 24$), family members ($n = 12$), and peer support specialists ($n = 12$). These interviews contained questions about general perspectives on functional independence and related activities. Participants reviewed representative audiovisual VRFCAT clips depicting the four domains assessed and responded to questions regarding each task's relevance for functional independence. Third, a codebook was developed for thematic analyses of transcripts from recorded interviews.

Results: Interviews were acquired from each of the targeted stakeholder subsamples (total $N = 48$). Qualitative analyses revealed that key stakeholders view the VRFCAT as assessing four functional capacity domains that are important for everyday functioning and independence. There was, however, some divergence among stakeholders in their perceptions of how the domains relate to independence. Caregivers saw these domains as important for minimizing dependence on the caregiver, whereas peer clinicians saw these functional domains as essential for pursuing higher-order goals (e.g., work) and patients emphasized practical aspects of functional skills, such as the avoidance of theft or loss. Regarding the specific skills assessed by the VRFCAT, high proportions of participants in each group rated the VRFCAT tasks as highly functionally relevant. Content analyses revealed similar themes across groups regarding why the four VRFCAT skill areas are important for functional independence.

Conclusions: This qualitative study provides confirmatory support that key stakeholders view the VRFCAT as assessing four domains that are important for independent functioning. Further, the specific tasks used in the VRFCAT to assess these domains

were uniformly viewed as functionally meaningful and important. The results also demonstrate that incorporating views from multiple stakeholder groups can provide complementary confirmatory evidence when assessing the content validity of a functional capacity measure in an indication like schizophrenia. This qualitative content validation evidence addresses a key requirement for measures evaluated in the FDA COA Qualification Program. Overall, the current qualitative results, along with quantitative evidence, provide convergent support that the VRFCAT is a functionally relevant, valid, and reliable co-primary to determine whether new drugs have a meaningful impact on cognition and on the lives of people with schizophrenia.

Keywords: Cognitive Impairment Associated With Schizophrenia, Functional Capacity, Clinical Trial Methodology

Disclosure: WCG VeraSci: Employee (Self).

P561. Generalized Slowing of Resting State Neural Oscillations in People With Schizophrenia

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Background: Neural oscillations are thought to reflect the flow of information through the brain. Recently, there has been interest in partitioning electroencephalography (EEG) recordings into periodic and aperiodic components. While both periodic and aperiodic components contribute to conventional measures of power within EEG bands (e.g., delta, theta, alpha, beta), the periodic aspect of EEG is thought to reflect true oscillatory behavior within neural systems while aperiodic activity captures sporadic brain activity. Given past evidence of resting state EEG power abnormalities in schizophrenia, we sought to determine if the periodic aspect of neural activity was aberrant in people with schizophrenia (PSZ) after removal of aperiodic activity.

Methods: EEGs were gathered during a resting state from 104 PSZ and 105 healthy control participants (HCs). We used the fitting-oscillations-and-one-over-f (FOOOF) toolbox was to remove aperiodic neural activity, and computed the cross-correlation between power spectra for individual participants and the mean power spectrum for HCs to quantify the relative speed of neural oscillations.

Results: Periodic activity in PSZ was shifted toward lower frequencies compared to HCs during eyes closed rest ($t(187.86) = -3.67$, $p < 0.001$). PSZ on average had a .50 hz shift toward oscillatory slowing compared to HCs across the frequency spectrum. Greater lag was associated with more symptoms of psychopathology and worse cognitive functioning.

Conclusions: Slowed periodic activity at rest is evident in schizophrenia. A slower pace of neural oscillations may limit the transmission of information within and across brain systems. Slowed neural oscillations may contribute to poor integration of low-level perceptual and high-level cognitive functions in people with the illness.

Keywords: Schizophrenia (SCZ), EEG/ERP Electrophysiology, Oscillations

Disclosure: Nothing to disclose.

P562. Striatal Dopamine Synthesis Capacity and Genetic Liability for Treatment-Resistant Schizophrenia in Healthy Adults

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Background: Pharmacotherapy with first-line dopamine D2¬ receptor antagonists remains the primary biological treatment modality in schizophrenia, yet treatment response varies considerably across patients. Among individuals presenting with first episode schizophrenia, a subgroup representing nearly 25% will prove to be considered treatment resistant, often requiring multiple antipsychotic trials for lack of efficacy and, ultimately, treatment with clozapine, a unique agent that is helpful in this subgroup. Accumulating evidence from molecular neuroimaging studies has suggested that the abnormally increased striatal dopamine synthesis capacity observed in some patients with schizophrenia is often not observed in those with treatment-resistant schizophrenia, who, in fact, tend to show the opposite pattern (i.e., abnormally reduced striatal dopamine synthesis). This has raised the possibility that treatment-resistant schizophrenia may not simply denote one extreme of a unitary dimension of illness severity in schizophrenia, but rather is characterized by distinct underlying neurochemical mechanisms that merit dedicated investigation. The neurobiological causes of treatment resistance remain unknown; however, recent work has identified an array of common genetic variants that appear to be associated with treatment resistance in schizophrenia, though the impact of these variants on the living human brain, even in health, remains unclear, and biological validation studies are still needed.

Methods: One-hundred and eighty-seven healthy individuals without psychiatric illness provided blood for genotyping and were studied with [18 F]-FDOPA positron emission tomography (PET; mean age 36 + /-11, 98 women) at the NIH Clinical Center in Bethesda, Maryland. For each individual, genotyping was conducted across the genome using Illumina SNP chips, and polygenic scores representing cumulative genetic risk burden were generated for schizophrenia generally and also for treatment-resistant schizophrenia specifically, based on genome-wide association study literature summary statistics. For neuroimaging, a 6-hour fast and 4-hour abstinence for caffeine and nicotine were required, and an oral dose of carbidopa was given one hour before injection of the tracer. Each individual's separately-collected T1-weighted anatomical MRI scan was segmented for a cerebellar reference region and was co-registered to each participant's native space, attenuation-corrected, and realigned PET data. After extraction of the reference region's time activity curve, PET data were normalized to MNI-space using ANTS software. Gaussian smoothing was implemented to improve signal-to-noise ratios. PMOD software was used to implement standard graphical linear modeling to estimate the specific uptake parameter, Ki. Mean specific uptake was calculated for each of three bilateral subdivisions of the striatum (associative, sensorimotor, ventral), which were then interrogated for association with polygenic scores for schizophrenia generally and, separately, with polygenic scores for treatment-resistant schizophrenia. These analyses were performed using R software and employed general linear models that controlled for age, sex, and, to account for subtle population stratification effects, genetic principal components.

Results: A significant positive association between tracer specific uptake and general schizophrenia polygenic risk score was found in the sensorimotor striatum ($p = 0.04$) though not in other striatal subdivisions. In contrast, a significant negative association between tracer specific uptake and treatment resistance polygenic risk score was found in all regions studied: associative striatum ($p = 0.04$), sensorimotor striatum ($p = 0.04$), and ventral striatum ($p = 0.02$).

Conclusions: Presynaptic dopamine synthesis capacity in the striatum shows normative variability that is linked to mental

health-related genetic risk burden, even in healthy individuals. Cumulative genetic risk for schizophrenia generally may predispose to elevated dopaminergic tone, whereas cumulative genetic risk for treatment-resistant schizophrenia may negatively bias dopaminergic tone. These divergent contributions to dopaminergic system variability follow expected directional effects based on observations from prior [18 F]-FDOPA PET imaging experiments in clinical cohorts. In conjunction with those prior experiments, this study provides novel insights into the unique biology of treatment-resistant schizophrenia genetic risk and has relevance for modern formulations of the dopamine hypotheses of schizophrenia. Future studies in patient populations will be important to understand the clinical implications of these associations, and further investigation of the distinct molecular mechanisms underlying risk for treatment resistance in schizophrenia is needed.

Keywords: Schizophrenia, Dopamine, Genetics, Treatment Resistance

Disclosure: Nothing to disclose.

P563. Clozapine Therapeutic Drug Monitoring: Investigating Tolerability and Redefining Thresholds for Safety

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Background: One third of schizophrenia patients do not respond to standard antipsychotic medication and are classed as treatment-resistant schizophrenia (TRS). Clozapine is the only evidence-based treatment for TRS, yet clozapine is underutilized, in part, because clinicians fear serious side effects. Laboratory alerts caution that serum total clozapine and norclozapine levels above 1200 ng/mL put patients at risk of adverse effects. In response to high levels, clinicians often decrease dose or discontinue treatment altogether overvaluing the risks compared to the treatment benefits for patients. The Glasgow Antipsychotic Side Effects Scale for Clozapine (GASS-C) is a psychometric instrument used by clinicians to measure clozapine-related side effects. The current study aimed to evaluate within-subject and between-subject changes in clozapine levels and GASS-C severity.

Methods: The GASS was administered to TRS outpatients prescribed clozapine, on a stable dose for a minimum of three months. All patients were followed at monthly intervals over three visits ($n = 152$). We applied 1200 ng/mL as the upper limit for clozapine values. Our sample included patients with clozapine levels crossing the threshold over the three follow up visits ($n = 20$) (mean age, 41.0 ± 10.9). We completed a secondary sensitivity analysis between patients with consistently below threshold values ($n = 106$) (mean age, 42.0 ± 12.6) versus patients with consistently above threshold values ($n = 26$) (mean age, 51.0 ± 16.2). We completed baseline descriptive analyses and mixed logistic regression models to assess changes in clozapine levels and side effect severity.

Results: Amongst all the patients whose levels crossed the threshold over the three visits, no changes in side effects were reported in 66.7%, improvement of side effects was reported in 22.2%, and a worsening of side effects was reported in 11.1%. Results indicate no significant association ($p = 0.8463$) between changes in clozapine levels and side effects. At each visit when a patient's level was below 1200 ng/mL, 50% reported no side effects and 50% reported moderate side effects. When a patient's level was above 1200 ng/mL, 42.9% reported no side effects and 57.1% reported moderate side effects. The sensitivity analysis mixed logistic regression model found higher clozapine levels were associated with increased odds of side effect severity (Odds ratio [OR] = 1.72, 95% Confidence Interval[CI] = 0.48-6.22, $p = 0.408$) within subjects. Higher clozapine levels were associated with increased odds of side effects severity between high and low subjects (Adjusted OR = 1.48, 95%CI = 0.253-8.686, $p = 0.662$).

Conclusions: In patients whose total serum clozapine levels cross the safety threshold of 1200 ng/mL, no significant differences in side effects are reported. Though reports of increased side effects were associated with higher levels, the results were not significant. Future studies are needed to confirm these results in larger cohorts to inform guidelines for clozapine safety thresholds and optimize clinicians' use of clozapine to improve the outcomes for patients with TRS clozapine.

Keywords: Clozapine, Treatment-Resistant Schizophrenia, Therapeutic Drug Monitoring

Disclosure: Nothing to disclose.

P564. Glutamatergic Target Engagement Biomarker in Schizophrenia

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Background: N-methyl-D-aspartate-type glutamate receptors (NMDAR) antagonists reproduce both the symptoms and the cognitive deficits of schizophrenia (Sz), suggesting that NMDAR modulators may be beneficial. These psychotomimetic effects in turn are mediated, at least in part, by stimulation of presynaptic glutamate release in frontal brain regions. In animal models, both the behavioral and neurochemical effects of ketamine administration and especially its effect on glutamate release in medial prefrontal glutamate/dorsal anterior cingulate cortex (mPFC/dACC) are blocked by agonists of metabotropic (mGluR2/3) glutamate receptors, which are localized to presynaptic glutamate terminals.

However, several glutamate-targeted medications predicated on these theories, such as the mGluR2/3 agonist POMA and the NMDAR modulator bitopertin, have failed in pivotal clinical trials despite robust effectiveness in preclinical models. A major barrier to effective glutamatergic treatment development is the absence of validated measures for target engagement that can identify effective compounds and guide dose selection. We present two ongoing target engagement studies.

Methods: In Study 1, using the R61 mechanism, we conducted a study to assess target engagement and the optimal dose (80 vs.100 vs. 120 mg/kg) of the NMDAR modulator D-serine combined with 3 sessions of a neuroplasticity-based auditory remediation program in Sz. Milestones were designed to confirm target engagement, and were assessed with three preplanned outcomes: plasticity, mismatch negativity (MMN) and theta oscillations, requiring at least a moderate effect size difference ($d = 0.5$) between D-serine and placebo groups.

In Study 2, we investigate the dose-response of ketamine in healthy volunteers, using ketamine-induced pharmacoBOLD (phBOLD) as a prelude to potential future target engagement studies with mGluR2/3 agonists in Sz. In our previous work, we chose a single dose of ketamine that produced a highly robust (0.23 mg/kg, $d = 5.4$) which have been near the peak of the dynamic range for BOLD activity. In Study 2, we assessed lower ketamine doses, predicting that doses that produce a more moderate phBOLD response may be optimally sensitive to the effects of glutamatergic agents.

Results: In Study 1, 45 Sz subjects were randomized, meeting our preplanned "n." The target engagement milestone required a moderate effect size difference ($d \geq 0.5$) D-serine vs. placebo

treatment. Across all 3 visits, there was a statistically significant treatment effect for plasticity improvement ($p = 0.014$). Significant plasticity improvement was seen in the ≤100 mg/kg dose-cohorts, starting with the 1st visit (10-13.9%, all $p < 0.001$, all $d > 0.67$). By contrast, placebo-treated participants showed non-significant changes across all visits (4-5%, n.s.). Target engagement was demonstrated by a larger MMN pitch ($p = 0.049$, $d = 1.0$) and larger []-ITC ($d = 0.5$) for the 100 mg/kg dose-cohort without safety concerns. A significant correlation was seen between plasticity improvement and auditory cognition ($r = 0.46$, $p = 0.036$), but not with other MCCB domains, suggesting specificity.

In Study 2, we present a preliminary analysis of the phBOLD response for the first 30 healthy volunteer subjects in the 0.04 mg/kg to 0.08 mg/kg bolus cohorts. As expected, ketamine produced a phBOLD response of ~1% signal change peaking at ~3 minutes. The preliminary effect size for scan 1 ($d = 1.1$), shows the expected drop off from our previous study using a 0.23 mg/kg bolus ($d = 5.4$). Similarly, the preliminary analysis of the available behavioral results shows the expected dose response. The BPRS increase in the present study remains significant ($p = 0.02$, $d = 0.61$).

Conclusions: Study 1 findings strongly support engagement of the NMDAR system by D-serine, as measured by behavioral plasticity and MMN. Sustained effects will be assessed in an ongoing R33. In Study 2, preliminary results support a ketamine dose response for pharmacoBOLD.

Keywords: Target Engagement, Auditory Mismatch Negativity, Glutamate Receptors, Schizophrenia, Clinical Trial

Disclosures: Guidepoint, Otsuka, Wedbush, Jefferies: Consultant (Self), Medincell, Karuna, Leal, Merck: Advisory Board (Self), Roche, Cerevance, Neurocrine, Click, Boehringer Ingelheim: Contracted Research (Self), Taisho: Other Financial or Material Support (Self).

P565. Dopamine D2 Receptor Blockade and the Risk of Death Due to Choking

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Background: Several studies have reported that patients with schizophrenia have an increased risk of death due to choking, and up to 3% of deaths in this patient population may be attributable to this cause of death. However, the role of antipsychotic medication has remained unknown.

Methods: We studied risk of death due to choking (adjusted Hazard Ratio, aHR) associated with schizophrenia, and with antipsychotics with differential degree of dopamine 2 receptor blockade during years 1998–2017 in a nationwide cohort of patient diagnosed with schizophrenia in Finland ($n = 59{,}916$, mean age 46.2 years, SD 15.8 years). Medication exposure periods were obtained by using data from national register of filled prescriptions. We compared with Cox model the risk of death due to choking during the use of weak D2-blocker antipsychotic (clozapine, quetiapine, aripiprazole), moderate D2-blocker antipsychotic (olanzapine), and strong D2-blocker antipsychotic (other antipsychotics) with no use of antipsychotic (reference). The hazard ratios were adjusted for age, sex, stroke, substance abuse, any neurological disorder, and time since schizophrenia diagnosis.

Results: A total of 287 choking deaths occurred during 817,000 patient-years, corresponding to standardized mortality ratio 20.5 (95% CI 17.1–23.9) compared with general population. A total of 268 deaths occurred during outpatient care (162 during use of strong D2-blocker antipsychotic, 30 during olanzapine, 21 during weak D2-blocker antipsychotic, and 53 during no antipsychotic use). The corresponding adjusted Hazard Ratio (aHRs) were 1.74 (95% CI 1.19–2.55) for strong D2-blockers, 1.59 (0.96–2.64) for olanzapine, and 1.04 (0.73–1.63) for weak D2-blockers.

Conclusions: Schizophrenia diagnosis is associated with a 20-fold risk of death due to choking. This risk is modified by the strength of D2-blockade of the used medication. While no incremental risk increase in addition to illness per se is observed for weak D2-blockers, the risk is substantially elevated during the use of strong D2-blockers.

Keywords: Schizophrenia (SCZ), Antipsychotic Treatment, D2 Dopamine Receptor, Choking

Disclosures: Eli Lilly, Janssen-Cilag: Grant (Self), HLS Therapeutics, Orion, WebMed Global: Consultant (Self), Eli Lilly, Evidera, Janssen-Cilag, Lundbeck, Mediuutiset, Otsuka, Sidera, Sunovion: Honoraria (Self).

P566. Increasing Endocannabinoid 2-AG Levels Reverses Amphetamine-Induced Prepulse Inhibition Deficits in Rats

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Background: There is much interest in the cannabinoid system as a potential therapeutic target for multiple psychiatric illnesses. The active component of marijuana, THC, has been studied for its ability to modulate behavioral functions relevant to psychiatric illness through binding of the cannabinoid receptors. There are two endogenous cannabinoid ligands - anandamide (AEA) and 2-arachidonoylglycerol (2-AG), termed endocannabinoids (eCB). AEA and 2-AG have separate synthesis and breakdown mechanisms, allowing for distinct targeting and alterations in the tone of each endocannabinoid individually. Multiple studies have focused on the functional effects of AEA; very little is known about the role of 2-AG. Hence, our study focused on the behavioral sequelae of elevating endogenous levels of 2-AG.

Methods: The overall design of this study was to test the compound JZL184, a potent inhibitor of monoacylglycerol lipase (MAGL). MAGL is the primary breakdown enzyme for 2-AG, and JZL184 administration increases 2-AG levels in the brain. We tested a range of JZL184 doses (administered intraperitoneally 30 min. prior to testing) in three behavioral paradigms that assess various core functions known to be disrupted in psychiatric disorders such as schizophrenia and post-traumatic stress disorder. These were prepulse inhibition of startle (PPI; assesses sensorimotor gating), elevated plus-maze (EPM; assesses anxiety-like states), and a behavioral observation paradigm (BOP; assesses ingestive, motor, and motivational responses). Separate cohorts of male and female rats were used for PPI ($Ns = 7$), EPM ($Ns = 22$), and BOP ($Ns = 6$). PPI and BOP experiments were within-subjects' designs, with each animal receiving all drug treatments in a counterbalanced order over multiple test days (four days between consecutive tests). PPI experiments contained the following experimental conditions: vehicle/vehicle; vehicle/AMPH; 8 mg JZL/vehicle; 16 mg JZL/vehicle; 8 mg JZL/AMPH; 16 mg JZL/AMPH. In BOP experiments, food-deprived rats were given JZL184 (vehicle, 8 mg/kg, or 16 mg/kg) and placed in test cages with sucrose pellets. Initially, a screen blocking access to sucrose pellets was present; after 15 min of recording rats' behaviors, the screen was removed (allowing access to the sucrose pellets), and behaviors were scored for an additional 45 min. Scored behaviors were duration and total bouts of: locomotion, rearing, eating, drinking, grooming, and screen approaches. For the EPM experiments, separate groups of rats received either vehicle (VEH) or 16 mg/kg JZL184. before being placed in the EPM for 5 min. The experimenter (blind to treatment condition) scored open arm entries and time, closed arm entries and time, center

entries and time, head dips, stretch-attends, and grooming. For all experiments, males and females were housed and tested separately to avoid exposure to olfactory cues of the opposite sex.

Results: To determine if 2-AG had modulatory effects on PPI, we first tested if JZL184 alone could alter PPI. When no deviations from basal PPI levels were seen with any dose of JZL184, we tested if JZL184 would reverse deficient PPI. We administered amphetamine (AMPH, 0 or 1.75 mg/kg), a psychotomimetic agent known to disrupt PPI, either with or without JZL184 pre-treatment. When JZL184 (at either dose) was co-administered with AMPH, males exhibited a reversal in the AMPH-induced PPI deficit; however, no reversal effect was seen in the females. The specificity of the AMPH-induced PPI deficit reversal in the males was further supported when no reversal in AMPH-induced startle was observed contrast to PPI, no measures in the EPM, ingestive, motor, and motivated approach behavioral paradigms were affected by the administration of JZL184 at any dose.

Conclusions: This set of findings indicates that the PPI-restorative effects of JZL184 were not due to non-specific alterations on baseline startle, mood, motor, or motivated behaviors. Thus, JZL184 shows targeted restoration of disrupted sensorimotor gating (as assessed by PPI). Moreover, this PPI-restorative effect of JZL184 appears to be sex-specific, as only males have normalized PPI levels after JZL184 pre-treatment. Together, these results demonstrate that eCBs, specifically 2- AG, can improve cognitive processes related to psychiatric disorders such as schizophrenia and post-traumatic stress disorder, where startle abnormalities like disrupted PPI are seen. To our knowledge, we are the first to show reversal in AMPH- induced PPI deficits using JZL184. Previous findings show that cannabinoid receptors are localized on norepinephrine-producing neurons and that 2-AG potently stimulates these receptors; moreover, stimulation of these cannabinoid receptors inhibits norepinephrine release. Given that AMPH is known to disrupt PPI at least in part by increasing norepinephrine release, one potential mechanism by which JZL184 reverses AMPH- induced PPI deficits could be the blockade of AMPH-induced norepinephrine release through enhanced levels of 2-AG.

Keywords: Cannabinoid, Endocannabinoids, Acoustic Startle Response, Prepulse Inhibition, JZL184

Disclosure: Nothing to disclose.

P567. Lack of Tolerance With Repeat Dosage of Dexmedetomidine in Rat for 21 Days as Measured by EEG

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Background: Igalmi is an FDA-approved treatment for agitation associated with schizophrenia or bipolar I or II disorder. Igalmi is a sublingual film formulation of dexmedetomidine (Dex), a selective, full agonist at alpha-2-adrenergic receptors (ADRA2A) expressed widely in the central nervous system. Dex activates the ADRA2A receptor and modulates norepinephrine release from locus coeruleus (LC) neurons, thereby reducing sympathetic hyperarousal. However, chronic activation of ADRA2A receptors, could also lead to tolerance, thereby reducing drug efficacy whereas discontinuation of repeated Dex dosing could result in withdrawal symptoms.

In this study, we report on a repeat-dosage study with Dex in rats to examine and quantify the putative development of tolerance. To this end we characterized a response to Dex using electroencephalogram (EEG) in conjunction with locomotor activity. We used this putative Dex EEG biomarker to follow and quantify Dex activity over time in the central nervous system (CNS).

Methods: Studies were carried out at a CRO (SynapCell, France). 24 Sprague-Dawley male rats aged 3 months were implanted with electrodes in the frontal and parietal cortex and connected to an intraperitoneal telemetry transmitter. Rats were dosed acutely with 5 or 10 µg/kg of Dex intramuscularly (IM) followed by a washout (1 week), repeat dosing for 22 days, 2-day washout and dosing with yohimbine. EEG signal was recorded for 3 hours and included a baseline (1 h), response following injection with compound (1 hr) and response to food administration (1 hr). EEG responses were quantified with time-frequency (TF) decompositions and fast Fourier transform (FFT) power spectra plotted relative to baseline values. Treatment groups are $n = 16$, and follow a balanced cross-over protocol with a 7 day washout. Averaged time points were tested for significance using 1-sided ANOVA.

Results: Acute dosing had several effects on overall behavior and EEG activity in rats. Vehicle (Veh) injection rapidly caused hyperlocomotion combined with a shift in EEG intensity (power) from the lower to higher EEG frequency bands. Dosing with Dex initially elicited a similar response but withing minutes significantly attenuated hyperlocomotion and the shift in EEG power. Instead, whereas EEG signals following Veh injections returned to baseline, Dex treated animals showed significant increases in the FFT power distribution between 1-30 Hz and decreases between 31 – 140 Hz. This shift in EEG power was accompanied by a significant decrease in locomotion.

Animals were food restricted. Following the response to Dex or Veh, food was added to the cage which caused excitement (locomotor activity) in the rats with significant increases in power between 100-140 Hz. In contrast to Veh, Dex treated rats did not show any increases in locomotion in response to food (although movement was not absent). Thus, responses to Dex, the injection, and food could be quantified and followed over time by the relative EEG FFT power distributions between 1-30 Hz, 31-99 Hz, and 100-140 Hz.

During repeat dosing, the Dex induced EEG-signal stayed robust and no significant changes were found overtime in the response-profiles between treatment groups. Similarly following the 2-day washout period, no significant differences were found between the 2 treatment groups. Thus, no evidence of increased locomotor activity or high frequency EEG activity was observed following discontinuation of Dex dosing. These results indicate that under our conditions Dex does not induce tolerance nor does discontinuation of Dex lead to hyperlocomotion or hyperexcitability.

We next investigated the effects of yohimbine, an ADRA2A receptor antagonist, with many effects opposite to Dex. If chronic dosing with Dex would build tolerance by downregulating ADRA2A receptor levels, responses to yohimbine would be attenuated. We quantified the EEG and locomotion response-profiles to yohimbine in the two treatment groups after the 2 day washout and again found no significant differences.

Conclusions: IM injection of Dex elicits rapid characteristic changes in the rat EEG power distributions and locomotion. Dex also differentially affects the EEG and locomotion-response profiles to evoked events such as the IM injection itself and addition of food to the cage. The EEG and locomotion response-profiles are quantitative, can be monitored over time and may represent a putative Dex CNS biomarker. Repeat dosing of animals for 22 days with Dex, did not induce any significant changes in the EEG and locomotion response-profiles over time, both to the injection as well as the excitability induced by food. Taken together these findings conclude that in rats, repeat dosing of Dex for 22 days does not cause tolerance as measured in CNS. Consistent with these observations we also found no evidence of hyperlocomotion or EEG excitability following Dex treatment

cessation. Assuming ADRA2A regulation is similar in rats and humans, these results suggest that Dex efficacy in reducing agitation should not diminish after multiple, consecutive doses.

Keywords: Dexmedetomidine, Agitation, Locus Coeruleus, alpha2-adrenergic, EEG Biomarkers, Acute Agitation

Disclosure: Nothing to disclose.

P568. Discovery and Evaluation of Allosteric Agonists for the Schizophrenia Risk Gene GPR52

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Background: GPR52 is an orphan G protein-coupled receptor and recently identified GWAS schizophrenia risk gene. This orphan is a Gs/olf class receptor that activates cAMP signaling and is primarily expressed in the ventral striatum of the human brain in dopamine D2 medium spiny neurons. The unique brain expression profile of GPR52 suggests the receptor may functionally regulate cAMP signaling to oppose the neural signaling of dopamine D2 receptors. This distinguishes GPR52 as an attractive, druggable target for psychiatric disorders including schizophrenia and substance use disorders. Here we report our efforts to elucidate GPR52 neuronal signaling and to create and evaluate selective activators for this receptor.

Methods: To elucidate the molecular signaling of GPR52, the human GPR52 receptor sequence was expressed in HEK293 cells, or cells lacking Gs/olf G proteins, and signaling was assessed using the Glosensor cAMP assay or Beta-arrestin TANGO assay. Medicinal chemistry and pharmacology were used in a small molecule discovery campaign to create novel GPR52 activators. GPR52 selectivity and off-target profiling was accomplished using competition radioligand binding at >30 CNS off targets and druglike pharmacokinetics and brain penetration was determined in rats. Molecular docking of novel ligands into a solved GPR52 crystal structure (PBD:6LI0) was done to explore receptor-ligand binding modes. A whole cell patch clamp study was performed by measuring evoked action potentials (AP) using mouse striatal slices ($n = 3$-6 cells analyzed per treatment group). A behavioral study tested vehicle or increasing doses of GPR52 agonists for antipsychotic-like activity in an amphetamine-induced locomotion study using c57BL6J mice ($n = 12$ animals for each 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 activator PW0787, which was eliminated by stable knockout of Gs/olf G proteins using CRISPR/Cas9 genome editing. Medicinal chemistry and pharmacology were subsequently used to modify an indoline carboxamide ligand and determine compound features crucial for GPR52 activation. Surprisingly, when the nitrogen containing ring of the indoline system was broken into more flexible variants, this greatly increased compound potency (EC50: ~40 nM) and efficacy, while retaining excellent target selectivity, plasma exposure and serum concentration. Unexpectedly conversion of the indoline to analine derivatives greatly reduced GPR52 activation of the Beta-arrestin pathway, resulting in the creation of several G protein biased activators. Molecular docking of the balanced compound PW0787 into the GPR52 crystal structure suggested compound binding with extracellular loop 2 (ECL2) and an allosteric site of action. PW0787 did not occupy a typical orthosteric site seen within GPCRs, but instead interacted within a narrow side pocket near ECL2, further suggesting an allosteric site binding mechanism. To assess the necessity of the ECL2 allosteric interaction for PW0787 activity, ECL2 was replaced with an equivalent span of alanine residues, which eliminated compound activity entirely. Notably, treatment of striatal slices with PW0787 increased the frequency and number of evoked action potentials in D2, but not D1, medium spiny neurons of the nucleus accumbens. Dose dependent testing of the optimized agonist PW0787 revealed 3 and 10 mg/kg treatments of mice significantly reduced amphetamine-induced hyperlocomotion, indicating antipsychotic-like activity.

Conclusions: Taken together, these findings indicate the schizophrenia risk gene GPR52, via Gs/Golf cAMP signaling, is an excitatory receptor selectively expressed in D2 medium spiny neurons. Our drug discovery effort has resulted in novel GPR52 activators with optimized potency and efficacy, including both balanced and functionally selective G protein biased activators. The novel balanced GPR52 activator PW0787 is a selective, orally bioavailable, brain penetrant allosteric agonist that excites D2 medium spiny neurons and also shows antipsychotic-like activity.

Keywords: Orphan Receptors, Drug Discovery/Development, cAMP Signalling, Schizophrenia- Novel Treatment, D2 Medium Spiny Neuron

Disclosures: New Atlas Biotechnology: Contracted Research (Self), PSYLO: Consultant (Self).

P569. Pimavanserin Treatment Increases Plasma Brain-Derived Neurotrophic Factor Levels in Rats

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Background: Pimavanserin, a serotonin 5HT-2A receptor inverse agonist, with no appreciable affinity to dopamine D2 receptors, is the first-line, FDA-approved treatment of hallucinations and delusions associated with Parkinson's Disease psychosis (PDP), which occurs in up to 50% of PD patients. The neurobiological mechanism underlying the therapeutic effectiveness of Pimavanserin in PDP remains unknown. A number of earlier studies, including reports from our laboratory, have shown that treatment with 5HT-2A antagonists and other drugs acting on the serotonergic system like SSRIs increase Brain derived neurotrophic factor (BDNF) levels in rodents. BDNF is synthesized as the precursor proBDNF, that undergoes cleavage intra or extracellularly to produce a mature BDNF (mBDNF) protein. mBDNF is believed to play an important role in neuroplasticity and neurogenesis. The present study tested the hypothesis that treatment with Pimavanserin is associated with higher and sustained elevations of mBDNF. PDP is a progressive neurodegenerative disorder and an increase in mBDNF may contribute to a neuroprotective effect.

Methods: Male Sprague–Dawley rats, weighing 280–320 g (6–8 weeks old; $N = 10$/group) were treated with Vehicle (0.9% saline), Pimavanserin (1 mg/kg/d) or Fluoxentine (10 mg/kg/d). Drugs were administered subcutaneously for 4 weeks (chronic) or 2 hours (acute). We have analyzed proBDNF and mBDNF in plasma, serum and key brain regions [prefrontal cortex (PFC) and hippocampus] by ELISA. Data were analyzed by One-way ANOVA followed by posthoc-Tukey's multiple comparisons test.

Results: In chronic treatment studies described above, both Pimavanserin and Fluoxetine significantly increased plasma levels of proBDNF as compared to vehicle group. However, a significant increase in plasma mBDNF was found in Pimavanserin-treated rats, but not in Fluoxetine-treated group. Both Pimavanserin and Fluoxetine significantly increased proBDNF levels in the PFC as compared vehicle group. No significant change in mBDNF levels was found in the PFC and hippocampus in any of the chronic treatment groups as compared to vehicle group. In acute

treatment studies, Pimavanserin significantly increased proBDNF levels in plasma and serum as compared to vehicle group. Fluoxetine treatment increased proBDNF levels in serum as compared to vehicle group. No significant difference was observed in peripheral mBDNF levels in any of the acute treatment groups as compared to vehicle group.

Conclusions: The observed increases in plasma mBDNF levels following chronic Pimavanserin treatment suggest the neuroprotective potential of Pimavanserin in the long-term treatment management of PDP in addition to its antipsychotic effects.

Keywords: Pimavanserin, BDNF, Parkinson's Disease Psychosis, Neuroprotection

Disclosure: Acadia Pharmaceuticals: Contracted Research (Self).

P570. Projection Specific Effects of mGlu3 Receptor Activation in Regulation of Schizophrenia-Like Nucleus Accumbens Pathophysiology and Sociability Deficits

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Background: Genetic variants in GRM3 (the gene encoding for the mGlu3 receptor) are associated with altered glutamatergic neurotransmission in schizophrenia and can predict improvement in symptoms following drug treatment. However, the mechanisms through which mGlu3 receptors modulate glutamatergic neurotransmission and pathophysiological changes that may be relevant for schizophrenia are still not clear.

Methods: The mGlu3 receptor-induced long-term depression (mGlu3-LTD) of excitatory neurotransmission in the nucleus accumbens was tested using whole-cell patch-clamp electrophysiology from the slices prepared from D1-TdTomato mice (5-6 mice/group). The mGlu3 receptor signaling was activated by bath application of mGlu2/3 receptor agonist, LY379268 in the presence of mGlu2 negative allosteric modulators. The projection-specific effects were evaluated using slice optogenetics experiments after virally expressing channelrhodopsin-2 in different brain areas. Schizophrenia-like symptoms were induced by treating mice (10-15/group) with phencyclidine (PCP, 10 mg/kg; s.c.) for 7 days followed by a 7-day washout. Sociability deficits in the mice were accessed using a 3-chamber social behavior test. Both male and female mice were used in the study. The effects of treatments on the excitatory transmission and behavior test were evaluated using one-way ANOVA followed by the Newman-Keuls post hoc test.

Results: Activation of mGlu3 receptors induces long-term depression (LTD) of excitatory neurotransmission onto D1 medium spiny neurons (MSNs) in the nucleus accumbens (NAc). Further, the mGlu3-LTD is expressed at the synapses from medial thalamus (mThal) afferents to the D1 MSNs of the NAc shell (Thal-D1 NAc shell), but not at synapses from basolateral amygdala inputs to the D1 MSNs of the NAc shell, indicating synapse-specific effects of mGlu3 receptor activation in the NAc. Interestingly, mGlu3 receptor activation normalized augmented glutamatergic signaling in the NAc slices of mice treated with PCP. It also decreased the PCP-induced potentiation of activity of thalamic afferents to the D1 MSNs of the NAc shell. Further, in vivo potentiation of mGlu3 receptor activation function rescued the PCP-induced impairments in the social novelty.

Conclusions: These data demonstrate that activation of mGlu3 receptors can rescue schizophrenia-like physiological and behavioral deficits and provide a mechanism by which polymorphisms in the GRM3 can contribute to the pathophysiology of schizophrenia. Therefore, targeting these receptors can provide a viable approach for developing new therapeutics for treating patients with schizophrenia.

Keywords: Metabotropic glutamate receptor 3 (mGluR3), Schizophrenia novel treatment, schizophrenia negative symptoms, Excitatory Synapses, Medium Spiny Neuron

Disclosure: Nothing to disclose.

P571. Targeting the FGF12:Nav1.2 Complex for CNS Probe Discovery

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Background: Voltage-gated sodium (Navs) channels play a critical role in action potential initiation and propagation. Structurally, the Nav channel features a pore-forming α subunit, of which nine isoforms have been described (Nav1.1-Nav1.9), that is tightly regulated through protein:protein interactions (PPI) with auxiliary proteins. One of the alpha subunits, Nav1.2, is of particular interest. Nav1.2's PPI with its auxiliary protein fibroblast growth factor 12 (FGF12), part of the intracellular fibroblast growth factor family (FGF11-14), occurs through interactions at its C-terminal domain (CTD). Specifically, FGF12 has been shown to interact with Nav1.2 to increase its fast inactivation kinetics and slow its recovery from fast inactivation. Notably, co-expression of FGF12 and Nav1.2 is enriched in the cerebral cortex, suggesting that FGF12:Nav1.2 interaction is a potential determinant of activity in this region. Various mutations in FGF12 have been implicated in either gain-of-function (GOF) or loss-of-function (LOF) regulatory effects on Nav1.2 channel activity in the context of autism spectrum disorder (ASD), and FGF12 has been identified as dysregulated in schizophrenia (SCZ) and major depression disorder (MDD). Thus, FGF12 ligands that could modulate the interaction of FGF12 with its binding partner, Nav1.2, could be therapeutically valuable for ASD, SCZ and MDD, disorders that have been associated with changes in FGF12 function.

Methods: We employed medicinal chemistry, chemoinformatics, the split-luciferase assay (LCA), surface plasmon resonance (SPR), patch-clamp electrophysiology in heterologous expression systems to validate the activity of hits and leads in in vitro and in-cell assays, and in cortical neurons derived from human-induced pluripotent stem cells (hiPSCs).

Results: Intracellular FGFs and Nav channels form PPI complex pairs that are sufficiently heterogeneous in critical binding regions to allow for specific pharmacological modulation. Based on this premise, we conducted an in-cell high-throughput screening against FGF14:Nav1.6, a PPI complex closely related to FGF12:Nav1.2, but functionally distinct in its ability to regulate Nav channels. Following development of a double-stable HEK293 cell line expressing split-luciferase complementation assay (LCA) constructs and assay optimization in 384-well plates, we screened ~50,000 small molecules, peptides and rationally-designed drug-like analogues against FGF14:Nav1.6. Compounds were ranked by a combination of % maximal luminescence and individual Z-scores, which were calculated based on the mean and SD of its respective plate controls (0.3% DMSO). Using cut-offs of $Z \leq -5$ and ≥60% reduction in complex formation for inhibitors and $Z \geq 3$ and ≥150% increase in complex formation for enhancers, we identified 960 primary hits. Of these, 640 compounds failed to achieve significance during triplicate validation screening, and counter-screening against full-length luciferase in transiently transfected HEK293 cells resulted in the exclusion of an additional 149 compounds due to significant inhibitory effects ($Z \leq -3$). Thus, the primary set of hits was reduced to 151 inhibitors and 20 enhancers, which were then stratified by structural and chemical parameters including predicted permeability (logP). From these compounds, we derived a sub-class of 50 molecules with ideal

drug-like properties. We then counter screened these 50 hit compounds against the FGF12:Nav1.2 complex, with the expectation that a unique set of ligands would exhibit specificity for FGF12. We have identified 3 lead compounds 7350, 0801, and 7827 that share common chemical scaffolds targeting the FGF12:Nav1.2 PPI interface. Ongoing studies are focusing on determining the potency, specificity, biophysical mechanisms and functional effects of these compounds in hiPSCs-derived cortical neurons.

Conclusions: This evidence suggests that pharmacologically targeting FGF12 represents a promising method of modulating Nav1.2 activity for a wide spectrum of neuropsychiatric disorders.

Keywords: Ion Channels, Action Potentials, Growth Factors

Disclosure: Nothing to disclose.

P572. 2,5-Dimethoxy-4-Propylamphetamine (DOPR) Increased Effortful Motivation in Mice

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Background: There is a significant gap in available pharmacological treatments for many neuropsychiatric disorders, including schizophrenia and depression. Identifying novel treatments and targets remains paramount. The lack of efficacy of many compounds in human trials despite positive preclinical data however, signals a clear need for improved translatability of preclinical studies of behavioral disorders. The progressive ratio breakpoint task (PRBT) measures effortful motivation and exhibits pharmacological predictive validity between humans and mice.

2,5-Dimethoxy-4-propylamphetamine (DOPR) is a relatively poorly understood compound with psychedelic properties. It is closely related to 2,5-Dimethoxy-4-iodoamphetamine (DOI), another psychedelic drug that may improve anhedonia-relevant symptoms. The psychoactive effects of DOI are well documented, particularly its ability to elicit the head twitch response (HTR), a response to hallucinogenic compounds seen in mice that is highly predictive of hallucinogenic effects in humans. Here, we investigated the effects of DOPR on effortful motivation in the PRBT and HTR. As positive controls, amphetamine was used for the PRBT and DOI in the HTR assay.

Methods: 40 C57BL/6 N mice (50% female) were trained in operant chambers. After initial training stages, mice were tested in the PRBT which required mice to increase their nosepokes in a progressive ratio for the same 40 µL strawberry milkshake reward. The point at which they stopped was defined as their breakpoint and was the primary outcome measure. After baseline assessment, mice were dosed with DOPR (0.0106, 0.032, 0.106, or 0.32 mg/kg, i.p.), saline, or amphetamine (0.3 mg/kg) in a within-subjects design. Breakpoint was analyzed using dose as a within-subjects factor, while sex and baseline performance (median-split) were between-subjects factors. C57BL/6 N male mice were assessed for HTR in response to either DOPR or DOI. Mice were administered with a single dose of DOPR or DOI (0.1, 0.32, 1, 3.2, or 10 mg/kg, s.c.) or vehicle ($n = 6$ per dose), and placed in an open field. Head twitches were counted manually by an observer blinded to the treatment condition. Binding of DOPR and DOI to the 5-HT2A receptor was also measured, as well as functional activity at the 5-HT2A, 5-HT2B, 5-HT2C, and 5-HT1A receptors. Receptor binding was measured by displacement of [3H]Ketanserin in HEK293 cells expressing receptors. Functional activity was measured via Ca ++ flux FLIPR assays.

Results: DOPR did not exert a main effect on breakpoint ($F(4,132)=1.3$, $p = 0.261$), but when analyzed by baseline, DOPR did not affect those with a high breakpoint ($F(4,68)=1.0$, $p = 0.410$), but increased the breakpoint of mice that had a low baseline ($F(4,64)=3.3$, $p = 0.016$) at 0.0106, 0.106, and 0.32 mg/kg doses, with a trend towards increased breakpoint seen at 0.032 mg/kg vs. vehicle. These effects were consistent with amphetamine, with no main effect ($F(1,33)=0.5$, $p = 0.495$), driven by a lack of effect in high performers ($F(1,17)=0.3$, $p = 0.620$), but improvement in low performers ($F(1,16)=8.9$, $p = 0.009$). No effect of sex was seen in response to either DOPR or amphetamine.

DOPR caused a dose-dependent increase in HTR ($F(5,30)=60.0$, $p < 0.0001$), with doses of 3.2 and 10 mg/kg passing a Bonferroni post hoc correction relative to vehicle ($p < 0.002$), and peak effect at 3.2 mg/kg. These results closely matched the effects of DOI ($F(5,30)=75.4$, $p < 0.0001$), with 1, 3.2, and 10 mg/kg passing a Bonferroni post hoc correction relative to vehicle ($p < 0.002$), and peak effect at 3.2 mg/kg. The binding affinity at 5-HT2A of the two compounds was similar, with DOPR having a Ki of 17.56 nM and DOI having a Ki of 14.51 nM. DOPR and DOI also show agonist activity at 5-HT2A (EC50 of 0.12 and 0.19 nM, respectively) and 5-HT2C (EC50 of 0.27 and 0.82 nM, respectively) receptors, with >25-fold lower potency at 5-HT2B receptors and no significant activity at 5-HT1A (both > 1,000 nM EC50).

Conclusions: The positive effect of DOPR on effortful motivation points to possible therapeutic applications in psychiatric illness states characterized by reduced effortful motivation as measured by the PRBT. The similarity of effects of DOPR to well-studied drugs such as DOI and amphetamine provides a useful reference point to interpret its pharmacological effects. Importantly, the doses needed to increase breakpoint in the PRBT were as low as 0.0106 mg/kg. While 0.1 mg/kg increased HTR, this effect was not significant, and maximal effect at 3.2 mg/kg, supporting the premise that low doses of DOPR may be therapeutic in anhedonia states without causing unwanted hallucinogenic side effects.

Keywords: Motivation, Psychedelics, Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P573. Evaluation of Selective Muscarinic M4 Agonists on Cortical Oscillations: A Sleep-EEG Study in Rats

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Background: One of the leading theories on the pathophysiology of schizophrenia is that an overactivity of dopamine in certain brain regions is closely associated with the prevailing psychotic symptoms. Current antipsychotics target a direct blockade of dopamine receptors, yet this can lead to significant side effects. An alternate approach is to indirectly modulate levels of dopamine. The presynaptic localization of the M4 receptors differentially expressed in the striatum may serve to balance acetylcholine and dopamine levels in the region of the brain primarily responsible for psychotic symptoms. Activation of M4 receptors decreases acetylcholine, which in turn decreases levels of dopamine without the direct receptor blockade Thus, selective activation of M4 may be an effective treatment of the neurobehavioral components such as psychosis, agitation, and cognitive deficits, that are associated with schizophrenia and other neuropsychiatric disorders.

Methods: A polysomnographic electroencephalography (sleep-EEG) study was carried out using radiotelemetry in unanesthetized Sprague Dawley rats following subcutaneous compound administration ($n = 8$ rats per treatment per crossover study). EEG was recorded and analyzed using conventional methods: spectral

power was determined using NeuroScore (DSI) for each 1 Hz bin for every 10 seconds and averaged into frequency bands, per temporal epoch, per sleep-stage, and by treatment group.

Results: Full and partial M4 agonists showed similar profiles on cortical oscillations. Both increased low frequency (delta) and suppressed high frequency (alpha, beta and gamma) activity.

Conclusions: These data confirm dose-dependent effects on cortical oscillations following M4 activation. Interestingly, whereas activation of dopaminergic receptors results in increases in higher frequencies such as alpha, beta and gamma oscillations, antipsychotics commonly decrease alpha and gamma oscillations. Data generated with these M4 activators align with an antipsychotic profile and suggest a putative role of M4 activation in mitigating psychotic symptoms.

Keywords: EEG, EEG Biomarkers, M4 Muscarinic Receptors, M1 and M4 Muscarinic Receptors, Antipsychotic

Disclosure: Cerevel Therapeutics: Employee (Self).

P574. TAAR1 Dependent and Independent Actions of the Potential Antipsychotic and Dual TAAR1/5HT1A Receptor Agonist SEP-383856

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Background: SEP-383856 (SEP-856) is a novel antipsychotic under clinical development. It displays a unique pattern of receptor interaction, with only weak (partial agonist) activity at dopamine D2 receptors, yet more potent agonist activity at the trace amine associated receptor (TAAR1) and 5-hydroxytryptamine 1A receptor (5HT1A). Nonetheless, these observations await independent confirmation and more detailed characterization of the in vitro and in vivo actions of SEP-856 at TAAR1 and 5HT1A receptors would be instructive.

Methods: We employed luminescence complementation technology in heterologous live cell systems, confocal microscopy, voltage clamp electrophysiology, behavioral readouts and TAAR1 knockout (KO) mice to study SEP-856 in further detail.

Results: We provide evidence for the ability of SEP-856 to activate TAAR1 at the surface plasma membrane, and show that this interaction results in Gαs recruitment (pEC50: 6.08 ± 0.22 EMAX: 96.41% ±15.26) and by extension, to G-protein inward rectifying potassium (GIRK) channel activation. Using TAAR1-KO mice, we find TAAR1 to be indispensable for SEP-856 control of body temperature, baseline locomotion reduction and for "antipsychotic-like" efficacy as characterized by a reversal of dizocilipine (MK-801) mediated disruption of pre-pulse inhibition. Conversely, the inhibition by SEP-856 of MK-801 induced locomotion was unaffected in TAAR1 KO mice. SEP-856 behaved as a low-potency, partial agonist at the 5HT1A receptor, while it partially inhibited recruitment of D2 receptor-coupled Gα and GIRK by DA and acted as a weak partial agonist with low potency at the same receptor when applied alone.

Conclusions: Our findings corroborate and extend previous observations on the molecular substrates engaged by potential antipsychotic, SEP-856, that could prove to have major advantages in the treatment of schizophrenia and other psychotic disorders.

Keywords: TAAR 1, Trace Amines, TAAR1 Knockout Mice

Disclosure: Nothing to disclose.

P575. Modulation of D1 Receptor-Expressing Spiny-Projection Neurons is Indicative of Antipsychotic Effect

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Background: Overactive dopamine transmission in psychosis is predicted to unbalance striatal output via D1- and D2-dopamine receptor-expressing spiny-projection neurons (SPNs). Antipsychotic drugs are thought to re-balance this output by blocking D2-receptors.

Methods: We used in vivo imaging to record D1- and D2-SPN Ca2+ dynamics in mice ($N = 7–11$ per group) under normal and hyperdopaminergic conditions using amphetamine treatment. We examined these conditions in the presence or absence of treatment with 10 different antipsychotic drugs or candidates. We evaluated the effects of drug treatment combinations on neural activity, open field locomotion, sensorimotor gating, and hallucination-like perception in both males and females. We also used chemogenetics to causally link drug effects on D1-SPN activity to the normalization of these behaviors.

Results: Initially we compared effective (clozapine, olanzapine, and haloperidol) antipsychotics to a candidate drug that failed in clinical trials (MP-10). All drugs normalized amphetamine-driven hyperlocomotion, but only MP-10 normalized D2-SPN hypoactivity. By contrast, the effective antipsychotics all normalized hyperdopaminergic D1-SPN dynamics. Chemogenetic inhibition of D1-SPNs was sufficient to normalize locomotion, sensorimotor gating, and hallucination-like perception under hyperdopaminergic conditions. Given the surprising correlation between clinical efficacy and D1-SPN modulation, we next evaluated compounds selectively targeted to D1-SPNs. D1R partial agonism, antagonism, or M4 cholinergic receptor modulation all normalized the levels of D1-SPN activity, locomotion, and sensorimotor gating. The antipsychotic drug candidate xanomeline (an M1/M4 muscarinic agonist) also normalized D1-SPN activity and behavior. Remarkably, SEP-363856, a trace amine-associated receptor 1 (TAAR1) agonist with antipsychotic efficacy, also normalized D1-SPN activity, even though it does not principally target the striatum or normalize behavior under hyperdopaminergic conditions.

Conclusions: In contrast to the prevailing view, our results show that modulation of D1 rather than D2-SPNs is sufficient and critical for antipsychotic drug efficacy. These findings underscore the utility of using neural dynamics to readout drug efficacy and suggest that D1-SPN activity is a more relevant therapeutic target than D2-SPN activity for the development of effective antipsychotics.

Keywords: Antipsychotic Drugs, M1 and M4 Muscarinic Receptors, PDE10A, D1 dopamine Receptors, D1 Agonist, D1-PAM

Disclosure: Nothing to disclose.

P576. Impaired Auditory Cortex Dynamic Range in First Episode Psychosis and Associated Disease Burden

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Background: There is a growing appreciation for the contribution of sensory disruptions to disease morbidity in psychosis. However, auditory dynamic range, the scaling of neurophysiological responses to stimulus intensity, is an attribute of high-fidelity sensory systems that remains understudied in psychosis. The goad of the present study was to assess auditory cortex (AC) dynamic range among individuals with a schizophrenia spectrum illness during their first psychotic episode (FESz) and examine its relationship to clinical outcomes.

Methods: Thirty-five FESz and 40 healthy controls (HC) matched for age, sex, parental socioeconomic status, and premorbid IQ participated in the study. Magnetoencephalography (MEG) was recorded during binaural presentation of 1KHz tones at 3 intensities (75 dB, 80 dB, and 85 dB). Structural MRIs were obtained to enhance cortical localization of MEG sensor activity. All participants completed the Wechsler Abbreviated Scale of Intelligence (WASI), the MATRICS cognitive battery (MCCB), and the Global Functioning: Role and Social scales (GFR/GFS). Patients were administered the Positive and Negative Syndrome Scale (PANSS). Primary outcomes included AC activity elicited by tone stimuli as well the dynamic range of this activity, defined as the increased AC activity from 75 dB to 85 dB, and its correlations with clinical assessment scores.

Results: FESz exhibited an overall reduced AC response to tones relative to HC ($p = 0.002$, partial $\eta 2 = 0.13$). Importantly, the enhancement of AC activity to tones of increasing intensity observed across groups ($p < 0.001$, partial $\eta 2 = 0.35$) was blunted in FESz relative to HC ($p = 0.03$, partial $\eta 2 = 0.05$). Reduced dynamic range among FESz was associated with lower GFS ($r = 0.62$, $p < 0.001$) and GFR ($r = 0.45$, $p = 0.006$) scores, worse MCCB performance ($r = 0.49$, $p = 0.003$), and increased PANSS Negative symptom subscale scores ($r = -.53$, $p < 0.001$).

Conclusions: Beyond an impaired sensory response to pure tones, FESz exhibit reduced AC dynamic range relative to HC. This impairment was correlated with various markers of disease morbidity including poorer community functioning as well as cognitive and negative symptoms, though the most robust association was observed with social functioning scores. The relationship with impaired social functioning may reflect the role of AC dynamic range in decoding the emotional content of language and highlights its importance to future therapeutic sensory remediation protocols.

Keywords: First Episode Schizophrenia, Magnetoencephalography, Auditory Cortex, Auditory Processing, Social Functioning

Disclosure: Nothing to disclose.

P577. Shared and Unique Abnormalities in Sleep and Rest-Activity Rhythms in Residential and Outpatient Schizophrenia Spectrum Disorder Patients

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Background: Sleep and rest-activity-rhythm (RAR) abnormalities are commonly reported in schizophrenia spectrum disorder (SSD) patients. However, an extensive characterization of RAR alterations in SSD patients relative to healthy control subjects is currently lacking. Furthermore, differences in RAR parameters between residential and outpatient SSD individuals, including their relationships with the SSD clinical symptoms, have not been thoroughly examined.

Methods: Two hundred and fifty participants, including one hundred and thirty-seven patients diagnosed with Schizophrenia Spectrum Disorders (SSD, seventy-nine residential patients, and fifty-eight outpatients) and one hundred and thirteen healthy comparison (HC) subjects, were recruited at ten different mental health centers in Northern Italy Ras as part of the DiAPAson project. To monitor habitual sleep-wake patterns, study participants were instructed to wear an ActiGraph GT9X on the non-dominant wrist for seven consecutive days. Data from 20 participants were excluded due to having either less than 3 days of actigraphy data or being detected as an outlier. Therefore, 68 residential SSD patients, 54 SSD outpatients, and 108 HC individuals were included in further analyses. RAR parameters, including M10, L5 relative amplitude (RA), intra-daily variability (IV), inter-daily stability (IS), alpha, beta, F-statistic (F-stat), and sleep parameters (i.e., total sleep time [TST], wake after sleep onset [WASO]) were computed for each study participant. Moreover, negative symptoms were assessed in residential and outpatient SSD patients with the Brief Negative Symptom Scale (BNSS). Analysis of covariance (ANCOVA) was performed to identify differences in RAR and sleep parameters between HC, outpatient SSD, and residential SSD groups after controlling for age and sex. Statistical significance was determined by applying Bonferroni's correction for multiple comparisons. For RAR/sleep parameters showing significant ANCOVA differences across the three groups, the Tukey HSD test was used for pairwise comparison, including differences between each SSD population with HC and between the two SSD samples. Finally, correlation analyses between BNSS scores and RAR parameters were performed.

Results: Among sleep parameters, TST ($F(2, 225) = 79.43$, $p < 0.001$), but not WASO, was different between groups after Bonferroni's correction for multiple comparisons. Furthermore, except RA and F-stat, all RAR parameters, including IV ($F(2, 225) = 8.35$, $p = 0.003$), M10 ($F(2, 225) = 31.13$, $p < 0.001$), L5 ($F(2, 225) = 7.91$, $p = 0.005$), alpha ($F(2, 225) = 46.092$, $p < 0.001$), beta ($F(2, 225) = 27.68$, $p < 0.001$), and IS ($F(2, 225) = 14.33$, $p < 0.001$) were significantly different across the three groups. Specifically, TST was higher in both SSD groups compared to HC ($t = 11.18$, $p < 0.001$, $t = 9.28$, $p < 0.001$; for residential and outpatients SSD vs HC, respectively). Both SSD groups showed also lower M10 (residential vs control: $t = -7.71$ and $p < 0.001$; outpatients vs control: $t = -4.2$ and $p < 0.001$) and L5 (residential vs control: $t = -3.79$ and $p < 0.001$; outpatients vs control: $t = -2.43$ and $p = 0.048$), along with higher alpha (residential vs control: $t = 7.43$ and $p < 0.001$; outpatients vs control: $t = 8.25$ and $p < 0.001$) compared to HC. Residential SSD patients had higher IV (residential vs control: $t = 2.98$ and $p = 0.010$), IS (residential vs control: $t = 5.35$ and $p < 0.001$), and beta (residential vs control: $t = 7.16$ and $p < 0.001$) relative to HC. In contrast, SSD outpatients showed no differences in any of those three measures compared to HC. We also observed that M10 ($t = 2.67$, $p = 0.024$) was higher in SSD outpatients compared to residential patients, whereas IV ($t = -3.95$, $p < 0.001$), beta ($t = -5.51$, $p < 0.001$), and IS ($t = -2.73$, $p = 0.020$) were higher in residential compared to SSD outpatients. Furthermore, residential patients had worse negative symptoms compared to outpatients ($t = 2.6299$, $p = 0.010$), and IS correlated with the severity of negative symptoms across all SSD patients ($R = 0.248$, $p = 0.024$).

Conclusions: In this study, we found that compared to healthy controls, residential and outpatient SSD individuals had both unique (IV, beta, IS) and shared (e.g., TST, M10, L5, alpha) abnormalities in RAR/sleep measures, and IS was associated with the severity of the SSD clinical symptoms.

Keywords: Actigraphy, Schizophrenia (SCZ), Circadian Rhythm, Brief Negative Symptom Scale (BNSS)

Disclosure: Nothing to disclose.

P578. Odor Discrimination and Identification in Schizophrenia: Relationship to MRNA in Lymphocytes and MATRCIS Battery Scores

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Background: Patients with schizophrenic have been reported to show deficits in various measures odor perception but odor discrimination has not been standardly assessed. DNA methylation and GABAergic input have been implicated in biochemical process controlling odor in animal studies, but this has not been investigated in human studies. Some studies have related cognitive deficits in schizophrenia to odor deficits but none have used the MATRICS battery to investigate this question.

Methods: In a study of DNA methylation and GABAergic mRNAs in lymphocytes we also measured odor identification and discrimination with the Sniff and Sticks battery in 58 patients with chronic schizophrenia (CSZ) and 48 non-psychiatric controls (NPC). mRNAs in lymphocytes were assessed by qPCR using TaQMan probes. Cognition was assessed by the MATRICS battery in CSZ and NPC and symptoms in CSZ were assessed by PANSS scale. The relationship of odor deficits to mRNA levels and MATRICS scores and symptoms was explored by correlation analysis.

Results: CSZ showed significant deficits compared to NPC in odor identification ($P = 0.011$, Cohen's $d = 0.50$)), but much larger deficits in discrimination ($P < 0.001$, $d = 1.01$). In step down regression analysis odor discrimination but not odor identification had significant β weight for classify patients into the CSZ vs NPC group. There were significant negative correlations ($r = -33$ to -.68) of odor identification with DNMT1 mRNAs, and significant negative correlations with odor discrimination and GABAergic mRNAs in CSZ subjects (-.38 to -.42). Odor discrimination scores correlated significantly ($P = 0.02$ to $P = 0.009$) with several Matrics Domain scores in CSZ subjects but not NPC; there was a sex effect and these correlations were stronger in female than male CSZ.

Conclusions: Odor discrimination deficits, which has not been consistently evaluated in schizophrenia studies, showed the strongest differentiation between patients with schizophrenia and controls. This is the first study to report relationship between odor deficits and DNMT and GABAergic mRNAs in human subjects. However, the negative correlations of odor scores with lymphocyte mRNA levels may not necessarily reflect neuronal processes.

Keywords: Odor Deficits, Schizophrenia, DNMT, DNMT

Disclosure: Nothing to disclose.

P579. Neurovascular Water Exchange and Negative Symptoms in Schizophrenia

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Background: Negative symptoms limit real-world functioning and treatment response in patients with schizophrenia spectrum disorder (SSD). Mounting evidence supports cerebrovascular contributions to negative symptoms but with unknown mechanism. The blood-brain barrier (BBB) is at the nexus of neural-vascular exchanges, tasked with regulating cerebral homeostasis. BBB abnormalities in SSD, if any, likely occur at subclinical levels and imaging measures used to assess large molecule BBB leakage in major neurological events may not be sensitive enough to directly examine clinical evidence of BBB abnormalities in SSD.

Methods: We tested the hypothesis that neurovascular water exchange (Kw) measured by non-invasive diffusion-prepared arterial spin label MRI is impaired in SSD and associated with negative symptoms. Peripheral vascular endothelial health was examined by brachial artery flow-mediated dilation ($n = 44$ healthy controls, $n = 37$ SSD) to assess whether centrally measured Kw is related to endothelial functions. The study design was cross-sectional and both sexes were included in the studies.

Results: Whole-brain average Kw was significantly reduced in SSD ($p = 0.005$), particularly in the right parietal lobe, including the right supramarginal gyrus ($p = 0.002$) and right postcentral gyrus ($p = 0.008$). Reduced right superior corona radiata ($p = 0.001$) and right angular gyrus Kw ($p = 0.006$) was significantly associated with negative symptoms. Peripheral endothelial function was also significantly reduced in SSD ($p = 0.0001$). Kw in 94% of brain regions in controls positively associated with peripheral endothelial function, although the trend was not observed in SSD patients, where the correlation was inversed in 52% of the brain regions. Conclusions.

Conclusions: This study provides initial evidence of negative symptoms in schizophrenia linked to subclinical neurovascular water exchange abnormality in specific brain regions.

Keywords: Blood-Brain-Barrier, Neurovascular, Schizophrenia Spectrum Disorders, Endothelial Function, Negative Symptoms

Disclosure: Nothing to disclose.

P580. Adolescent Development of Cortical Circuits for Predictive Coding is Sex-Dependent and Relates to Maturation of Prefrontal Cortex

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Background: Although not diagnostic, difficulties in the processing of sensory information in the context of past and present stimuli are common in a number of neuropsychiatric disorders, serving to ultimately undermine how affected individuals perceive and interpret their world. For example, this has been quantified in people with schizophrenia (SZ) as altered sensory cortical responses in the EEG to unexpected stimuli during "oddball" sequences— i.e. reduced "mismatch negativity" (MMN). Understanding the essential cell and circuit mechanisms generating healthy and impaired MMN remains a key goal in neuropsychiatry given the strong translational potential of this biomarker.

Our past work in mice has shown that, during a visual oddball paradigm, long-range projections from prefrontal brain regions, such as the anterior cingulate area (ACa) in mice, may modulate activity in primary visual cortex (V1) via tonic input at oscillating at ≈10 Hz. We found that serves to enhance the activity of V1 VIP-positive interneurons while suppressing SST-positive interneurons. This SST-suppression effectively disinhibits ensembles of pyramidal neurons to release prediction-error-like "deviance detection" responses (DD) to unexpected stimuli (i.e. the MMN-like response).

Because many neuropsychiatric diseases onset during adolescence in a sex-dependent manner, and because past work has shown that human EEG indices of predictive coding (MMN, P300) mature across adolescence, we sought to understand i) how such MMN-like responses develop in adolescence at the cellular level, and ii) how this development corresponds to the development of top-down inputs from prefrontal cortex.

Methods: For part 1, awake mice (male ($n = 8$) and female ($n = 10$)) underwent two-photon calcium imaging at p28 pre-adolescence, p42 mid-adolescence, and p84 adult while viewing a visual oddball and a control sequence. Sex and developmental effects on DD was assessed with age by sex by stimulus type (deviant vs control) on cell-level responses. For part 2, local field potentials (LFP) were recorded in prefrontal (PFC; area ACa) and visual cortex (V1) in male and female mice aged p28 and p84 ($n = 14$ mice) to assess interregional synchrony (phase-locking factor- PLF). A one-way ANOVA was carried out on PLF to assess developmental effects of PLF.

Results: Results show a sex by age interaction on deviance detection ($F(2,2756)=21.11$). Males did not develop DD until adulthood (p84) while females exhibited DD at all ages. Closer analysis shows that, regardless of sex, the DD present in pre-adult ages (p28, p42) was strongly predicted by simple feature selectivity in the local (<300μm3) network (i.e. the average orientation selectivity of neurons; $r = 0.66$, $p < 0.01$) while this relationship was not present in adulthood ($r = 0.30$). This suggests a more complex, network-driven predictive coding emerges during adolescence. Our LFP recordings in PFC and V1 were consistent with this interpretation, showing that prefrontal-visual synchrony increases strength from p28 to p84 ($f(1,12)=9.51$, $p < 0.01$).

Conclusions: Altogether this suggests that difficulties in sensory-context processing may involve long-range cortical circuits which develop in late adolescence, corresponding to the onset of multiple neuropsychiatric diseases. Juvenile females may exhibit a form of DD which is more independent of prefrontal input, buttressed by stronger local feature selectivity in sensory regions, suggesting a potential protective factor explaining lower rates of adolescent onset psychotic disorders.

Keywords: Biomarker, Adolescence, Mismatch Negativity, Two-Photon Calcium Imaging, LFP

Disclosure: Nothing to disclose.

P581. Comprehensive and Simultaneous 3-D Imaging of Interneuron Subtypes in CA1 Depicts Deficits in Interneuron Activity Resulting in Microcircuit Imbalance in a Mouse Model for the 22q11.2 Deletion Syndrome

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Background: Individuals with the 22q11.2 deletion syndrome (22q11.2DS), one of the strongest genetic risk factors for schizophrenia, demonstrate cognitive impairments, including episodic memory (EM) dysfunction. Our group previously showed that EM is impaired in a mouse model for the 22q11.2DS (Df(16) A + /-). Place cells, cellular representations of EM, are under strong inhibitory control by heterogeneous subtypes of GABAergic interneurons, which have been implicated in the pathophysiology of schizophrenia. In this study, we examined the contribution of pyramidal cells and hippocampal interneuron subtypes to local microcircuit dysfunction in CA1.

Methods: 2-photon imaging of CA1 pyramidal neuron population dynamics were performed in vivo to characterize plasticity during novel context exposure in a virtual environment in Df(16) A + / − and WT mice ($n = 2$ Wt, $n = 2$ Df16). Wild-type and Df(16) A + /− mice performed goal-oriented learning, random foraging and reversal learning tasks on a cued belt while undergoing large-scale, unbiased 3D GCaMP-Ca2+ imaging of in vivo CA1 interneuron dynamics ($n = 6$ Wt, $n = 5$ Df16). Molecular identification of major interneuron subtypes was performed post-hoc utilizing immunohistochemistry. Interneuron subtype activity was assessed through Pearson cross-correlations with velocity and through peristimulus time histograms around behavioral indicators.

Results: In Df(16)A + / − mice we observe a significant decrease in CA1PC somatic bursting rate during context switch compared with WTs, suggesting that plasticity is suppressed in vivo ($n = 2832$ WT, $n = 1732$ Df(16)A + / − cells, $p < 0.001$). Interneurons exhibit subtype-specific alterations in activity during locomotion, and a significant overall decrease in place preference ($n = 8$ Wt, $n = 7$ Df(16)A + / − , $F = 2.111$, $p = 0.045$).

Conclusions: Results examining CA1 principal neuron dynamics and plasticity collected in vivo and in vitro suggest that inhibitory circuits are either over-compensating in vivo or are intrinsically deficient themselves. We identify subtype-specific alterations in interneuron dynamics contributing to microcircuit dysregulation, ultimately resulting in a less stable microcircuit in CA1 during learning.

Keywords: Cognitive Impairment Associated With Schizophrenia, Interneurons, 22q11.2 Deletion Syndrome, Memory and Learning

Disclosure: Nothing to disclose.

P582. Perineuronal Nets Degradation in the Ventral Hippocampus of Adult Rats Partially Recreates an Adolescent-Like Phenotype of Stress Susceptibility

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Background: The onset of some psychiatric disorders, such as schizophrenia, often occurs in late adolescence and/or early adulthood, which is a period of greater vulnerability to socio-environmental factors. Evidence suggests that stress during this period may affect brain structures that are important in regulating stress response, such as the ventral hippocampus (vHipp), leading to a dopamine system overdrive, which has been associated with psychotic symptoms. Parvalbumin (PV) interneurons in the vHipp are proposed as a site of vulnerability to adolescent stress. Here, we assessed the long-lasting effects of exposure to stress during adolescence or adulthood on behavior, the activity of dopamine neurons in the ventral tegmental area (VTA), and the maturation of PV interneurons and their associated perineuronal nets (PNNs) in the vHipp. We also tested whether the removal of PNNs in the vHipp in adult rats, which is proposed to reset PV interneurons to a juvenile-like state, recreates an adolescent-like phenotype of stress susceptibility.

Methods: Male Sprague-Dawley rats were exposed to a combination of stressors, consisting of daily footshock (1.0 mA, 2 s, randomized every 60 ± 20 s) for 10 days (adolescence: PND 31-40; adulthood: PND 61-70), and three 1-hour restraint stress sessions (days 1, 2 and 10), right after the footshock session. Between 2-3 weeks after the end of the stress, the animals were submitted to different behavioral tests to evaluate anxiety [elevated plus-maze (EPM) and light-dark box], sociability (social interaction test), cognitive function [novel object recognition (NOR) test], anhedonia (splash-test), and hyperresponsivity to psychostimulants (locomotor response to the NMDA receptor antagonist MK-801). The impact of stress on in vivo electrophysiological activity of dopamine neurons in the VTA and the number of PV + and PNN + cells in the vHipp were also studied. Only males were used in this study since we have previously found that female rats were resistant to present behavioral and electrophysiological changes after exposure to the same stress protocol. In a second set of experiments, adult animals received intra-vHipp bilateral infusion of chondroitinase ABC (0.05U/μL, 700 nL), which degrades PNNs, or penicillinase (control – an enzyme inert in mammals). One week later, animals were submitted to stress and tested 2-3 weeks post-stress.

Results: Adolescent stress exposure ($n = 12$ naïve and 12 stressed) produced in adult rats anxiety responses in the light-dark box (decreased the time in the light compartment, $t(22) = 4.4$; $p = 0.002$), decreased social interaction ($t(22)=3.1$; $p = 0.005$), impaired cognitive function in the NOR test ($t(22) = 4.6$; $p < 0.0001$), and increased locomotor response to MK-801 ($t(22)=2.5$; $p = 0.02$). Furthermore, adolescent stress increased the number of spontaneously active VTA dopamine neurons (t(10)

=4.3; p = 0.001), along with a decrease in the number of PV + (t(10)=2.5; p = 0.03), PNN + (t(10)=7.6; $p < 0.0001$), PV + / PNN + cells (t(10)=4.9; p = 0.0006) in the vHipp. Correlation matrix analysis indicated that increased VTA dopamine system activity was highly correlated with anxiety behaviors, impairments in social interaction and cognitive function induced by adolescent stress. In contrast, adult stress ($n = 12$ naïve and 12 stressed) did not induce long-lasting changes in behavior, electrophysiology and the number of PV + , PNN + , PV + /PNN + cells in the vHipp. However, preliminary data indicate that the infusion of ChABC into the vHipp ($n = 8$-6/group) causes the adult stress to produce cognitive deficits in the NOR test and increase VTA DA neuron population activity ($p < 0.05$ vs. penicillinase-naïve), similar to that induced by the adolescent stress. The degradation of PNNs in naïve animals did not produce any change.

Conclusions: Our findings indicate that adolescent stress induced a schizophrenia-like hyperdopaminergic state which was highly correlated with long-term anxiety-like behaviors, cognitive and social interaction impairments, suggesting that adolescence may be a period of higher vulnerability for the development of schizophrenia in adulthood. In addition, degradation of perineuronal nets in the vHipp of adult rats partially recreates an adolescent-like phenotype of stress susceptibility, further suggesting that PNNs play an important role in protecting PV + interneurons from the deleterious effects of stress. Financial support: Sao Paulo Research Foundation (18/17597-3; 21/03391-7).

Keywords: Stress, Parvalbumin Interneurons/Perineuronal Nets, Ventral Hippocampus

Disclosure: Nothing to disclose.

P583. Cholinergic Modulation of Hallucination-Like Perception

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Background: Psychosis involves excessive dopamine release in the striatum. Cholinergic interneurons are crucial for controlling striatal dopamine release and are reduced in numbers in psychotic disorders. However, the role of striatal acetylcholine in psychosis and its interplay with dopamine are not well understood, mainly because psychosis is challenging to study in animal models.

Methods: Here, we employed a recently established task to measure hallucination-like perception in mice. Using optogenetics and dual-colour photometry, we tested how the activity of striatal cholinergic neurons relates to hallucination-like perception and to dopamine release.

Results: We found that optogenetic inhibition of striatal cholinergic transmission significantly increased hallucination-like perception (F(1,3)=21.0, p = 0.02). Preliminary results indicated that activity of cholinergic neurons peaked before hallucination-like perception and was inversely related to dopamine release.

Conclusions: These results suggest that reduced striatal cholinergic transmission might give rise to psychotic experiences via increasing dopamine release. If our findings are confirmed, novel antipsychotic treatment strategies could target striatal cholinergic interneurons.

Keywords: Hallucinations, Psychotic Disorders, Acetylcholine, Striatum, Mouse Model

Disclosure: Nothing to disclose.

P584. What is Odd about the Oddball: A Time-Frequency Deconstruction of Auditory P300 Deficits in Schizophrenia

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Background: The auditory P300 (P3) event-related potential (ERP) is among the best established biomarkers of cognitive dysfunction in schizophrenia (Sz). Sz-like deficits in P3 generation may be induced by ketamine, potentially implicating N-methyl-D-aspartate receptor (NMDAR)-medicated neurotransmission as well as by psychedelics such as psilocybin and LSD. However, the neural mechanisms underlying impaired P3 generation in Sz remain poorly understood at both local- and distributed- circuit levels. Here, we used a combined temporal dynamic and resting-state functional connectivity (rsFC) approach to evaluate both distributed and local circuit mechanisms underlying impaired P3 generation in Sz. Time-frequency deconstruction of the P300 allowed interrogation of distinct underlying processes reflected in alpha (thalamocortical input), theta (cortico-cortical interactions), and delta (neural entrainment) frequency bands.

Methods: Twenty healthy controls (HCs) and twenty outpatients with Sz were enrolled in the study. Tone-matching thresholds were measured to assess baseline auditory pitch discrimination function. Participants completed an adaptive auditory oddball task during EEG acquisition that normalized difficulty across individuals. Both ERP and time-frequency analyses (TFAs) were conducted. Hierarchical mixed-model regressions with confirmatory mediation modeling of responses to standard and target stimuli were performed to elucidate relationships between TFA measures and P3 amplitude. In addition, participants underwent resting-state fMRI scans to measure functional connectivity (FC) between surface-based resting-state networks defined in the Gordon et al. 2016 atlas, which parcellates the cortical surface based on FC data. Mixed model regressions with FC to the auditory network and group as fixed factors and EEG delta entrainment as a dependent measure were performed.

Results: Tone-matching thresholds were nearly identical between HCs and Sz patients (p = 1.0) suggesting the two groups demonstrated intact pitch discrimination. Mean mismatch negativity (MMN) amplitudes also did not differ between groups (p = 0.93), validating the normalization of oddball task difficulty. As expected, patients demonstrated deficits in P3 amplitudes compared to HC ($p < 0.01$). P3 generation was mediated by a combination of delta entrainment to standards ($p < 0.01$) and by early alpha activity to targets ($p < 0.01$). There were deficits in both delta entrainment ($p < 0.001$) and early alpha activity ($p < 0.01$) in patients. Delta entrainment strength was associated with FC of visual ($p < 0.01$) and sensorimotor hand ($p < 0.001$) networks to the auditory network. Furthermore, differential associations between FC to the auditory network and delta entrainment were observed between HCs and patients to sensorimotor hand ($p < 0.001$), default ($p < 0.01$), and cingulo-opercular ($p < 0.05$) networks in group by FC interactions.

Conclusions: P3 generation may be conceptualized primarily as a prediction error involving the delta-oscillatory system. In Sz, deficits are observed in both the initial alpha-frequency cortical response to targets and to stimulus-induced delta entrainment to standards, which, in turn, predict impaired delta responses to targets. All components of this response are impaired in Sz, suggesting impaired input to auditory cortex. Delta entrainment represents a key component of "active sensing" during active auditory processing. Both ERP and resting-state fMRI findings suggest that individuals with Sz may need to use active top-down mechanisms to compensate for impaired function of low-level auditory systems.

Keywords: Schizophrenia (SCZ), Neuronal Oscillations, Resting State Functional Connectivity, P300, EEG/ERP Electrophysiology

Disclosure: Nothing to disclose.

P585. Steady State and Harmonic Responses: Cross-Species Similarities and Differential Responses to the NMDA Antagonist, Memantine

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Background: Translational biomarkers are critical for psychiatric drug discovery, to prioritize lead molecules, signal entry of the drug into the brain, and to demonstrate a functional change to help dose selection. Biomarkers that index circuit level processes may bridge cellular changes with functional and behavioral outcomes. Our investigative group has studied electroencephalographic (EEG) measures of the Auditory Steady State Response (ASSR) to a 40 Hz click train as a potential cross-species biomarker for treatment development for schizophrenia (SZ). When spectrally complex click trains elicit ASSR, in addition to entrainment at the driving frequency, time-locked EEG harmonics produce a Steady State Harmonic Response (SSHR); ASSR and SSHR exhibit distinct characteristics and support distinct cognitive functions and thus may have different predictive properties as biomarkers. While the ASSR at 40 Hz is viewed as a potential biomarker of NMDA receptor functioning, the SSHR has not been evaluated as a target engagement measure. Here we report on the ASSR 40 Hz response and its 80 Hz SSHR in rodents and humans, and their sensitivity to acute pharmacologic challenge with the non-competitive NMDA antagonist, memantine (MEM).

Methods: Ten Sprague-Dawley rats (age 6-8 weeks) were implanted with epidural temporal (recording) and bilateral cerebellum (ground / reference) electrodes. After at ~ 2-weeks, rats were tested 90 min after MEM (0, 0.3, 1 and 3 mg/kg sc) in a repeated measures within-subject crossover design, with > 3 d between tests. EEG was recorded in individual Plexiglas cylinders equipped with a video camera and a house speaker. Data were acquired as 5-s sequential sweeps; click train used 5 mV monophasic, 1-ms square waves, 40/1 s, ~ 65 dB. Evoked ASSR and SSHR responses were band-pass filtered (35–45 and 75-85 Hz) and averaged from ~ 75 consecutive trials. Evoked Power (EP) and inter-trial coherence (ITC) were calculated using the 0.2-1.0 s data. Eighty-two human subjects, including 44 patients with schizophrenia (SZ) and 38 healthy subjects (M:F = 48:34; age = 33.4 ± 9.7 (mean±s.d)) were included. EEG was continuously recorded via established methods starting 385 min after MEM (0 or 20 mg, po) in a repeated measures within-subject crossover design, with 7 d between tests. The ASSR paradigm utilized 1 ms, 85 dB clicks presented in 500 ms trains at a frequency of 40 Hz. A total of 250 click trains were played through insert earphones with an inter-train interval of 0.5 s. For EP, the averaged epochs across the click trains were transformed into power spectrum by means of fast Fourier transform (FFT) using a bin width of 2 Hz. ASSR and SSHR ITC were calculated from wavelet coefficients obtained from continuous wavelet transformation; ITC was estimated by extracting and averaging across the 35–45 Hz and 75-85 frequency layers.

Results: In rats, 40 Hz driving stimuli evoked both an ASSR at 40 Hz and an SSHR at 80 Hz. For ASSR, ANOVA of EP showed significant effects of frequency ($p < 0.0001$; 40 Hz > 80 Hz) and treatment ($p = 0.03$); post-hoc tests showed significant EP augmentation by the 3 mg/kg MEM dose ($p = 0.001$). ANOVA of ITC showed a significant frequency x treatment interaction ($p = 0.015$); post-hoc testing showed significant augmentation by the 3 mg/kg MEM dose ($p < 0.05$). For the 80 HZ SSHR, no significant effects of MEM were detected on either EP or ITC. In humans, 40 Hz driving stimuli evoked both an ASSR at 40 Hz and an SSHR at 80 Hz. For ASSR, ANOVAs of EP and ITC showed significant main effects of frequency (p's < 0.0001; 40 Hz > 80 Hz); for EP at 40 Hz ASSR, there was a significant main effect of diagnosis and a significant diagnosis x treatment interaction (p's < 0.05); post-hoc testing revealed that the interaction was driven by MEM-induced enhancement of EP in SZ patients. Similar trends were observed for ITC at 40 Hz ASSR. For the 80 Hz SSHR, no significant effects of MEM were detected on either EP or ITC (both ns).

Conclusions: Across species, auditory stimulation by complex stimuli evokes EEG entrainment at both the stimulus driving frequency (ASSR) and its harmonics (SSHR). These two responses may be regulated by distinct neural substrates and may in turn regulate different cognitive processes. Compared to the 40 Hz ASSR, relatively less is known about the harmonic response to 40 Hz stimulation. Here, we report that, in rodents and humans, the 40 Hz ASSR exhibits greater power and inter-trial coherence than its 80 Hz second harmonic. Furthermore, across species, the 40 Hz ASSR power and coherence are enhanced by the non-competitive NMDA antagonist, MEM, while the SSHR is insensitive to MEM at the doses tested. In parallel studies, Digavalli et al. (in preparation) found that the 40 Hz SSHR to a 20 Hz driving frequency was disrupted by the NMDA antagonist, MK801, while the 20 Hz ASSR response was unaffected. The present results suggest that ASSR and SSHR: 1. can be studied across species; 2. produce different levels of evoked power and coherence (here, at 40 Hz, ASSR » SSHR), and these differences are maintained across species; 3. exhibit differential drug sensitivity, with MEM increasing 40 Hz ASSR but not its SSHR, and this difference is maintained across species. In total, ASSR and SSHR appear to be robust, non-redundant EEG signals suitable for cross-species analyses and potential biomarker development.

Keywords: Cross-Species Translation, Biomarker Development, Schizophrenia Therapeutics

Disclosure: Nothing to disclose.

P586. State-Dependent Hippocampal Hyper Synchronous Activity in a Reverse Translational Psychosis Model Mouse

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Background: Hyperactivity in the hippocampal (HPC) region was first identified as one of the prominent biomarkers in psychotic individuals, initially using PET brain imaging in early 2000's, drawing significant interest in its underlying mechanisms. Moreover, the hyperactivation of the HPC has been identified as an informative human biomarker of schizophrenia especially in young patients. Recent studies of human schizophrenia post-mortem brain tissue have shown decreased expression of NMDAR subunit GluN1 in the dentate gyrus (DG) with evidence of hyperactivity downstream in the hippocampus downstream Cornu Ammonis (CA) subregions. There is however little insight into the emergence of such hyperactivities in the HPC subregions and the time course of their development. Therefore, our group has reverse translated this feature into a selective DG-inhibited mouse model and conducted immunohistochemistry, behavior tests and long-term in vivo electrophysiology studies to determine the dynamics of the hippocampal hyperactivity in both adolescent and adult conditions.

Methods: 4 weeks old (for adolescent condition) and 8 weeks old (for adult condition) male C57BL/6 J mice were purchased from Jackson Labs. To specifically manipulate DG in a temporally controlled manner, we expressed an inhibitory DREADD (Designer Receptors Exclusively Activated by Designer Drugs, pAAV-CaMKIIa-hM4Di-mCherry) in the granule cells of the entire DG at 5 and

9 weeks of age, and started inhibiting the DG with Compound 21 (C21) for 21 days in adolescent (6 week) and adult (10 week) C57BL/6 J mice. For control, pAAV-CaMKIIa-eGFP was used. At 5th or 9th week of age, a 32 channel silicon linear array electrode was chronically implanted in the dorsal HPC to monitor the DG - CA1 axis. The daily 1 hour recording session started a few days before the start of the C21 administration to obtain baseline data and continued up to 2 months including the three weeks of C21 exposure between 6 to 9 weeks of age for the adolescent and 10 to 13 weeks of age for the adult conditions. Following removal of C21, behavior testing was conducted with social memory (SM) test and standard contextual and cued fear conditioning (CFC). One week after cessation of C21 exposure, brains were taken and assessed for cFos-positive cell density within the hippocampal subfields. For the in vivo electrophysiology, both wide-band local field potentials (LFPs) and multi-unit spike activity (MUA) were recorded at 32 kHz sampling rate. The LFPs were then band-pass filtered into sub-bands for all 32 recording points. The multi-unit spiking data was temporally aligned with LFPs and spike timing modulation in relation to the LFP amplitude were analyzed.

Results: When the DG was suppressed by the DREADD system during adolescent period, the cFos-positive (+) cell density was significantly increased in both CA3 and CA1 subregions ($N = 7$/group, $P < 0.05$), but not in DG itself. However, when the DG was inhibited during adulthood, cFos+ cells decreased in DG as well as in CA3 ($N = 6$/group, $P < 0.001$, $P < 0.01$ respectively), but not in CA1. We interpret that the significant increase in cFos expression, which indicates the hyperactivity in both CA3 and CA1 during adolescent period, mimics the HPC hyperactivity that was reported earlier in human ScZ. Using this system, we then subjected the mice to the SM test and the CFC test. In both behavior testing paradigms, only the adolescent DREADD mice group showed memory phenotypes ($N = 13$, SM: $P < 0.001$, CFC: $P < 0.05$), but not in the adult DREADD mouse group ($N = 18$), suggesting that there is a risk time window to exhibit memory impairments with the abnormal level of hyperexcitation in the HPC. Next, we analyzed the LFP data ($N = 5$ adolescent pairs and $N = 3$ adults). While we monitored the activities on a day-to-day basis, short-lived large irregular potentials that are accompanied by a large population of MUA (dubbed Hyper-Synchronous Events: HSEs) emerged at around day 7 at extremely low occurrence (once every 5 minutes) while animals were in either quiet awake or slow-wave sleep states, but not during awake state. Subsequently, the HSEs became more and more obvious as the days went by, and by day 14, HSE became extremely frequent (once every 10-15 sec). Among 5 adolescent pairs, we observed epileptiform/seizure-like events developed in two DG-DREADD mice during quiet wake and/or slow-wave sleep periods. During high HSE occurring periods, the spontaneous homecage behavior deteriorated in such a way that their nest in the homecage became disorganized. Moreover, the reduced-level of HSEs (once every minute or so) persisted even after the C21 treatment, i.e. the DG inhibition period, was over. This HSE development was observed neither in the control adolescent mice group nor in the adult mice and was specific to the adolescent DG-DREADD mice group suggesting that the HSE-like abnormal electrical discharges might also be occurring in the human ScZ HPC.

Conclusions: Adolescence age in mice appears to be a critical period in which decreased DG activity can induce hippocampal hyperactivity including epileptiform-like neural activity in the HPC, an outcome not observed in adult mice. This hippocampal hyperactivity originates as a transient increase in activity in CA3, and persists in CA1, defined by the presence of HSEs. Moreover, a psychosis-like behavioral phenotype tracks with CA3 activity. This suggests a sensitive period of development in which the brain is sensitive to alterations in hippocampal activity and can result in psychosis-like behavioral outcomes.

Keywords: Psychosis, Hyperactivity, Hippocampus, In Vivo Electrophysiology

Disclosure: Nothing to disclose.

P587. Associations Between Structural Stigma and Allostatic Load: Results From the National Health and Nutrition Examination Survey

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Background: Structural forms of stigma and discrimination are associated with a host of adverse outcomes across numerous stigmatized groups. Yet, the biological mechanisms underlying this relationship remain inadequately understood. To address this gap, the current study assessed the relationship between indicators of structural stigma surrounding lesbian, gay, and bisexual (LGB) individuals (i.e., state-level policies) and allostatic load indices representing physiological dysregulations.

Methods: Pooled data from the continuous 2001-2014 National Health and Nutritional Examination Survey was analyzed ($N = 21{,}774$). Ten state-level LGB-related policies (e.g., employment non-discrimination protections, same-sex marriage rights) were used to index structural stigma. A sex-specific allostatic load index representing immune, metabolic, and cardiovascular biomarkers was calculated. Multi-level regressions adjusting for covariates were used.

Results: Sexual minority men living in states with more policies protecting sexual minorities experienced significantly lower allostatic load ($\beta = -.45$, $p = 0.02$) than those living in states with fewer protective policies.

Conclusions: This is the first study to assess structural stigma at the state level in association with allostatic load among members of a stigmatized group. Our novel findings provide a mechanistic understanding of how the social environment can 'get under the skin and skull' for sexual minority Americans, with potential implications for other marginalized groups. This work also has social policy implications by demonstrating direct impacts of structural stigma on indices of physiological dysregulation.

Keywords: Allostatic Load, Sexual Orientation, Structural Stigma

Disclosure: Nothing to disclose.

P588. Effect of Lemborexant on Sleep Onset and Maintenance in Patients With Comorbid Insomnia Disorder and Mild Obstructive Sleep Apnea

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Background: Insomnia and obstructive sleep apnea (OSA) are common sleep disorders that are frequently comorbid, a clinical entity referred to as comorbid insomnia and sleep apnea (COMISA). Studies of hypnotics in patients with OSA often focus on the impact of the drug on respiratory variables such as the apnea-hypopnea index (AHI), since commonly prescribed hypnotic drugs may exacerbate pre-existing respiratory dysfunction. Additional concerns about the selection of a hypnotic for concomitant therapy for OSA include residual sedation in a somnolent patient population and the known risks of hypnotics for the treatment of insomnia. Lemborexant (LEM), a dual orexin receptor antagonist, is approved in multiple countries for the

treatment of insomnia disorder. Respiratory safety studies in patients with untreated mild-severe OSA indicated no negative impact on either the AHI or peripheral oxygen saturation following single or multiple doses of LEM 10 mg (LEM10), the higher approved dose (Cheng et al., J Sleep Res 2020). A post-hoc analysis was conducted to evaluate the effects of LEM, which has demonstrated respiratory safety in OSA, for the treatment of insomnia in patients with mild OSA.

Methods: Study E2006-G000-304 (Study 304; NCT02783729) was a 1-month, randomized, double-blind, placebo (PBO)-controlled and active-comparator (zolpidem tartrate extended release 6.25 mg [ZOL]) study of LEM 5 mg (LEM5) and LEM10 (1006 randomized). Subjects (male and female) age ≥55 y who met criteria for insomnia disorder per DSM-5 and with verified sleep maintenance problems were enrolled; subjects were not required to have difficulty with sleep onset. A screening polysomnogram (PSG) with respiratory montage was used to rule out moderate to severe OSA. Subjects whose AHI was <15 events/h of sleep (mild OSA) were eligible, assuming other criteria were met. Sleep onset (latency to persistent sleep [LPS]); sleep maintenance (sleep efficiency [SE] = total sleep time / time in bed); wake after sleep onset (WASO); and WASO in the second half of the night (WASO2H) were assessed at Days 1/2 and 29/30 using PSG and averaged across consecutive nights. Pairs of PSG during the PBO run-in served as the baseline. Change from baseline (CFB) was analyzed using mixed-effect model repeated measurement analysis. CFB means are reported (rounded to the 10th decimal) as least squares geometric mean ratio: visit/baseline for LPS and least squares means for SE, WASO, and WASO2H. These post-hoc analyses focused on the subpopulation of patients with both insomnia disorder and mild OSA.

Results: The Full Analysis Set (FAS) included 1006 subjects, of whom 410 (40.8%) had mild OSA. In the mild OSA subpopulation, median age was 65 y, 83.9% were female, and median body mass index was 27.56 (29.8% had BMI > 30); AHI (mean [SD]) on screening PSG was 9.33 (2.9) events/h. CFB for SE (%) was larger and statistically significant (increases = improvements) for both LEM5 and LEM10 (herein LEM5-LEM10) (13.1-15.8) vs PBO (3.4, $P < 0.0001$) and ZOL (11.2, $P < 0.05$) on Days 1/2; similar results were observed on Days 29/30 (LEM5-LEM10 12.8-14.0; PBO 4.4, $P < 0.0001$; ZOL 8.7, $P < 0.0001$). CFB for LPS, WASO, and WASO2H was larger and statistically significant (decreases = improvement) for LEM5-LEM10 vs PBO on Days 1/2 (LPS [ratio]: LEM5-LEM10 0.58-0.68 and PBO 0.89, $P < 0.005$; WASO [min] LEM5 LEM10 −48.8 to −57.6 and PBO −11.3, $P < 0.0001$; WASO2H: LEM5-LEM10 −28.6 to −36.1 and PBO −4.7, $P < 0.0001$) and Days 29/30 (LPS [ratio]: LEM5-LEM10 0.47-0.58 and PBO 0.80, $P < 0.005$; WASO [min]: LEM5-LEM10 −44.4 to −45.2 and PBO −16.0, $P < 0.0001$; WASO2H [min]: LEM5-LEM10 − 27.0 to −28.7 and PBO −9.2, $P < 0.0001$). LEM10 produced larger CFB vs ZOL for LPS, WASO, and WASO2H at Days 1/2 (ZOL LPS: 0.72, $P < 0.01$; WASO: −41.9, $P < 0.001$; WASO2H: −22.2, $P < 0.0001$) and Days 29/30 (ZOL LPS: 0.83, $P < 0.0001$; WASO: −33.1, $P < 0.01$; WASO2H: −18.7, $P < 0.005$), and LEM5 ($P < 0.02$ for all assessments) on days 29/30. LEM was well tolerated, with a safety profile in the subpopulation consistent with its known safety profile.

Conclusions: These results demonstrated the effectiveness of LEM compared with both PBO and ZOL in an older patient population with untreated COMISA, characterized by sleep maintenance complaints meeting insomnia disorder criteria and mild OSA. These data and those from the respiratory safety studies suggest LEM may be an appropriate choice for the treatment of insomnia in patients with mild OSA.

Keywords: Comorbid Insomnia Obstructive and Sleep Apnea (COMISA), Obstructive Sleep Apnea, Apnea-Hypopnea Index, Lemborexant, Dual Orexin Receptor Antagonist (DORA)

Disclosure: Eisai Inc.: Employee (Self).

P589. The Acute and Chronic Effects of Zolpidem and Lemborexant on Sleep Oscillation Expression in Older Adults With Insomnia

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Background: Insomnia symptoms increase in prevalence with age and are among the most common sleep disturbances reported in older adults. Two biological systems targeted to treat insomnia include the GABAergic and orexigenic systems. It remains unknown how use of medications that target these systems to treat insomnia differentially impact the expression of sleep oscillations supportive of the physical and mental health functions of sleep, particularly among older adults with insomnia. Here, we compare the acute and chronic effects of zolpidem and lemborexant, in comparison to placebo, on quantitative sleep electroencephalography (EEG) among older adults with insomnia.

Methods: One hundred sixteen older adults (71.01 ± 4.81 years, 78.67% female) with insomnia (Insomnia Severity Index 18.73 ± 3.13) completed a randomized, phase III clinical trial (E2006-G000-304; NCT02783729) comparing the effects of placebo ($n = 29$), zolpidem tartrate extended release (6.25 mg, $n = 26$; ZOL), and lemborexant (5 mg, $n = 29$ [LEM5]; 10 mg, $n = 32$ [LEM10]) on polysomnography(PSG)-recorded sleep across baseline (before), acute (1-2 days) and chronic (29-30 days) treatment. Spectral analysis was performed using the multitaper method on artifact-free EEG data during non-rapid eye movement (NREM) sleep epochs. Changes in absolute spectral power within canonical frequency bands (i.e., slow-oscillation [0.5-1 Hz], delta [1-4.5 Hz], theta [4.5-7.5 Hz], alpha [7.5-11 Hz], slow-sigma [11-13 Hz], fast-sigma [13-16 Hz], beta [16-28 Hz] and gamma [28-35 Hz]) from baseline were compared between groups at averaged frontal electrodes (i.e., F3, F4) across acute and chronic treatment phases using repeated measures ANOVA (RMANOVA) models with Fisher's Least Significant Difference post hoc testing (fixed factors: visit, frequency; between factor: drug intervention). In addition, changes from baseline in objective measures of sleep quality [SE: sleep efficiency, SOL: sleep onset latency, WASO: wake after sleep onset] were calculated. Further, the calculated sleep measures at chronic were used in a multivariate ANOVA to test for difference in change in sleep quality measures from baseline after chronic use of medications.

Results: Examining the effect of treatment on objective sleep quality, a MANOVA analysis model predicting objective sleep quality was significant ($p = 0.02$), with a significant intervention effect on WASO ($p = 03$) and SOL ($p = 0.04$) being detected. Post hoc comparisons revealed that chronic treatment impacted objective sleep quality differentially by treatment group. Compared to PBO, sleep quality improved significantly more following both LEM5 [SE $p = 0.05$, WASO $p = 0.01$] and LEM10 [SE $p = 0.05$, SOL $p = 0.03$, WASO $p = 0.04$] treatment. In addition, following chronic treatment, sleep quality, compared to ZOL, improved more for LEM5 [WASO $p = 0.04$, SOL $p = 0.05$] and LEM10 [SOL $p = 0.02$].

Examining the effect of treatment on absolute power, a 3-way RMANOVA revealed 1) a significant main effect of frequency [$F(7,784)=8.05$, $p < 0.001$], 2) a significant visit×frequency interaction [$F(7,784)=2.68$, $p = 0.009$], 3) a significant frequency×intervention interaction [$F(21,784)=4.99$, $p < 0.001$], and 4) a significant visit×frequency×intervention interaction [$F(21,784)=2.67$, $p < 0.001$].

Post hoc testing revealed a greater acute suppression in power for ZOL compared to the PBO [delta $p < 0.001$, theta $p < 0.001$, alpha $p < 0.001$], LEM5 [delta $p = 0.00$, theta $p < 0.001$, alpha

$p < 0.001$], and LEM10 [delta $p = 0.01$, theta $p < 0.001$, alpha $p = 0.001$] treatment groups. Post hoc testing also revealed chronic suppression in power for ZOL compared to the PBO [delta $p < 0.001$, theta $p < 0.001$] and LEM10 [slow oscillation $p = 0.04$, delta $p < 0.001$, theta $p = 0.004$] groups. However, chronic ZOL treatment was also associated with greater increases from baseline in power compared to LEM5 [slow-sigma $p < 0.001$, fast-sigma $p = 0.006$, beta $p = 0.06$-trend] and LEM10 [slow-sigma $p = 0.008$] groups. Further, following chronic treatment, LEM5, compared to PBO, showed a suppression of delta ($p = 0.02$), theta ($p = 0.04$), alpha ($p = 0.04$), and slow sigma ($p = 0.05$).

Conclusions: In older adults reporting moderate to severe insomnia symptoms, chronic treatment with lemborexant showed greater improvement in objective sleep quality relative to zolpidem and placebo. In addition, chronic treatment with lemborexant and zolpidem had distinct effects on sleep oscillation expression, with zolpidem suppressing low frequency activity and increasing high frequency activity, lemborexant 5 mg suppressing both low and high frequency activity, and lemborexant 10 mg showing no significant effect. Future studies should examine the functional consequences of these differential effects on sleep oscillation expression in insomnia patients.

Keywords: Insomnia Disorder, Sleep Oscillations, Older Adults

Disclosure: Nothing to disclose.

P590. Sleep Disturbance Associated With Suicide Risk for Children in the Adolescent Brain Cognitive Development Study

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Background: Suicide risk in children is a growing concern. During the past decade, the pediatric suicide rate has nearly tripled (Curtin and Heron, 2019) and a recent meta-analysis estimated the prevalence of suicidal ideation in children younger than 12 years of age is 7.5% (Geoffroy, et al., 2022). There is a critical unmet need to identify risk factors associated with suicidal thoughts and behaviors (STB) during childhood to design developmentally appropriate preventative strategies. Sleep disturbances including insomnia, hypersomnia, and nightmares have been linked to risk for suicide in studies of adults and adolescents (Harris et al., 2020; Goldstein, Bridge, and Brent, 2008) yet, few studies have examined sleep disturbance and associated risk for suicide in children. The purpose of this study was to examine the relationship between sleep disturbance and STB in children.

Methods: This study utilized baseline data (data release 4.0) from 11,869 participants in the Adolescent Brain Cognitive Development℠ Study (ABCD Study®), a longitudinal study that follows nine- and ten-year-old children through late adolescence to examine factors that influence developmental trajectories. Youth STB was assessed by the Kiddie Schedule for Affective Disorder and Schizophrenia (KSADS) suicide module completed separately by the parent and the youth. Recent child STB included any suicidal thoughts or attempts reported by the parent or child during the previous two weeks. Parents completed the Sleep Disturbance Scale for Children (SDSC) which assessed youth sleep disturbance in the previous 6 months including disorders of initiating and maintaining sleep, sleep-disordered breathing disorders, disorders of arousal, sleep-wake transition disorders, disorders of excessive somnolence and sleep hyperhidrosis. Generalized additive mixed models were used to examine the relationship between STB and sleep disturbance which modeled family nested within site as random effects controlling for age, sex, parent education, household income, and current symptoms of depression.

Results: At baseline, participants had a mean age of 9.9 and the sample included 6188 (52%) males and 5681 (48%) females. 330 youth had recent STB and 11,324 youth did not have recent STB. 63% of children with recent STB slept less than 9 to 11 hours compared to 52% of children without recent STB. Children with recent STB had higher scores on total sleep disturbance ($p = 0.01$; Cohen's $d = 0.13$), disorders of initiating and maintaining sleep ($p < 0.001$; Cohen's $d = 0.22$), and disorders of excessive somnolence ($p = 0.019$; Cohen's $d = 0.12$) compared to youth without recent STB. Forty-nine percent of children with recent STB had clinically significant sleep disturbance scores compared to 26% of children without recent STB had clinically significant sleep disturbance scores. There were no significant associations between recent STB and sleep breathing disorders, disorder of arousal, sleep-wake transition disorders.

Conclusions: Overall, our study revealed that in this large sample of youth between 9 and 10 years old, children with recent STB had greater sleep disturbance, particularly difficulty falling and staying asleep and excessive somnolence. These findings have important implications, as insufficient sleep and poor sleep patterns have become a common issue for children and adolescents worldwide (Gariepy, et al., 2020). Sleep disturbance is associated with decreased attention and inhibition and may be related to difficulty controlling thoughts of suicide and regulating emotions thereby increasing risk for suicide. The current study is cross sectional and cannot address whether sleep plays a causal role in STB; however, studies with adolescents have shown sleep disruption predicted future STB. Disturbed sleep is modifiable and sleep interventions may inform suicide prevention efforts. Future research should examine the longitudinal relationship between sleep disturbance and risk for suicide.

Keywords: Suicidal Behavior, Suicidal Ideation, Sleep Disturbances

Disclosure: Nothing to disclose.

P591. Acute Disruption of Affect and Daytime Alertness in Premenopausal Women Undergoing Experimental Sleep Fragmentation and Estradiol Withdrawal to Mimic Menopause

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Background: Animal studies show the adverse impact of sleep perturbation and estradiol withdrawal on both affective state and arousal and regulatory systems in females. However, few experimental studies translate these findings to humans, for whom middle-of-the-night sleep interruption and reproductive-hormone changes commonly co-occur with hot flashes (HF) during the menopause transition. Given the potential to intervene on HF-related sleep interruption as a modifiable target in order to improve the highly prevalent reduction in psychological well-being and daytime alertness among midlife women, we examined the acute effects of sleep fragmentation and estradiol withdrawal on these neuropsychological symptoms, and the moderating role of HF, using an experimental paradigm mimicking menopause.

Methods: We studied 27 premenopausal healthy female volunteers (age 28.7 ± 5.6 years) in two 5-night inpatient studies in 1) the mid-to-late follicular phase (estrogenized) and 2) following hypoestrogenism induced by a gonadotropin-releasing hormone agonist that provoked HF (detected objectively using

skin conductance methodologies) in 56% of study subjects. Each inpatient study had 2 nights of unfragmented sleep followed by 3 nights of experimentally induced sleep fragmentation to achieve approximately one hour of wake after sleep onset without reducing total sleep time. The following were assessed daily during the daytime of the unfragmented and fragmented sleep conditions: 1) affect on the Positive and Negative Affect Schedule (PANAS) to capture positive (PosAff) and negative affect (NegAff) (range 10-50 each), and, in a subset ($n = 16$), 2) daytime arousal and regulatory state through objective measurement of sustained attention using the Psychomotor Vigilance Task (PVT) mean reaction time (RT) in milliseconds and attentional failures (number of RT > 500 ms), as well as subjective measurement of sleepiness (Karolinska Sleepiness Scale, range 1-9). Acute effects of sleep condition (after one night of sleep fragmentation) and of estradiol withdrawal on PosAff, NegAff, PVT-RT, number of PVT-attentional failures, and sleepiness were examined using Generalized Linear Mixed Models testing interactions with HF.

Results: One night of sleep fragmentation acutely and adversely disrupted affective state (PosAff and NegAff), PVT-RT, and sleepiness (all $p \leq 0.039$) independent of estradiol state and HF. Estradiol withdrawal also had an adverse impact on PosAff, PVT-RT, and PVT-attentional failures (all $p \leq 0.001$), but not on NegAff or sleepiness. The effect size for most outcomes was small to medium (Cohen's d range 0.18–0.56). While HF did not independently influence these outcomes, HF were a robust effect modifier of the adverse impact of sleep fragmentation on both affective and arousal states (both 2-way interactions $p \leq 0.030$), such that those with both HF and sleep fragmentation had the lowest PosAff and the most attentional lapses. In 3-way interactions, HF were a robust effect modifier of the impact of estradiol-dependent effects of sleep fragmentation: those with HF appeared to be most sensitive to the effects of sleep fragmentation on PVT-attentional lapses, whereas their subjective perception of sleepiness was attenuated after estradiol was withdrawn and HF emerged (both interactions $p \leq 0.033$).

Conclusions: Experimental studies in humans demonstrate acute adverse effects of both menopause-pattern middle-of-the-night sleep interruption and estradiol withdrawal on psychological well-being and daytime alertness. Having HF influence the deleterious impact of sleep fragmentation on these outcomes, notably with worse affect and more objectively measured attentional lapses despite acclimation to the perception of sleepiness after a period of exposure to HF and hypoestrogenism. These findings highlight the potential therapeutic benefit of consolidating sleep, especially in combination with HF suppression, as a strategy to improve both affective state and sustained attention during the daytime in women undergoing the menopause transition.

Keywords: Women's Mental Health, Sleep Disturbance, Menopause, Estradiol, Hot Flashes

Disclosures: National Institutes of Health, Merck, Pfizer: Grant (Self), Bayer, Eisai, Hello Therapeutics: Consultant (Self), Merck: Other Financial or Material Support (Spouse), Arsenal Biosciences: Employee (Spouse).

P592. Effects of Electronic Noise Masking Ear Buds on Objective and Subjective Sleep Parameters in Health Care Providers

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Background: The importance of sleep amongst health care workers is undeniable, as good health and alertness are essential for the delivery of care to patients. The COVID-19 pandemic has had detrimental effects on sleep quality among health care workers, prompting the need to explore new interventions. The present study evaluated the effect of Bose Noise-Masking Sleepbuds(TM) in health care workers with reported sleep difficulties using both objective and subjective measures.

Methods: Seventy-seven ($n = 46$ females, $n = 31$ males) hospital-based health care workers, aged 20-50, completed this pre-post efficacy study. Participants had to self-identify as light or moderate sleeps with self-reported difficulty falling or staying asleep, and score at least an 8 ("subthreshold insomnia") on the Insomnia Severity Index. Objective sleep architecture data was obtained using the DREEM headband in-home sleep monitoring device, which records EEG signals, heart rate, movement and sound. Subjective measurements included perceptions of sleep onset latency, wake after sleep onset, total sleep time and scores on the Insomnia Severity Index. Participants first completed a 3-night baseline assessment wearing just the DREEM headband, followed by a 4-night adjustment period of wearing only the Sleepbuds(TM), and then a final 3-night intervention assessment wearing both the Sleepbuds(TM) and the Dreem headband. Primary outcomes were analyzed for statistical significance using mixed-effect linear regression and random intercept for each participant to model repeated measures over time.

Results: Use of the Sleepbuds(TM) was associated with a significant decrease in perceived sleep onset latency compared to baseline, with a 6 minute decrease from the baseline mean to intervention mean (21% faster, 95% CI: 8%-35%, $p = 0.003$, Cohen's $d = -0.38$). There was also a significant decrease in Insomnia Severity Index scores from baseline ($p < 0.001$) with a mean change score of -7.7 (4.4) points. There were no significant changes in objective sleep measures, including objective sleep onset latency, wake after sleep onset, number of awakenings or % time in various sleep stages (all p's > 0.05).

Conclusions: Compared to baseline assessments, the use of noise masking Sleepbuds(TM) in health care workers improved subjective sleep quality as indicated by a decrease in perceived sleep onset latency and decreases in Insomnia Severity Index scores. Lack of changes in objective sleep measures are likely due to the healthy sample population with lack of overt sleep pathology. Overall, the results suggest that noise masking ear bud technology holds promise as a non-pharmacological intervention to improve sleep in health care workers.

Keywords: Technology, Sleep, Insomnia

Disclosure: Nothing to disclose.

P593. Conversion of Suvorexant to Lemborexant and Study Under Environmental Conditions

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Background: Insomnia negatively affects physical and mental health and also increases the risk of traffic and workplace accidents. Approximately 20% of Japanese adults have chronic insomnia, and the rate is currently increasing. Patients are more likely to experience insomnia during hospitalization due to environmental changes, noises such as alarms, and changes in sleep habits. Suvorexant was the first approved dual orexin receptor antagonist (DORA). However, it was reported to be more effective in treating difficulties in maintaining sleep rather than in initiating sleep and was found to be effective in only 70%–75% of patients. A new DORA, lemborexant, is reported to have a numerically larger effect size on sleep measures compared with

placebo and suvorexant. This study aimed to examine the effects of a change in medication from suvorexant to lemborexant among patients with insomnia. The differences in sleep improvement effects between inpatient and elderly care facility environments are also examined.

Methods: Medical records of patients from three medical centers who received suvorexant in January 2021, including inpatients at the Mental Care Center of Showa University Northern Yokohama Hospital and patients at two clinics, were retrospectively studied. The patients visiting the two clinics resided in a long-term care facility.

Patients with chronic insomnia persisting for ≥3 months and who had been taking suvorexant for ≥3 months were selected. Participants were divided into two groups: the "modified" and "nonmodified" groups. When a drug change from suvorexant to lemborexant was required, informed consent was obtained from the patients and their families. The modified group was further divided into inpatient and facility groups. Four subtypes of insomnia (difficulty initiating sleep, difficulty maintaining sleep, early-morning awakening, and nonrestorative sleep) were investigated. Logistic regression was used to investigate improvements in both groups after 12 weeks.

The study design was approved by the Showa University Yokohama City Northern Hospital Ethics Committee.

Results: Of the 77 participants, 43 and 34 patients were included in the modified and nonmodified drug groups, respectively. In the comparison of sleep disorders between the two groups, we found significant improvement after 12 weeks in the modified drug group in terms of difficulty initiating sleep compared with the nonmodified drug group (odds ratio = 0.036, p = 0.008, confidence interval = 0.003–0.415). However, no significant differences were found between the two groups in terms of difficulty maintaining sleep, early-morning awakening, and nonrestorative sleep. There were only six and four cases of side effects in the modified and nonmodified drug groups, respectively.

Of the 43 patients in the modified drug group, 12 were in the inpatient group and 31 were in the facility group. There were no significant differences between the two groups in any insomnia disorder subtype.

Conclusions: Orexin recepter 2 (OX2R) has a stronger effect on maintaining arousal and circadian rhythm than orexin recepter 1 (OX1R). Similar to suvorexant, lemborexant shows an antagonistic inhibitory effect on both OX1R and OX2R, and lemborexant has a higher selectivity for OX2R than suvorexant. Therefore, compared with suvorexant, lemborexant increases non–rapid eye movement sleep and promotes sleep efficiency, resulting in shortened sleep latency and increased sleep maintenance effect. In this survey, the modified drug group showed significant improvements in difficulty initiating sleep compared with the nonmodified drug group. In addition, because suvorexant 15/20 mg is considered to be equivalent to lemborexant 5 mg, the average dose of lemborexant after 3 months was 6.750 (±2.750) mg. Lemborexant administration at ≥5 mg, as opposed to suvorexant at 20 mg, could have led to the significant improvement in the modified drug group.

In this study, only six and four cases of side effects were observed in the modified and nonmodified drug groups, respectively. Moreover, no serious side effects were observed, and no significant difference was observed between the two groups. Lemborexant 10 mg reportedly has a higher risk of somnolence than suvorexant 15/20 mg. Therefore, the dosage of lemborexant can be increased up to 10 mg in cases in which the effect is insufficient at 5 mg; however, because the risk of side effects increases at high doses, caution is required.

Sleep disorders can be treated by alleviating difficulties in initiating sleep by switching from suvorexant to lemborexant. In inpatient settings, where sleep disorders are more likely to occur, the effects were similar to those observed in facilities. In addition, it was confirmed that the change in drugs caused no serious side effects and that it was highly safe and tolerated. Therefore, the results of this study suggest that conversion to lemborexant is a promising and safe option for improving insomnia disorders in patients using suvorexant for difficulty initiating sleep.

Keywords: Insomnia Disorder, Lemborexant, Sleep Disorders, Dual Orexin Receptor Antagonist

Disclosure: Nothing to disclose.

P594. A Novel Orally-Available Selective Orexin 2 Receptor Agonist, E2086 Enhances Wake-Promotion and Treats Narcolepsy-Like Symptoms in Narcolepsy Model Mice

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Background: Orexin neurons in the hypothalamus are critical regulators of sleep/wakefulness states, and their loss is associated with narcolepsy type 1 (NT1). In patients with NT1, characterized by reduced orexin levels in cerebrospinal fluid (CSF), excessive daytime sleepiness and cataplexy are observed, both of which are diagnostic for NT1. Orexins act through two classes of G-protein coupled receptors, the orexin-1 receptor (OX1R) and the orexin-2 receptor (OX2R). Although both OX1Rs and OX2Rs are related to sleep-wake regulation, OX2Rs contribute more to sleep-wake regulation. Greater awake/non-rapid eye movement (NREM) sleep episode fragmentation and reduced duration of wakefulness in hypnograms have been seen for OX2R-knockout and double-receptor knockout mice compared with wild-type (WT) and OX1R-knockout mice. Therefore, an OX2R-selective agonist is expected to act as a wake-promoting drug. E2086 has demonstrated strong wake-promoting and cataplexy reduction in nonclinical studies. Here we report the in vitro and in vivo profiles of this novel, orally available OX2R-selective agonist.

Methods: Four nonclinical studies were conducted. (1) Lenti-X™ 293 cell lines that constitutively and stably express either human, mouse, or rat OX2R or OX1R were used to assess OX2R or OX1R-agonistic activity via calcium influx assay. (2) To confirm selectivity of E2086 on OX2R, in vitro binding assays on 86 off-targets were conducted at 1 and 10 uM. (3 and 4) To evaluate E2086-mediated wake-promoting effects in (3) WT mice and in (4) orexin-neuron deficient mice (orexin/ataxin-3 hemizygous mice), EEG/EMG recordings were conducted with E2086 or vehicle treatment during the active phase.

Results: (1) E2086 (0.03 – 10,000 nmol/L) activated human OX2R (EC50: 2.3 nmol/L) in the calcium influx assay without considerable species differences or activity on the human OX1R (EC50: 4700 nmol/L). (2) E2086 (1 and 10 uM) had no significant affinity (>50% inhibition in binding assay) on 86 receptors, transporters, and ion channels associated with important physiologic functions. (3, 4) Single dose oral administration of E2086 (0.3, 1, and 3 mg/kg) promoted wakefulness in both WT mice and orexin-deficient transgenic mice. Additionally, in orexin-deficient mice, latency of direct transitions from wake to REM sleep, which is a murine analog of human cataplexy, was also prolonged. After 14 days of repeated dosing in orexin-deficient mice, E2086 (3 mg/kg) significantly prolonged wakefulness duration and prevented cataplexy-equivalent episodes without tolerance throughout the treatment period.

Conclusions: These results suggest that E2086 is a selective and potent OX2R agonist, with wake-promoting and anti-cataplexy effects. Thus, these results support the potential for E2086 to improve wakefulness and other orexin deficiency-related

symptoms like cataplexy in patients with orexin network hypofunction such as narcolepsy Type 1 and suggest that E2086 may also improve wakefulness in patients with excessive daytime sleepiness across a range of orexin levels.

Keywords: Orexin, Narcolepsy, Wake-Promoting

Disclosure: Eisai, Co., Ltd.: Employee (Self).

P595. Inhibition of Kynurenic Acid Synthesis Protects Learning and Memory Despite Prolonged Sleep Deprivation in Rats

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Background: Sleep is highly conserved physiological process critical for learning and memory consolidation. Kynurenic acid (KYNA), a metabolite of the kynurenine pathway (KP) of tryptophan catabolism, has been shown to play a major role in sleep regulation, learning, and memory formation. KYNA is predominately synthesized by kynurenine aminotransferase II (KAT II) from its biological precursor kynurenine. KYNA released from astrocytes acts as an endogenous antagonist of N-methyl-D-aspartate and alpha7-nicotinic acetylcholine receptors, thereby hypothesized to modulate glutamatergic and cholinergic circuits involved in the regulation of sleep and cognition. Of clinical significance, elevated KYNA levels are found in brains of individuals with schizophrenia and bipolar disorder, who frequently experience sleep disturbances and cognitive impairments. We presently sought to determine if pharmacological inhibition of the KYNA synthesizing enzyme, kynurenine aminotransferase II (KAT II), may serve as a potential avenue to overcome sleep disturbances.

Methods: To obtain further insight into an interplay between brain KYNA, sleep, and cognitive function, adult male Wistar rats ($N = 4 - 12$ per group) were sleep deprived (SD) during the latter half of the light phase, Zeitgeber time (ZT) 6 to ZT 12 for 5 consecutive days, using our novel sleep restriction chamber. During this period, we assessed spatial and reversal learning in the Barnes maze during 4 acquisition trials and 1 reversal learning trial conducted at ZT 6. Vehicle or PF-04859989 (30 mg/kg, s.c.), brain-penetrable KAT II inhibitor that reduces brain KYNA, were administered at ZT 6 immediately after each trial, and prior to the onset of SD (ZT6 - ZT12). Upon completion of behavioral testing on day 5, tissues were collected from rats at ZT 8. A separate cohort of animals was implanted with telemetric devices to acquire polysomnographic recordings that combine electroencephalography (EEG) and electromyography (EMG) to evaluate efficacy of our automated sleep restriction chamber across 5 consecutive days and sleep recovery with vehicle or PF-04859989 (30 mg/kg, s.c.) treatment.

Results: Automated sleep restriction chamber effectively eliminates rapid eye movement (REM) sleep in rats during the SD period ($^*P < 0.05$). In the Barnes maze, vehicle-treated controls had a significantly reduced distance traveled between acquisition days 1 and 4, demonstrating learning in the behavioral paradigm ($^*P < 0.05$), while learning did not occur in vehicle-treated SD subjects. Importantly, PF-04859989-treated SD subjects also had reduced distance traveled across days 1 and 4 ($^*P < 0.05$). Further, reducing KYNA with PF-04859989 attenuated reversal learning deficits in SD animals (latency: $^{**}P < 0.01$; distance traveled: $^*P < 0.05$; errors: $^{**}P < 0.01$). Prolonged SD significantly increased both plasma tryptophan and KYNA ($^{**}P < 0.01$), while KAT II inhibition prevented these increases, yet elevated plasma kynurenine ($^{**}P < 0.01$) in SD rats.

Conclusions: Taken together, we demonstrated for the first time that KAT II inhibition may attenuate learning deficits during extended sleep loss in rats. The present and future complementary experiments provide mechanistic value to understanding the role of KYNA in modulating sleep behavior and demonstrate that KAT II inhibition may serve as a potential therapeutic avenue for improving neurocognitive dysfunction associated with prolonged sleep loss.

Keywords: Sleep Deprivation, Schizophrenia and Cognition, Alpha7 Nicotinic Acetylcholine Receptor, Electroencephalography, REM Sleep

Disclosure: Nothing to disclose.

P596. Sleep Oscillations in Insomnia and Aging

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Background: Sleep EEG oscillations that subserve sleep functions such as learning, memory, and removal of toxins are affected by aging as well as by sleep disorders. The studies reported here identify relationships between changes in sleep oscillations and insomnia-related sleep state misperception, sleep-dependent learning, and Alzheimer's disease (AD) biomarkers in older adults at risk for AD.

Methods: Study 1: Sleep oscillations related to sleep state misperception were determined in subjects with insomnia and normal controls ($n = 21$) who underwent sleep recordings with high density (256 channel) EEG (hdEEG) for nights where they were awakened repeatedly during sleep and asked whether they had been awake or asleep.

Study 2: hdEEG sleep and sleep dependent learning were assessed in cognitively intact older adults enriched for AD risk ($n = 58$) and correlated with insomnia, as well as inflammatory and AD biomarkers in CSF fluid.

Study 3: PSG was recorded from older adults with insomnia disorder ($n = 106$) who participated in a randomized phase III clinical trial (E2006-G000-304; NCT02783729) examining the acute (1-2 days) and chronic (29-30 days) impact of zolpidem extended release (ER) and lemborexant relative to placebo on subjective and objective sleep expression.

Results: Study 1: Subjects reported being awake despite the presence of EEG sleep prior to the awakening ~10% of the time, mostly during non-rapid eye movement (NREM) sleep stages N2 and N3; this was associated with increased alpha power (in the 10.5-12.5 Hz range) in posterior and mid-central brain regions.

Study 2: Similarly, in older adults at risk for AD, insomnia was associated with increased alpha (7.5-11.5 Hz) and theta (4.5-7.5 Hz) power, albeit in more frontal regions.

Study 3: Greater decreases in subjective wake after sleep onset (sWASO) were associated with greater increases in fast sigma activity (13-16 Hz) and slow oscillation power (0.5-1 Hz) following chronic zolpidem ER and lemborexant treatment. In addition, in chronic treatment with zolpidem ER, greater decreases in Alpha activity (7.5-11 Hz) were associated with greater decreases in subjective sleep onset latency (sSOL).

Conclusions: Insomnia, particularly characterized by sleep state misperception, may be due in part to abnormal, local intrusion of waking EEG activity (alpha and/or theta) during sleep in both young and older adults, including those with increased risk of AD. Subjective insomnia measures are associated with sleep oscillation expression.

Keywords: Sleep Oscillations, Insomnia Disorder, Preclinical Alzheimer's Disease, Sleep-Dependent Learning

Disclosures: Eisai: Grant (Self), Idorsia, Merck, Jazz: Consultant (Self), Genomind, Sage: Advisory Board (Self).

P597. Effects of Endogenous Orexin and Dynorphin Corelease on Ventral Tegmental Dopamine Neuronal Activity

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Background: Dopamine neurons in the ventral tegmental area (VTA) respond to motivationally relevant cues and are key targets of addictive drugs. Orexins (ox; also known as hypocretin) and dynorphin (dyn) are co-expressed lateral hypothalamic (LH) neuropeptides that project to VTA. While LHox promotes drug-seeking behavior, dynorphin inhibits drug-seeking behavior. Furthermore, these peptides have opposing effects on the firing activity of VTA dopamine neurons. Previous work in our lab implicated that exogenously application of ox and dyn, modulate different VTA dopaminergic projections. However, it is unknown if dynorphin inhibition of these circuits in opposition to LHox is driven by the LHox/dyn input, rather than other sources. This study sought to determine the effects of endogenous LHox/dyn release on VTA dopamine neuronal activity.

Methods: We expressed a cre-dependent channel rhodopsin2 selectively in LHox/dyn neurons of orexin-cre mice and photostimulated terminals in the VTA while recording VTA neuronal firing using patch clamp electrophysiology. VTA dopamine neurons were labeled with biocytin during recordings and posthoc imaged for tyrosine hydroxylase expression. Projection targets were identified using retrobeads injected into the nucleus accumbens lateral (lNAcSh) or medial shell (mNAcSh) as well as the basolateral amygdala (BLA). In some experiments, morphine tolerance was induced by delivering two daily injections of escalating morphine doses (5-100 mg/kg) over 5 days. Control mice were injected with saline.

Results: We showed a diverse response of LHox/dyn photostimulation on dopamine neuronal firing rate. A 30-Hz stimulation, increased firing in 7/19 neurons and decreased firing in approximately 9/19 of lateral VTA dopamine neurons, an effect persisted in the presence of synaptic transmission blockers. SB334687, an ox1 receptor inhibitor or NorBNI, a kappa receptor inhibitor reversed the potentiation or inhibition of firing, respectively. Morphine tolerance reduced the number of neurons that had inhibitory response to optical stimulation of LHox/dyn terminals. Of lNAcSh projecting dopamine neurons, optical stimulation of LHox/dyn neurons increased firing in 6/10 and decreased firing in 4/10 neurons, whereas mNAcSh projecting neurons had 4/11 neurons increase firing and 7/11 neurons decrease firing. BLA projecting dopamine neurons were mostly inhibited (8/11) by optical stimulation of LHox/dyn terminals.

Conclusions: Our findings provide evidence that LHox/dyn co-release tune the output of the VTA by simultaneously inhibiting and activating different VTA projection neurons. Morphine tolerance may lead to a shift in the balance of excitatory and inhibitory effects of LH ox/dyn co-release.

Keywords: Dopamine, Orexin, Dynorphin, Opioid Tolerance, Slice Electrophysiology

Disclosure: Nothing to disclose.

P598. Cell-Type-Specific Epigenetic Priming of Gene Expression in Nucleus Accumbens by Cocaine

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Background: Cocaine use disorder (CUD) is an intractable syndrome and rising overdose deaths represent an enormous public health crisis. The urgent need for better mechanistic insight into this chronic relapsing brain disorder is driven by sharp increases in cocaine use and the lack of effective treatments. Chronic cocaine exposure induces persistent changes in gene regulation in the brain's motivation and reward circuitry, coupled to neuroplasticity within the brain and vulnerability to relapse. Susceptibility to relapse is believed to involve stable changes in chromatin in the nucleus accumbens (NAc), a brain region that controls motivated behaviors, that alter transcription during long-term drug withdrawal. However, the molecular events that underlie maladaptive gene activity in cocaine addiction and other substance use disorders remain incompletely understood. A fundamental challenge is determining which neuronal subtypes are responsible: the NAc is composed primarily of two opposing types of medium spiny neurons (MSNs), the D1 and D2 dopamine receptor-expressing subtypes, which exhibit dramatic differences in activity and effects on drug reward. In these distinct subtypes, we examined how chronic cocaine modifies chromatin structure and characterized immediate versus persistent changes in gene regulation.

Methods: The NAc primarily comprises (>90%) two functionally distinct subtypes of MSNs, making the cell-type-specific identification of epigenetic changes critical. In recent years, the assay for transposase-accessible chromatin using sequencing (ATAC-seq) has become a fundamental tool of epigenomic research. It is employed to assess chromatin structure genome-wide to detect "open" chromatin regions indicative of active gene transcription or priming. Here, we defined chromatin accessibility in D1 and D2 MSNs using fluorescence-activated nuclei sorting (FANS) coupled to ATAC-seq and RNA-seq. Combined with unbiased histone modification profiling by mass spectrometry and ChIP-seq, we distinguished acute versus long-term alterations in chromatin and gene expression. Specifically, we characterized persistent alterations in circuit-specific chromatin composition with prolonged withdrawal (30d) after chronic exposure to cocaine (10d cocaine i.p.).

Results: We discovered that chronic cocaine persistently alters chromatin structure in D1 MSNs, involving dramatic depletion of the histone variant H2A.Z – a recently identified memory suppressor – at key neuronal genes related to synaptic plasticity. Genome accessibility is prominently increased at these genes even after prolonged withdrawal, linked to aberrant gene expression upon drug relapse. The histone chaperone ANP32E promotes the removal of H2A.Z, and we demonstrate that D1 circuit-selective ANP32E knockdown prevents cocaine-induced H2A.Z depletion and effectively blocks cocaine-conditioned place preference. In contrast, the D2-specific knockdown of ANP32E enhances cocaine-related reward learning in this animal model.

Conclusions: Here, we describe a novel epigenetic mechanism whereby chronic cocaine exposure causes lasting chromatin and downstream transcriptional modifications in the NAc. We link prolonged withdrawal from cocaine to the depletion of the histone variant H2A.Z, coupled with increased genome accessibility and latent priming of gene transcription, in D1 MSNs that relate to aberrant gene expression upon drug relapse. The histone chaperone ANP32E removes H2A.Z from chromatin, and we demonstrate that D1 MSN-selective Anp32e knockdown prevents cocaine-induced H2A.Z depletion and blocks cocaine's rewarding actions. By contrast, very different effects of cocaine exposure, withdrawal, and relapse were found for D2-MSNs. These findings establish histone variant exchange as an important mechanism

and clinical target engaged by drugs of abuse to corrupt brain function and behavior.

Keywords: Withdrawal, Drug Relapse, Epigenetics, Gene Priming

Disclosure: EpiVario, Inc: Founder (Self).

P599. Effects of Long-Term Intermittent Access to Alcohol on Glutamatergic Basal Forebrain Neurons During Aversion-Resistant Drinking

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Background: Compulsive alcohol use is a major contributor to alcohol use disorder's intractable and persistent nature. Prior research has shown that neuroadaptations in corticostriatal projections to nucleus accumbens are critical for the development of compulsive-like alcohol use behaviors, including "aversion-resistant" drinking, in which animals continue to consume alcohol despite its adulteration with bitter quinine. Yet it remains unclear how alcohol-induced changes in nucleus accumbens can impact sensitivity to aversive outcomes during consumption and seeking of alcohol. The nucleus accumbens projects to glutamatergic neurons in the ventral pallidum, lateral preoptic area, and lateral hypothalamus, which are implicated in aversive processing and constraint of reward-seeking behaviors. Our goal here is to examine the effects of long-term intermittent access to alcohol and aversion-resistant drinking on cell-type specific markers and activity in the ventral pallidum, lateral preoptic area, and lateral hypothalamus from rats tested for aversion-resistant alcohol consumption.

Methods: Adult Long Evans rats ($n = 68$, $n = 33$ females, 35 males), were assigned to long-term (LTA, $n = 38$) and short-term (STA, $n = 24$) alcohol access groups, or to a no ethanol exposure group ($n = 6$). For 14 weeks, LTA rats had access to a bottle of 15% ethanol, for 24-hour periods every other day, whereas STA rats had access to an additional water bottle, but not ethanol. Rats then underwent testing for consumption of 15% ethanol with 0, 45, and 90 mg/L quinine. Brains were extracted immediately after a final 30-minute test with access to ethanol, ethanol plus quinine, or no solution. Brains were then processed via in situ hybridization with RNAscope for mRNA markers of neural activity (Fos), as well as markers for glutamatergic (Slc17a6 [[Vglut2]) and GABAergic neurons (Gad1).

Results: Our preliminary results indicate that, in rats that have undergone a final test with quinine-adulterated alcohol, long-term intermittent access to alcohol reduces the percentage of vGlut2 positive cells that are positive for Fos mRNA. This suggests that alcohol exposure blunts the activation of glutamate neurons during aversion-resistant drinking. Additionally, we found that long-term access increased the percentage of vGlut2 neurons that are also Gad1 positive, suggesting that alcohol exposure may alter the downstream effects of these neurons in areas like the lateral habenula. Female rats also had a higher proportion of vGlut2 neurons that were Gad1 positive.

Conclusions: Our results indicate that intermittent access to alcohol can alter the expression of cell-type specific mRNA markers and neural activity in the glutamatergic basal forebrain. Specifically, long-term access increased the colocalization of markers for glutamate and GABA neurons, and reduced Fos expression in glutamate neurons. Given that glutamatergic basal forebrain neurons have been implicated in aversive processing, increased colocalization could alter the processing of both rewards and punishment during conflict. Reduced activity and increased GABA release by neurons that normally respond to aversive events could be two mechanisms by which alcohol exposure alters sensitivity to negative outcomes.

Keywords: Alcohol, Aversion, Glutamate GABA, Compulsive Drug Intake, Ventral Pallidum

Disclosure: Nothing to disclose.

P600. Endocannabinoids Influence Representations of Interval Timing in the Nucleus Accumbens

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Background: Cannabinoids disrupt timing by interfering with dedicated brain timing circuits. The ability to perceive and respond to environmental information in the time domain is critical for adaptive survival, and striatal circuits play a central role in timing behavior. Our previous work demonstrated that phasic dopamine release in the nucleus accumbens (NAc) encodes interval timing and that CB1 receptor activation accelerates the perception of time and shifts temporally-engendered patterns of phasic dopamine release.

Methods: Using in vivo fiber photometry and neuronal ensemble recordings, we examined how endocannabinoid signaling orchestrates timing-mediated NAc network dynamics in male mice.

Results: We found that interval timing was encoded by bidirectional ramping activity of NAc ensembles and sustained levels of phasic dopamine release. Increasing levels of the endocannabinoid 2-AG via the MAGL inhibitor JZL184 (18 mg/kg, ip) resulted in an acceleration of time estimation and attenuation of interval encoding in a CB1 receptor-dependent manner.

Conclusions: These results reveal a significant role for endocannabinoids in ventral striatal network dynamics associated with interval timing and may have important implications for the use of pharmacotherapies targeting the endocannabinoid system and for the recreational use of plant-based and synthetic cannabinoids.

Keywords: Endocannabinoids, Timing, Dopamine, In Vivo Electrophysiology

Disclosure: Nothing to disclose.

P601. The Interaction of Biological Sex, Early-Life Stress, and Mesolimbic Dopamine Function on Vulnerability to Heroin Self-Administration

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Background: The scale and severity of the opioid epidemic in the United States cannot be overstated. With the recent restriction on prescription opioids, there has been a shift to greater abuse of illegal drugs such as heroin. To combat the opioid crisis effectively, we need a deeper understanding of the factors that drive vulnerability to develop opioid use disorder (OUD). Chronic psychosocial stress has been linked to increased risk for a host of negative outcomes, including depression, anxiety, and substance use disorders (SUDs). Exposure to these stressors during adolescence drives an even higher risk for future psychiatric disorders that may engender or worsen SUDs in adulthood. Our group and others have shown that chronic adolescent social isolation (aSI) stress in male rodents leads to persistently increased drug responsiveness and negative affective behaviors when

compared to adolescent group-housed (aGH) control. The robust impact of aSI on drug and alcohol self-administration (SA), coupled with strong evidence that aSI-induced alterations in the dopamine system potently regulate vulnerability to drug-seeking, lead us to predict these effects would apply to opioids as well. Here, we will assess the impact of aSI on heroin self-administration.

Methods: Male ($n = 112$) and female ($n = 40$) Long-Evans rats were housed in groups (4/cage) or isolation (1/cage) from postnatal day (PND) 28-70 or 21-70, respectively. Following the housing paradigm, all rats were assayed for anxiety-like behavior and response to a novel environment, implanted with jugular catheters, and individually housed in operant chambers that serve as both the home cage and SA chamber. Following recovery, rats were given access to a lever and trained to self-administer heroin (FR1, 0.025 mg/kg/infusion). After acquiring heroin SA, rats were tested for dose-responsivity (FR1), motivation for heroin seeking using the progressive ratio (PR), and escalation of intake using a long access paradigm (unlimited infusions, 6 hr/session, FR1). Next, the combination of cue- and stress-induced reinstatement responses using the pharmacological stressor, yohimbine (1.25 mg/kg, IP), was evaluated following the extinction of responding. A group of aSI and aGH were given sham surgeries to serve as heroin-naïve controls. To measure dopamine alterations after chronic heroin exposure (or LgA), we utilized ex vivo fast-scan cyclic voltammetry (FSCV) to measure dopamine release and uptake kinetics and terminal receptor functioning in brain slices containing the nucleus accumbens (NAc). RNAscope in situ hybridization (ISH) was used to quantify the expression of D2 mRNA and D3R mRNA. Lastly, negative affect elicited by heroin withdrawal was assessed by recording ultrasonic vocalizations (USVs) before the last LgA session.

Results: In these studies, we found that male and female aSI rats have increased anxiety-like behavior and locomotor response to a novel environment. Our SA results revealed that aSI rats have increased rates of heroin SA acquisition, escalation of heroin responding on LgA, responding during extinction sessions, and cue- and stress-induced reinstatement responding. However, female aSI and aGH rats displayed increased responses during LgA compared to male aSI and aGH rats. In addition, we found that aSI has increased responding to various doses of heroin on a PR schedule of reinforcement. Using FSCV, we found that heroin-naïve aSI rats have increased electrically stimulated dopamine release and uptake rats, indicating increased DA system functioning. Following LgA, stimulated dopamine release was reduced in both aSI and aGH rats, however; dopamine uptake rates were only reduced in heroin-exposed aSI rats, compared to their respective heroin naïve counterparts, suggesting a greater downregulation of the dopamine system in heroin-exposed aSI rats. In addition, we found that heroin aSI rats had increased activity at D3, but not D2, autoreceptors, which may play a role in the profound decrease in NAc dopamine terminal function in aSI rats after heroin. This finding was supported by the RNAscope ISH showing increased expression of D3 mRNA in the NAc of aSI rats. Further, analysis of USVs revealed increased 22 kHz calls in heroin aSI rats compared to heroin naïve aSI and heroin-exposed aGH rats. Consistent with our results demonstrating downregulated dopamine functioning in the NAc, this data suggests that heroin-exposed aSI rats experienced greater negative affect during withdrawal from heroin, which may, in part, drive the increased heroin seeking exhibited during SA.

Conclusions: Our results demonstrate that exposure to chronic psychosocial stress during adolescence results in robust behavioral and neurobiological adaptions that lead to increased vulnerability to opioid seeking. In addition, the intersection of adolescent stress, biological sex, and dopamine functioning heroin vulnerability may be linked to greater heroin vulnerability.

Keywords: Heroin Self-Administration, Dopamine (D2, D3) Receptors, Dopamine, Early Life Stress (ELS), Nucleus Accumbens

Disclosure: Nothing to disclose.

P602. Transcriptional Correlates of Drug-Associated Memories in the Nucleus Accumbens

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Background: Background: Substance use disorders exemplify a maladaptive imbalance wherein drug seeking persists despite negative consequences or drug unavailability. This maladaptation correlates with neurobiological alterations that hijack reward seeking and consequently withdrawal, extinction, and relapse processes. Extinction, a form of learning in which drug-seeking responses are attenuated by repeated cue exposure in the absence of the drug, represents a valuable tool to suppress drug-associated memories at the behavioral and molecular levels. While there is increasing evidence linking addiction phases to faulty epigenetic and transcriptional modifications in brain reward regions such as the nucleus accumbens (NAc), there is a pressing need to characterize these molecular events in a phase, subregion, and cell-specific manner.

Methods: Methods: Here, we used cocaine self-administration (SA) in rats combined with RNA-sequencing (RNAseq) of NAc subregions (core/shell) to transcriptionally profile the impact of extinction learning on counteracting drug memories in different contexts.

Results: Results: As expected, we first observed that rats receiving extinction training in the original SA context (cues/no drug) significantly reduced active lever pressing when compared with rats receiving force abstinence in either their home cages or in the SA context (no cues/no drug). Further analysis revealed that rats undergoing withdrawal in the original drug context increased drug seeking and incubation. As for the animals receiving extinction in a different context (ABA), a heterogonous distribution of rats showing either extinction or renewal was observed. Consistent with these behavioral features, RNAseq data reveal specific transcriptional patterns that correlate with these distinct phenotypes and the influence of extinction training on modulating the transcriptional burden upon withdrawal. Subsequent motif and pathway analyses will identify hub genes that encode these phenotypes to manipulate these critical targets, virally. Complementary to these datasets, with the goal of subsequent cell-specific characterizations, we are using RNAscope, electrophysiology, chemogenetic and fiber photometry approaches on transgenic rats expressing Cre-recombinase selectively in D1 or D2 NAc medium spiny neurons.

Conclusions: Conclusion: Together, these approaches will provide unprecedented evidence of how extinction, withdrawal, and renewal reprogram the transcriptome of the NAc to identify novel avenues to prevent drug relapse.

Keywords: Addiction Phenotypes, Extinction, Transcriptomics, Cocaine

Disclosure: Nothing to disclose.

P603. Cocaine Taking and Craving Drives Differential Gene Expression in Midbrain Dopamine Neurons

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Background: Repeated cocaine use induces long-lasting gene expression changes throughout the brain reward system. Of particular interest, understanding the gene expression changes that occur in midbrain dopamine (mDA) neurons during cocaine taking and craving could provide insights into regulation of the negative affective states associated with drug craving and relapse. To explore this question, we combined a cell subtype-specific nuclear tagging approach with a mouse model of cocaine intravenous self-administration (IVSA), and bioinformatics analyses to identify mDA-specific changes in gene expression during cocaine taking and cocaine craving.

Methods: Male Dat-Cre heterozygous mice (8-12 weeks) received bilateral intra-VTA injections of a Cre-inducible virus expressing a nuclear membrane-bound HA tag (KASH-HA) to selectively label mDA nuclei, and were then implanted with a jugular catheter for cocaine IVSA studies. To this end, mice completed a food training protocol under a fixed ratio 5, time-out 20 (FR5TO20) schedule of reinforcement, in which 5 active lever presses (FR5) resulted in delivery of a food pellet and presentation of a 20-second cue light, indicating a time-out (TO20) period where lever pressing was recorded but had no effect. Mice were allowed to establish food training over consecutive daily 1 hr session for 7 days, at which point the reinforcer changed from food pellets to cocaine infusions [0.3 mg/kg/infusion] for 5 consecutive days, and then 1.0 mg/kg/infusion for 10 consecutive days. Following cocaine IVSA, mice underwent 21 consecutive days of forced abstinence (craving) in their home cages, during which mice did not have access to the drug. VTA samples were collected and enucleated following either food training ($N = 5$), cocaine maintenance ($N = 7$) or cocaine craving ($N = 7$). To obtain a pure mDA nuclear population, the suspended nuclei were immunostained for HA and sorted via fluorescence assisted nuclear sorting (FANS). RNA was extracted from both mDA and non-mDA populations, and nuclear RNA-sequencing libraries (pooled $N = 2$-3 samples per group) were prepared and sequenced. Differential gene expression analysis was then conducted using DESeq2, followed by Gene Ontology (GO) pathway analyses to evaluate the biological context for gene expression changes.

Results: RNA-sequencing of mDA nuclei showed an enrichment in dopamine identity genes, Th, Dat, Vmat2, Ddc, as well as depletion of glial genes when compared to non-labeled nuclear samples, ensuring the purity of our sequenced samples ($|Log2FC| > 1.3$ and padj<0.1). Three-way comparison between food training ($N = 5$), cocaine maintenance ($N = 7$), and cocaine craving ($N = 7$) showed that cocaine craving mice had the highest variation in differentially expressed genes, especially in direct comparison with food-trained controls ($p < 0.05$). Further, gene ontology pathway analyses revealed a pattern in which extracellular matrix (ECM) genes were upregulated during cocaine maintenance and then downregulated during cocaine craving ($p < 0.05$, $q < 0.1$), with ECM genes such as Bgn, Col1a1, Fn1, and Hspg2 showing near complete depletion (normalized gene count<1) during cocaine craving.

Conclusions: While preliminary and only performed in males, upregulation of ECM genes during cocaine taking and subsequent downregulation during cocaine craving supports the role of the ECM in synaptic remodeling and plasticity. More broadly, these IVSA stage-specific and cell-specific gene expression changes suggest that mDA neurons differentially regulate their transcriptional profiles to adapt to environmental changes (i.e. presence or absence of a drug). As the nuclear capture method, we developed and employed retrieves both nuclear mRNA and chromatin, follow-up studies (chromatin profiling) will determine the transcriptional regulators underlying these changes.

Keywords: Cocaine Self-Administration, Cocaine Craving, Dopamine Neuron, Cell-Specific, Transcriptome

Disclosure: Nothing to disclose.

P604. Distinct Ensembles Support Early Vs Late Stages of Cocaine Memory Encoding

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Background: Learned associations between the rewarding effects of drugs and the context in which they are experienced are decisive for precipitated drug-seeking and relapse in addiction. These associative memories are stored in sparse and highly discriminative populations of concomitantly activated neurons defining drug-recruited ensembles. In this study, we explore the dynamics and molecular mechanisms of both the recruitment of these ensembles upon initial drug exposure and their contribution to the encoding, strengthening and ultimately expression of drug-associated memories. Additionally, we explore the intrinsic and acquired cellular properties favoring the allocation of specific cells to these ensembles and/or predicting their further reactivation.

Methods: Capitalizing on the activity-dependent labeling in Arc-CreERT2 mice (Denny et al., 2014), we captured and permanently tagged (fluorophores, channel-rhodopsin) cocaine-activated ensembles in the nucleus accumbens for further characterization, optogenetics, and nuclei sorting. Cocaine-context associative memories have been assessed in a cocaine-conditioned place preference paradigm. Ensemble reactivation has been measured as the overlap between ensemble-specific fluorophore tag and the recent Arc activation. Tagged nuclei were isolated with Fluorescence Activated Cell Sorting to analyze their molecular signature.

Results: We identified distinct subsets of neurons activated at both early and late stages of drug exposure and show that the reactivation of an initial ensemble correlates with behavioral sensitization. Similarly, re-exposure to a cocaine-paired context in a conditioned place preference (CPP) paradigm triggered cocaine ensembles' reactivation. Using optogenetics-mediated artificial reactivation, we found that populations recruited at early versus late stages of drug exposure had opposite roles in CPP expression. In addition, these two distinct ensembles exhibited significantly different levels of endogenous reactivation following re-exposure to either a cocaine challenge or a cocaine-paired context. Single nucleus RNA Sequencing was then performed on FACS-isolated tagged neurons, and we successfully isolated a cluster of reactivated cells within the initially activated ensemble.

Conclusions: By tracking ensemble reactivation at different stages of cocaine exposure, this work provides new insights into the fate of neurons recruited at early phases of cocaine-related memory formation. Together, this ensemble-specific approach represents a pivotal step in identifying highly specific cellular processes involved in the encoding of pathological memories associated with addiction.

Keywords: Neuronal Ensembles, Arc, Reward Learning, Nucleus Accumbens, Addiction

Disclosure: Nothing to disclose.

P605. Loss of Microglial MyD88 Induces Aversion Resistant Drinking in Adolescent Male Mice

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Background: Binge drinking and heavy alcohol use are a massive public health burden and have increased in the United States in the last few years. There is limited drug development and treatment therapy to effectively reduce alcohol use disorder (AUD) and its societal impact. Further elucidation of biological mechanisms involved in AUD could enhance prevention, treatment, and disease progression of AUD. Recently, neuroimmune responses have been described as potential therapeutic targets for AUD. Studies suggest that alcohol signaling through innate immune toll-like receptors (TLRs) on microglia, the brain's primary immune cells, to stimulate the release of immune signaling molecules may be critical. Microglia also use neuroimmune molecules to interact closely with other cells in the brain, such as neurons and their synapses, providing a mechanism through which alcohol impacts neuroplasticity and behavior. MyD88 is an essential co-adaptor protein for nearly every TLR, and therefore is important for broad inflammatory signaling.

Methods: Our lab previously developed and characterized a mouse line that allows for a microglia-specific MyD88 deletion (Cx3cr1-CreBT-MyD88f/f), thereby blunting inflammatory TLR signaling. MyD88 was removed from microglia using Bacterial Artificial Chromosome (BAC) Transgenic mice with Cre recombinase under the Cx3cr1 promotor (Cx3cr1-CreBT) crossed with MyD88f/f mice. To study the role of TLR signaling in drinking during brain development, we employed a drinking in the dark (DID) binge-drinking paradigm in adolescent male and female mice + /- microglial-specific MyD88 deletion. Mice were weaned from mothers between P26-P28 and group housed for 1-3 days before transfer to single housing. Water bottles were replaced with 30% ethanol for 2 hours for 3 days and 4 hours on the fourth day for two weeks. Tasting aversion quinine was added to the bottles of some groups at week four. Following adolescent DID, adult mice were sacrificed, and cerebellum and brain regions associated with addiction were punched and protein processed for analysis. (Two-way ANOVA used for data analysis)

Results: MyD88 signaling alters alcohol consumption in adolescent mice in a sex dependent manner. At weeks 1 and 2 on binge drinking day, Cre+ males consumed more alcohol than females (Week 1, $F(1,17)=6.340$ $P = 0.200$; Week 2, $F(1,21)=9.173$ $P = 0.0064$. Surprisingly, loss of microglial Myd88 induced aversion resistant drinking in adolescent males ($F(1,21)=0.3574$ $P = 0.3574$). Preliminary data shows that alcohol consumption and MyD88 signaling alters expression of proteins in the cerebellum and various brain regions involved in addiction, decision making and memory. Following DID, microglia maker P2RY12 protein expression is decreased in the cerebellum of both Cre- and Cre+ males compared to females. Loss of microglial Myd88 increases protein expression of cerebellar parvalbumin in both male and female mice. Alcohol consumption reduces Iba-1 expression in the cerebellum. However, Iba-1 is elevated in the hippocampus of DID Cre+ males and females. Lastly, we observed lower protein expression of TMEM119 in the prefrontal cortex, nucleus accumbens and hippocampus of Cre- females compared to males following adolescent DID. (Alcohol consumption Cre- female $n = 5$, Cre+ female $n = 5$, Cre- male $n = 6$, Cre+ male $n = 9$); (Western Blot $n = 1$ per group).

Conclusions: These data show an immune inflammatory TLR signaling component involved in excessive alcohol consumption. Ongoing studies will further examine the role of alcohol induced microglia activation, PVIs and PNNs in AUD.

Keywords: Adolescent Binge Drinking, Microglia, Sex-Specific, Myd88

Disclosure: Nothing to disclose.

P606. Distinctions in the Prefrontal Cortex Protein Profile of Rats Exhibiting Incubated Cocaine- Versus Sucrose-Seeking: Implications for Pharmacotherapy

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Background: Craving is a cardinal feature of both drug and food addiction that is elicited by re-exposure to reward-associated cues/contexts. Insidiously, the intensity of both cue-elicited drug and food craving incubates during protracted withdrawal and their parallel behavioral trajectories have led to the hypothesis that common time-dependent neuroadaptations may drive both phenomena. Over the past several years, our laboratory has sought to determine the functional relevance of protein correlates within the ventromedial prefrontal cortex (PFC) of incubated cocaine-craving induced by a history of long-access (6 h/day) cocaine self-administration procedures in rats and identified increased PI3K/Akt/mTOR signaling within the prelimbic (PL) subregion as necessary for the expression of incubated cocaine craving, while reduced mGlu1 and mGlu5 subtypes of metabotropic glutamate receptors impair the consolidation of extinction learning and an imbalance in Homer1 vs. Homer2 expression facilitates the reinstatement of cocaine-seeking following extinction. Whether or not the incubation of cocaine-craving induced by shorter-access cocaine-self-administration procedures or the incubation of food-craving is associated with comparable, behaviorally relevant, changes in protein expression is not known. Such knowledge would identify common and unique molecular adaptations important for the appropriate pharmacological targeting in cocaine use and over-eating disorders.

Methods: At the outset of our studies, we hypothesized that if the incubation of cocaine- and food-craving involved similar mechanisms, then our results from a model of incubated sucrose-craving should parallel those observed in cocaine-incubated rats, regardless of their cocaine self-administration history. In our study of incubated cocaine-seeking, rats were trained to self-administer intravenous cocaine (0.25 mg/0.1 ml/infusion) under operant-conditioning procedures during which each cocaine infusion was paired with a 20-sec tone-light stimulus. Two different short-access self-administration procedures were probed; one cocaine group was trained for 2 h/day for 10 days (2-Hour), while the other cocaine group was trained for 6 h on Day 1, and 2 h/day for the remaining 9 days (Mixed). In the first study of incubated sucrose-craving, male and female rats were trained to self-administered 45 mg banana-flavored sucrose pellets for 6 h/day for 10 days, with each pellet delivery paired with the tone-light stimulus. On either withdrawal day (WD) 1 (sucrose) or 3 (cocaine) and on WD30, rats were tested for cue-elicited craving in the absence of the sucrose/cocaine reinforcer. In a second sucrose incubation study, rats were orally infused with either 1.0 mg/kg of the mTOR inhibitor Everolimus or vehicle prior to the test for craving to determine if this pretreatment, which effectively blocks incubated cocaine-seeking, would also reduce incubated sucrose-seeking. Following cue testing, PFC subregions were dissected out for immunoblotting in all studies.

Results: Relative to cocaine-trained rats on WD3, the Mixed cocaine self-administration procedures elicited more robust incubation of cocaine-craving on WD30 than the 2-Hour procedure [Group X Withdrawal: $F(2,63)=4.053$, $p = 0.023$] and

the incubated craving of the Mixed group was associated with a time-dependent increase in PL Homer2a/b [interaction: $F(2,63) = 4.19$, $p = 0.02$] and phospho-CaMKII expression in the PL [interaction: $F(2,59) = 6.243$, $p = 0.004$], although both cocaine groups exhibited elevated phospho-Akt within this subregion [interaction: $F(2,64) = 8.12$, $p = 0.001$]. No incubation-related changes in either mGlu receptor or any of the AMPA or NMDA receptor subunits examined were detected in either subregion (p's > 0.10). Relative to sucrose-trained rats on WD1, we observed a robust incubation of sucrose-craving in both male and female rats on WD30 [Withdrawal effect: $F(1,59) = 14.72$, $p < 0.0001$]. However, opposite cocaine incubation, sucrose incubation reflected lower phospho-Akt expression within the PL, but this effect was selective for females [Sex X Withdrawal: $F(1,51) = 5.456$, $p = 0.024$]. No other protein changes were detected in the PL of sucrose-incubated rats. In the IL, only male sucrose-incubated rats exhibited increased mGlu5, phospho-Akt and phospho-mTOR expression [for all proteins, $F(1,55) > 6.22$, p's<0.01]. However, pretreatment with 1.0 mg/kg Everolimus did not block the sucrose-incubated state in either male or female rats [Group effect: $F(2,42) = 9.118$, $p = 0.001$, SNK post-hoc tests].

Conclusions: Our cocaine data to date indicate that while the PFC protein profile of cocaine-incubated rats varies as a function of their cocaine history, elevated indices of Akt/PI3K/mTOR signaling within the PL is a common molecular adaptation that may be key to driving the cocaine-incubated state. Our sucrose data indicate a clear sex difference in the protein profile of sucrose-incubated rats, particularly with respect to Akt/mTOR activation. However, we failed to detect any effect of oral pretreatment with Everolimus on incubated sucrose-seeking in rats of either sex, indicating that unlike incubated cocaine-craving, incubated sucrose-craving does not require mTOR activity. Taken together, our body of work argues that the incubation of cocaine- and sucrose-seeking reflect distinct molecular changes within PFC subregions of relevance to not only the appropriate targeting of pharmacotherapies to mitigate cue-induced craving for sweet food versus drug, but also their potential to induce off-target effects.

Keywords: Incubation of Cocaine Craving, Incubation of Sucrose Craving, mTOR, Medial Prefrontal Cortex, mGluR5 Receptors

Disclosure: Nothing to disclose.

P607. Exploring a Role for GalR1-MOR Heteromers in Modulating the Cellular Effects of Opioids in the VTA

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Background: Opioids exert their rewarding effects by binding to mu opioid receptors (MORs) on GABAergic neurons and inhibiting GABA release onto ventral tegmental area (VTA) dopamine (DA) neurons. The endogenous neuropeptide galanin is thought to oppose the dopaminergic effects of opioids by signaling through galanin receptor 1 (GalR1) – MOR heteromers. While these heteromers represent an intriguing target for therapeutic intervention, their distribution in the brain and cellular effects on DA neurons remain unknown. Because visualizing G-protein complexes in vivo is technically challenging, we characterized GalR1 and MOR RNA co-expression in the mouse brain as an indicator of which brain regions and neuronal subtypes might be capable of assembling the heteromer. We examined GalR1 and MOR RNA expression and co-expression levels in regions that exert GABAergic control over VTA DA neurons: rostromedial tegmental nucleus (RMTg), nucleus accumbens (NAc), and the VTA itself. We also used slice electrophysiology to determine whether galanin alters morphine-induced disinhibition of VTA DA neurons through a GABAergic mechanism.

Methods: RNAscope fluorescent in situ hybridization was performed on brain tissue from C57BL/6 J mice (males and females) using probes for GalR1, MOR, and GAD1 as a cell type-specific marker for GABAergic neurons. Confocal images were acquired, and a nuclear-based analysis of GalR1 and MOR expression among GAD1 + and GAD1- neurons was performed using Cell Profiler software. Whole-cell patch clamp studies were conducted using mouse VTA sections. GABAergic inputs to the VTA were electrically stimulated, and the resulting inhibitory postsynaptic current (IPSC) was recorded from VTA DA cells. IPSCs were recorded at baseline and following bath application of galanin, morphine, or both.

Results: We found that the pattern of GalR1 RNA expression was similar across the three brain regions; ~25% of GABAergic cells co-expressed GalR1 and MOR mRNA, compared with only ~5% of non-GABAergic cells. Electrophysiology studies revealed that, as expected, morphine attenuated GABAergic IPSCs onto VTA DA neurons. Subsequent application of galanin had no effect, but pre-incubation of the slices with galanin prevented the effects of morphine.

Conclusions: Our findings indicate that a significant fraction of GABAergic neurons that provide input to VTA DA neurons co-express GalR1 and MOR in mice, and are thus capable of containing GalR1-MOR heteromers. By contrast, most non-GABAergic cells within these brain regions do not co-express these receptors. Our electrophysiological results indicate that galanin opposes the GABA-mediated effects of opioids on VTA DA neurons under certain conditions. Future studies will examine how GALR1-MOR heteromer disruption impacts galanin's ability to exert these effects.

Keywords: Opioids, Galanin, Ventral Tegmental Area (VTA)

Disclosure: Nothing to disclose.

P608. Adenosine 2a Receptor Agonism Decreases Nicotine Seeking and Associated Accumbens Microglial Activation

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Background: Neuroimmune and glutamatergic mechanisms within the nucleus accumbens core (NAcore) are involved in nicotine-motivated behaviors. We found profound NAcore microglia activation and increased expression of the glutamate NMDA receptor subtype, GluN2B, by chronic nicotine self-administration (SA). Microglia are brain immune cells, and play a critical role in regulating neuronal activity and synaptic plasticity. Agonism of adenosine 2a receptors (A2aRs), located on microglia to regulate their proliferation and survival, is neuroprotective in disease models. Prior studies show a beneficial effect of modulating A2aRs on nicotine withdrawal. Thus, NAcore adenosine signaling may play a critical role in driving nicotine seeking behavior and associated microglial activation.

Methods: Here we examined the ability of A2aR agonism to decrease cue reinstated nicotine seeking and associated NAcore microglial activation ($N = 11$-13/group). Rats underwent nicotine (0.06 mg/kg/infusion) or saline SA, extinction, and reinstatement, receiving either chronic systemic treatment with an A2a agonist (CGS21680; 0.4 mg/kg, IP), antagonist (SCH58261, 0.4 mg/kg, IP), or vehicle (saline, IP). Microglial morphology (via 3DMorph) or mRNA (via RT-qPCR) was then quantified from the NAcore. Linear mixed effects modeling (LME) was used for analysis.

Results: Chronic systemic treatment with the A2a agonist significantly decreased cue-induced nicotine seeking as compared to both vehicle and antagonist treatment ($p < 0.05$). Antagonist treatment also significantly increased reinstatement-induced microglial activation compared to saline SA, as shown by 5 measures of cell morphology (p's < 0.05 for each measure) and no differences were found between agonist-treated nicotine and saline SA rats. Agonist treatment decreased expression of both the A2aR coding gene, Adora2a, and the GluN2B coding gene, Grin2b, compared to vehicle and antagonist.

Conclusions: These results suggest that rebalancing of A2a and GluN2B receptor expression may be involved in decreasing nicotine seeking. Together, our results indicate that A2aR agonism may be protective against NAcore microglial activation induced by nicotine seeking, and may thus be a viable therapeutic treatment strategy for smoking cessation.

Keywords: Activated Microglia, Adenosine A2A Receptor, Nicotine Addiction, Cue Reinstatement, Nucleus Accumbens Core

Disclosure: Nothing to disclose.

P609. Molecularly-Defined Cell Types Within the Septal Complex and Their Putative Role in Opioid Dependence and Withdrawal

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Background: The lateral septum (LS) is a limbic brain area that has been recognized as both a site of reinforcement and as a regulator of fear expression. Its connectivity with key brain structures in reward learning and in anxiety underlies the LS' involvement in diverse behaviors. While there is evidence for the LS' role in addiction-related behaviors, it is unclear how chronic drug use impacts genetically-defined cell types within the LS, and how disrupting these neurons may contribute to drug withdrawal, a highly aversive experience that facilitates drug seeking.

Methods: We used single-cell RNA-sequencing (scRNAseq) to identify LS cell types that are involved in opioid dependence and withdrawal. To this end, we employed a morphine binge paradigm, in which both male and female C57/bl6 mice received a 7-day escalating dose of morphine (MOR, 10-70 mg/kg, 6 mice). Control mice received saline (SAL, 6 mice). Upon completion of the MOR schedule, some MOR mice received naloxone to precipitate opioid withdrawal (NAL, 1 mg/kg, 6 mice). Two additional groups of mice receiving only one dose of morphine (one-MOR, 6 mice) and solely a dose of naloxone (NAL-only, 6 mice). Septal tissue was then harvested and underwent single cell capture and sequencing via the 10X Genomics platform and Illumina HiSeq 4400. Analysis was carried out using Seurat v3.0.

Next, we used hybridization chain reaction (HCR), a highly multiplexed in situ method, to assess the topography of each molecularly-defined cell type within mouse septal tissue (8 mice) and the induction of immediate early genes (IEGs) following NAL (3 groups, 4 mice each). We then assessed how Nts+ neuronal activity in vivo changes over MOR and NAL treatment using 2-photon deep brain imaging. To selectively image from Nts+ neurons in the LS, we injected male and female NTS-cre mice with a cre-dependent AAV delivering GCamp6s, a genetically-encoded calcium indicator. We also implanted a GRIN lens in the LS. 4 weeks following lens implantation, mice underwent the binge and withdrawal paradigm described above, where 2 mice received SAL and 2 mice received MOR. Both groups received NAL. During MOR and NAL administration, both spontaneous and evoked activity were recorded. Mice received random presentations of 10% sucrose (positive valence) and 20 psi air puffs (negative valence).

Results: Our scRNAseq experiment yielded ~50,000 total cells across our 5 conditions, and over half of these cells were putative neurons (~26,000), determined by the expression of Stmn2 and Thy1. We identified 14 bioinformatically-defined cell clusters, 12 of which were GABAergic. 2 clusters were GLUergic. Among these neurons were both canonical LS cell types (e.g. Nts +, Sst +, Crhr2+), as well as novel, undescribed populations within the septum (e.g. Met +, Pax6+). Overall, chronic MOR admin profoundly alters gene expression across all LS neurons, enhancing genes involved in GLUergic signaling and depleting protein synthesis pathways. We trained a classifier to determine which cell cluster was the most transcriptionally perturbed in each condition. In doing this, we identified Nts+ neurons to be highly disrupted by MOR and NAL, whereas Met+ neurons to be the most altered by NAL and not chronic MOR.

We selected genetic markers representative of 12 cell clusters and ran HCR. In situ mapping of selected cell types indicated that although there is genetic heterogeneity within the septum, septal cells co-express genes in a gradient-like fashion. For example, Nts is co-expressed with up to 65% of Col15a1 +, 49% of Met +, 41% of Onecut2 +, 38% of Sst+ cells. Despite extensive overlap, LS Nts + neurons form a distinct layer across the A-P and D-V axes. We repeated HCR with mice treated with SAL, MOR and NAL to measure the expression of IEGs altered by MOR and NAL. Here, we discovered that although NAL induces Fos in Nts+ neurons (K-S test, $D = 0.356$, $p < 0.0001$, median diff of 16 copies), Fos is more highly induced in cells co-expressing Nts and Met (K-S test, $D = 0.564$, $p < 0.0001$, median diff of 25 copies). For deep-brain imaging experiments, we targeted the intermediate portion of the LS, where this co-expression occurs. 2-photon imaging of LS Nts+ neurons during MOR admin and NAL precipitated withdrawal revealed that NAL increases the number of Nts+ activity peaks during the first minute of NAL exposure (Two-way ANOVA, $F(1,122) = 3.910$, $p < 0.05$).

Conclusions: Our scRNAseq results revealed that although chronic MOR transcriptionally alters all LS cell types, Nts+ neurons are among the most genetically perturbed by both chronic MOR and NAL, indicating that these neurons may play some role in addiction-related behaviors. In situ mapping of cell types identified in scRNAseq showed that there are gradient-like overlaps among each population. Further analysis suggested that cells co-expressing Nts and Met, a novel genetic marker, are the most predictive of LS activation during withdrawal. Finally, we showed using 2-photon imaging that Nts neurons are in fact activated by NAL in vivo, and we currently have ongoing work attempting to determine whether and how MOR and NAL disrupt evoked Nts+ activity patterns. Together, these results highlight a novel role for LS Nts+ neurons, an underappreciated cell type in limbic brain circuitry.

Keywords: Opioid Abuse, Single-cell RNA Sequencing, Lateral Septum, 2-photon Techniques, Opioid Withdrawal

Disclosure: Nothing to disclose.

P610. Carbonic Anhydrase 4 Disruption Prevents Synaptic and Behavioral Adaptations Induced by Cocaine Withdrawal

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Background: Cocaine use followed by withdrawal induces synaptic changes in nucleus accumbens (NAc), which are thought to underlie subsequent drug-seeking behaviors and relapse.

Previous studies suggest cocaine-induced synaptic changes depend on acid-sensing ion channels (ASICs).

Methods: Here we investigated potential involvement of carbonic anhydrase 4 (CA4), an extracellular pH-buffering enzyme. We examined effects of CA4 in mice on ASIC-mediated synaptic transmission in medium spiny neurons (MSNs) in NAc, as well as on cocaine-induced synaptic changes and behavior.

Results: We found CA4 is expressed in the NAc and present in synaptosomes. Disrupting CA4 either globally, or locally, increased ASIC-mediated synaptic currents in NAc MSNs and protected against cocaine withdrawal-induced changes in synapses as well as cocaine-seeking behavior.

Conclusions: These findings raise the possibility that CA4 might be a novel therapeutic target for addiction and relapse.

Keywords: Alcohol and Substance Use Disorders, Synaptic Plasticity, Drug Seeking

Disclosure: Nothing to disclose.

P611. Synapse-Selective Association of Nucleus Accumbens Astrocytes Affects Synaptic Plasticity at Baseline and After Heroin Use

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Background: Astrocytes regulate excitatory activity at striatal synapses via gliotransmission, glutamate uptake, and spatial buffering of glutamate spillover at nearby spines and extrasynaptic receptors. Astrocyte insulation of synapses in the nucleus accumbens core (NAcore) is dynamic during extinction of addictive drug use and during drug seeking, and a high degree of synaptic insulation by NAcore astroglia serves to suppress cued drug seeking.

Methods: To determine how astrocyte morphology impacts excitatory synaptic activity in the striatum, we used confocal microscopy to quantify astrocyte adjacency to the two main synapse types in the NAcore, D1 and D2 receptor-expressing synapses on medium spiny neurons (D1- and D2-synapses). We next used whole cell patch clamp electrophysiology to determine the impact of synaptic insulation by astroglia on D1- and D2-synapses in the NAcore. We compared tissue treated with an ezrin-targeted morpholino oligo, which induces astroglia retraction from synapses, with control oligo-treated tissue, to determine how synapses responded to electrical stimulation in the presence or absence of astroglial insulation.

Results: We found that striatal astrocytes exhibit a bias in their synaptic association, with increased adjacency to D2-, vs. D1-synapses at baseline. After extinction of heroin seeking, this pattern is reversed, and astrocytes increase their insulation of D1-synapses, while simultaneously retracting from D2-synapses. By comparing traces from control vs. ezrin oligo-treated tissue, we found that astrocyte retraction from both D1- and D2-synapses reduced synaptic depression elicited by high frequency stimulation. Notably, high frequency stimulation-induced synaptic depression was more likely at D2- vs. D1-synapses. We also found that synaptic release probability was increased onto D1-, but not D2-synapses, in the absence of astrocytes insulation.

Conclusions: Based on these data, we predict that astrocyte-synapse adjacency serves to dampen synaptic activity in the striatum, perhaps via different mechanisms at D1- and D2-synapses. Given the differences in astrocyte-synapse association at baseline and after drug withdrawal, we predict that astrocyte-synapse adjacency predicts the likelihood of synaptic plasticity at different striatal subcircuits relevant to drug seeking behavior.

Keywords: Excitatory Synapses, Astrocyte-Neuron Interaction, Heroin Self-Administration, Short-Term Synaptic Depression

Disclosure: Nothing to disclose.

P612. Sex-Specific Effects of Oxycodone Withdrawal and Abstinence on Brain Region-Specific Transcriptomes in Heterogeneous Stock Rats

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Background: The prevalence of opioid use disorder and opioid dependence have increased dramatically over the recent decade. Despite current treatments, more than 90% of people being treated for opioid use disorder or dependence, eventually relapse. To improve treatment and to discover new, more effective therapeutics, a greater understanding of the molecular mechanisms contributing to dependence, and withdrawal and craving during abstinence is necessary. The individual differences in the vulnerability to opioid dependence and severity of withdrawal is due to a myriad of factors, including genetic diversity. Further understanding of the molecular mechanisms that contribute to this vulnerability within the context of individual differences will provide new insights into potential genetic factors that underlie the molecular and behavioral plasticity accompanying chronic opioid use. Powerful tools for investigating the genetics underlying opioid use include the population of Heterogeneous Stock (HS) rats. HS rats are derived from an eight-way cross of inbred and outbred rat strains to generate the most genetically diverse rat population. The recombination of these genomes results an expansion of phenotypic heterogeneity driven by enhanced genetic diversity. Leveraging the Oxycodone Biobank of HS rats at University of California San Diego, we investigated the transcriptional alterations associated with opioid use, withdrawal, and abstinence in male and female HS rats in several brain regions related to opioid use disorder and dependence.

Methods: HS male and female rats underwent oxycodone self-administration followed by acute withdrawal and abstinence. Brain tissue was collected following oxycodone intoxication, acute withdrawal, and abstinence, from separate cohorts of HS male and female rats. An additional cohort of untreated, naïve HS rats were also used for comparison. Subregions of the nucleus accumbens (NAc), including the "core" and "shell" areas, along with the prefrontal cortex (PFC) and suprachiasmatic nucleus (SCN) were micropunched from frozen brain tissue sections then processed for RNA extraction. RNA-sequencing was conducted on NAc shell and core, PFC, and SCN from male and female HS rats ($n = 6$ group/sex/region, total n, 172 samples). Comparisons between treatment groups and sex were conducted using DESeq2 (Bioconductor) with the full model including interaction terms (treatment x sex). Differentially expressed (DE) transcripts were at log fold-change of plus/minus 0.26 and $p < 0.01$ thresholds. For DE transcripts, various enrichment analyses included pathways and gene set enrichment. Rank-rank hypergeometric ordering (RRHO) analyses compared changes in transcriptional patterns between oxycodone intoxication, withdrawal, and abstinence within and between sexes and brain regions.

Results: Within each brain region, analyses investigated the interaction between sex and opioid treatment condition (i.e., intoxication, withdrawal, and abstinence) to identify DE transcripts that were altered in male and female HS rats. In DLPFC, there were 267 DE transcripts altered by sex and opioid treatment condition, along with 156 and 182 DE transcripts significantly altered in NAc

shell and core, respectively. In SCN, 143 DE transcripts were identified. In the DLPFC and SCN, the majority of these transcripts were significantly changed by sex during abstinence (109 transcripts, DLPFC; 99 transcripts, SCN). Enrichment analyses showed pathways related to circadian rhythms and protein among others during oxycodone abstinence in DLPFC specifically in male HS rats. In NAc core, pathways were enriched for circadian rhythms and protein processing, including acetylation during both intoxication and abstinence, specifically in female HS rats. During withdrawal, pathways were mostly associated with growth factor signaling, mRNA splicing, and glial cell activation. Pathways enriched in the SCN were associated with corticosteroid signaling and extracellular matrix organization, also with sex-specific effects specific to oxycodone withdrawal and abstinence.

Conclusions: Our findings begin to identify the sex-specific transcriptional alterations in brain circuitry relevant to opioid use disorder and opioid dependence. Together, our data also suggests associations between circadian rhythm pathways, extracellular matrix signaling, and others in oxycodone withdrawal and abstinence consistent with recent findings in human brain.

Keywords: Oxycodone, Transcriptome Biology, Heterogeneous Stock Rats, Behavioral Genetics

Disclosure: Nothing to disclose.

P613. Simultaneous and Sequential Cocaine + Alcohol PSU Alters Neurocircuitry of Cocaine Seeking

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Background: While many medications reduce the reinstatement of cocaine-seeking in animals, these agents show little clinical efficacy at preventing relapse in humans. This is possibly due to the fact that an estimated 60-90% of cocaine users also use alcohol, but animal models of polysubstance use (PSU) are seldom used. Here we developed a rat model of simultaneous cocaine +alcohol self-administration for the investigation of the neurobiology of drug seeking in a PSU condition.

Methods: Male and female rats underwent IVSA of cocaine alone ($n = 21$) or a cocaine+alcohol solution ($n = 21$) for 2 hr/day for 5 consecutive days, then 6 hr/day, 2 days/week, for 5 weeks. Two doses of cocaine were tested (0.25 and 0.5 mg/kg/infusion) with and without alcohol (12.5 or 25 mg/kg/infusion). At the conclusion of IVSA, rats were tested on a progressive ratio schedule for 2-3 days, followed by tests for cued cocaine-seeking (relapse) at 1 and 30 days of abstinence.

Results: For the low dose of cocaine, the addition of alcohol to the intravenous solution had no effect on cocaine intake during self-administration, breakpoint for self-administration, or cued relapse after 30 days of abstinence. For the high dose of cocaine, the addition of alcohol to the intravenous solution resulted in increased cocaine intake during self-administration ($p = 0.0201$), increased breakpoint for self-administration ($p = 0.0026$), and increased cued relapse after 1 day of abstinence ($p = 0.0359$).

Conclusions: Simultaneous cocaine+alcohol self-administration increases cocaine intake and the motivation to seek drug and drug-associated cues in a dose-dependent manner. Ongoing work is examining the effects of alcohol on the neurocircuitry of cued cocaine-seeking. Sequential cocaine and alcohol uniquely alter patterns of neuronal excitation in the infralimbic cortex and ventral tegmental area relative to monosubstance cocaine use.

Keywords: Polysubstance Abuse, Cocaine Seeking, Alcohol Use Disorder and Drug Addiction

Disclosure: Nothing to disclose.

P614. Determining Parabrachial Nucleus Physiology and Function During Naloxone-Precipitated Opioid Withdrawal

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Background: The drive to relieve aversive somatic and affective symptoms that accompany opioid withdrawal is a contributor to opioid relapse and the progression of opioid use disorder (OUD). Understanding the neurophysiological regulation of neural circuits involved in opioid withdrawal will give us an opportunity to identify novel mechanisms to dampen these aversive experiences of withdrawal and possibly put a break on the cycle of OUDs. Calcitonin gene-related peptide (CGRP, gene name: Calca) of the parabrachial nucleus (PBN) have been demonstrated to be critical in relaying aversive spinovisceral information to brain substrates for appropriate behavioral response to threat or harm. Here, we seek to understand whether PBN CGRP neurons are involved in opioid withdrawal and the neurophysiological mechanisms that may be altered throughout the opioid exposure cycle.

Methods: We performed patch-clamp ex vivo electrophysiology and in situ hybridization to profile the baseline intrinsic properties and potential neurophysiological regulators of PBN CGRP neurons in non-dependent mice. We next performed ex vivo single cell calcium imaging of PBN CGRP neurons to determine how this cell population responds to pharmacological activation of mu-opioid receptors. In order to determine how the PBN is involved across the opioid exposure cycle, we used a regimen of twice-daily i.p. injections of increasing concentrations of morphine across 5 days (20 mg/kg, 40 mg/kg, 60 mg/kg, 80 mg/kg, 100 mg/kg) to induce opioid-dependence mice. To precipitate opioid withdrawal, a 100 ug/kg dose of naloxone was administered (i.p.) 5 hours following the morphine injection of 100 mg/kg of morphine. Control mice were administered saline instead of morphine in the same frequency. Following this treatment, one cohort of mice was used to perform in situ hybridization for Fos, an immediate early gene and a marker of increased neural activity, in PBN tissue. In a separate cohort of mice, we performed in vivo fiber photometry of calcium dynamics in mice undergoing morphine treatment and naloxone precipitated withdrawal.

Results: We were able to profile the spontaneous activity, intrinsic excitability and presence of M-Current and Hyperpolarization-activated current in PBN CGRP neurons. We were also able to determine the responsivity profiles of PBN CGRP neurons following pharmacological activation of mu-opioid receptor in ex vivo PBN slices. We found increased Fos expression on PBN CGRP neurons in morphine-dependent mice following naloxone-precipitated withdrawal ($p < 0.05$). Finally, using fiber photometry of calcium dynamics, we found that morphine reduces PBN CGRP neuron population activity ($p < 0.05$) and naloxone-precipitated withdrawal increase activity ($p < 0.05$).

Conclusions: Based on the Fos and fiber photometry results in morphine dependent mice, PBN CGRP neurons are regulated by naloxone-precipitated opioid withdrawal. Future studies will determine the causal role these neurons may play in mediating opioid withdrawal symptoms.

Keywords: Opioid Withdrawal, Parabrachial, Neurophysiology

Disclosure: Nothing to disclose.

P615. Midbrain Mechanisms in Fentanyl Use and Relapse

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Background: Rates of opioid use and overdose death continue to skyrocket in the United States. Synthetic opioids such as fentanyl contribute to >70% opioid-related deaths, but synthetic opioid use remains broadly understudied. 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, and altered NAc and VTA activity 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.

Methods: All experiments were conducted in 8–10-week-old mice using sex as a biological variable (defined gonadally). Mice were trained to self-administer fentanyl (1.5 µg/kg/infusion IV, 5 days FR-1, 5 days FR-2) in operant chambers equipped with nose-pokes. To assess NAc->VTA projections in fentanyl relapse, 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. To assess molecular adaptations in the VTA, mice received transynaptic AAV-Cre in the NAc prior to fentanyl or saline self-administration training ($n = 8$-12/sex/condition). VTA nuclei were isolated from half the mice (pooled 4 mice/sex/condition) for single nuclei RNAseq with the 10X Genomics platform. Brains from the remaining mice were flash frozen and 16 µm sections containing the VTA were processed with RNAscope and imaged on a confocal microscope.

Results: We found female mice self-administered more fentanyl compared with male mice (Day x Sex $F(1,31)=3.5$, $p = 0.007$), and female mCherry controls exhibited greater fentanyl-seeking behavior compared with male controls (Sex $F(1,31)$, $p < 0.001$). Increased Gq signaling in NAc->VTA neurons abolished fentanyl seeking in both sexes (Virus $F(2,31) = 29.6$, $p < 0.001$; female: $p < 0.0001$; male, $p = 0.002$; Dunnet's post-hoc). Increased Gi signaling in NAc->VTA neurons showed a trend towards decreased fentanyl seeking in female mice ($p = 0.059$). We detected transsynaptic Cre from NAc in 2% of all VTA nuclei, with the most enrichment in cluster 3 (36% of Cre+ nuclei). Cluster 3 nuclei had more expression of dopamine neuron markers—Ddc, Th, Slc6a3, Slc18a2, Drd2 – in addition to enrichment for multiple GABA receptor subunits (Gabrb2,Gabrb1, Gabbr2, Gabrb3, Gabrg3) and vesicular glutamate transporter 3 (Slc17a8). We identified numerous differentially expressed genes in the VTA between fentanyl vs saline self-administering mice: 56 upregulated and 64 downregulated. We also identified sex-specific changes in gene expression: Female--13 down, 9 up; Male—277 down, 135 up. Gene Ontology analysis revealed enrichment in neuronal morphology GO terms (e.g. actin cytoskeleton reorganization, axon extension) neurotransmission terms (e.g. neurotransmitter secretion, synaptic vesicle exocytosis), and potassium channel terms.

Conclusions: First, our data in mice replicate findings in rats 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. Third, NAc likely targets both dopaminergic and GABAergic VTA neurons. Finally, fentanyl self-administration alters expression of genes important for neuronal structure and physiology in the VTA. On-going work aims to validate and expand the snSeq findings with RNAscope, and manipulate expression of molecular candidates.

Keywords: Fentanyl, Self-Administration, Single-Nucleus RNA Sequencing, DREADDs

Disclosure: Nothing to disclose.

P616. Single Cell Sequencing Reveals Cell Type Specific Transcriptional Responses to Oxycodone and Buprenorphine by iPSC-Derived Brain Organoids From Patients With Opioid Use Disorder

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Background: Oxycodone is one of the most prescribed opioid medications in the United States, whereas buprenorphine is currently the most prescribed medication for opioid use disorder (OUD) pharmacotherapy. This study was designed to identify gene expression profiles associated with drug treatment with oxycodone or buprenorphine drug treatment in induced pluripotent stem cell (iPSC)-derived brain organoids from patients with OUD. Our data showed cell type specific transcriptional responses to oxycodone and buprenorphine in iPSC-derived forebrain organoids. We then performed functional genomic studies of genes, transcription factors, and pathways identified during this series of experiments. Our findings enhance the understanding of drug mechanism(s) of action and the underlying pathophysiology responsible for opioid addiction. These results may provide novel mechanistic insight into drug action at single-cell resolution.

Methods: We performed single nuclei RNA-sequencing (snRNA-seq) using iPSC-derived forebrain organoids from three male subjects with OUD. The forebrain organoids were treated with vehicle, oxycodone or buprenorphine for seven days. The concentrations of oxycodone (50 ng/mL) and buprenorphine (2 ng/mL) used to perform these experiments were selected to fall within the range of blood drug concentrations in patients taking standard clinical doses of these two drugs. Functional validation was performed using iPSC-derived forebrain organoids and neural cells.

Results: We hypothesized that oxycodone and buprenorphine might regulate somewhat similar biological pathways in iPSC-derived brain organoids from patients with OUD. We began by performing bulk RNA-seq using iPSC-derived brain organoids to test that hypothesis and observed that oxycodone and buprenorphine displayed surprisingly distinct gene expression profiles. Specifically, we identified 279 and 3333 genes for which expression was significantly altered (FDR < 0.05) after exposure to oxycodone or buprenorphine treatment, respectively, as compared to vehicle treatment. The most common and most highly affected biological pathways in the presence of oxycodone differed from buprenorphine. However, bulk RNA-seq cannot provide detailed insight into the molecular mechanism of drug action, i.e., specific cell type alternation. As a result, we took a step further by performing single-cell transcriptomics using the same samples.

We analyzed 25787 nuclei using snRNA-seq after quality control filtering. The single-cell sequencing data revealed 16 transcriptionally distinct clusters containing treated and untreated organoids from three subjects. As a first step, we set out to determine the cell-type specific and drug-specific gene expression profiles in iPSC-derived forebrain organoids. Differential gene expression analysis was performed for each cluster to determine the effect of the drugs on iPSC-derived forebrain organoids in a cell-type specific fashion. Our results showed that oxycodone and buprenorphine displayed distinct gene expression profiles. Specifically, oxycodone affected transcriptional response primarily

in neurons, whereas buprenorphine significantly influenced transcription regulation in glial cells. Pathway analysis showed that oxycodone induced the type I interferon signaling pathway in neural cells, whereas the mTOR signaling pathway was the most commonly affected pathway in response to buprenorphine treatment.

We pursued functional genomic studies to confirm that IFNγ concentrations were induced by oxycodone in iPSC-derived forebrain neurons and forebrain organoids. However, buprenorphine had no effect on IFNγ concentrations. We next constructed a protein-protein interaction network using the differentially expressed genes in the interferon signaling pathways, and found that STAT1 appears to interact with several genes in the interferon signaling pathway, which could be activated by oxycodone but not buprenorphine. Strikingly, we also found that, at baseline, STAT1 expression in iPSC-derived forebrain neurons was significantly higher in patients with OUD as compared with unaffected control subjects. Even more striking, STAT1 expression was significantly induced by oxycodone only in patients with OUD but not in unaffected control subjects.

We subsequently cultured iPSC-derived neurons and exposed them to fludarabine, a STAT1 inhibitor that causes a specific deletion of STAT1 but not other STATs. Of importance, a series of genes involved in the interferon signaling pathway significantly altered their mRNA expression in response to fludarabine treatment in a dose-dependent fashion. These results suggest that upregulation of STAT1 might be associated with OUD, and that STAT1 might play a role in transcriptional regulation in type I interferon signaling in response to oxycodone.

Conclusions: Our results revealed cell type specific transcriptional responses to oxycodone and buprenorphine by iPSC-derived brain organoids from patients with OUD. We demonstrated that elevation of STAT1 expression associated with OUD might have a role in transcriptional regulation in response to oxycodone but not buprenorphine. Our results imply that different opioids do not share the same biological effects or mechanisms. This series of studies identified a novel layer of molecular regulation associated with OUD that might potentially open new avenues for future drug development to treat and/or prevent OUD.

Keywords: Opioid Addiction, Oxycodone, Buprenorphine, Single-cell RNA Sequencing, Brain Organoids

Disclosure: Nothing to disclose.

P617. Insulin-Like Growth Factor 1 and Its Receptor in Prefrontal Cortex Regulates Heroin Addiction-Induced Maladaptation

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Background: Opioid use disorder (OUD) is a chronic relapsing psychiatric disorder with an enormous socioeconomic burden. Relapse is one of the most difficult challenges during recovery. None of the current treatment is effective to prevent relapse. Therefore, it is urgent to further understand the neurobiology of OUD.

Clinical studies show that the neuronal responses to stimuli in the prefrontal cortex (PFC) from individuals with OUD are disrupted. Consistently, preclinical data also report opioid-induced synaptic dysfunction in the PFC. Given the critical role of PFC in regulating opioid-related behaviors, it is vital to investigate the molecular mechanisms underlying opioid-induced PFC dysfunction and its role in shaping opioid-induced behavioral plasticity. Increasing studies have shown that insulin-like growth factor 1 (IGF1) and IGF1 receptor (IGF1R) regulate synaptic transmission, but the involvement of IGF1/IGF1R in opioid addiction-induced synaptic dysfunction remains unknown. Here we used a mouse heroin self-administration (SA) model to investigate the role of IGF1/IGF1R on heroin-induced maladaptation in the PFC.

Methods: Current study used both male and female C57BL/6 J mice. All the procedures are approved by the Institutional Animal Care and Use Committee. 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 age of P60, mice were trained to self-administer water on a fixed ratio schedule. After water training, animals underwent jugular surgery. After recovery, mice underwent 10 days of heroin SA (50 ug/kg/infusion, 3 h/session), followed by 14 days of forced abstinence. Then animals were sacrificed for testing IGF1 level in the PFC. Another set of animals received IGF1 infusion in the PFC during the last 5 days of abstinence, then were subjected to 1 hour of cue- and context-induced drug seeking test. To study the IGF1-dependent synaptic and molecular mechanisms that underlying heroin-induced behavioral plasticity, a different set of animals that received IGF1 infusion during abstinence were sacrificed without cue re-exposure for electrophysiology recording and RNA-sequencing.

Results: We found that IGF1 in PFC was significantly decreased after prolonged abstinence from heroin SA ($n = 6$/group, $t10 = 3.3$, $P < 0.01$, t-test). Moreover, intra-PFC IGF1 administration attenuated ($n = 7$-8/group, $t13 = 2.8$, $P < 0.05$, t-test) while IGF1R selective knockdown in PFC pyramidal neurons potentiated ($n = 8$-10/group, $t16 = 2.2$, $P < 0.05$, t-test) heroin-seeking behavior. Furthermore, we used whole-cell patch-clamp method to detect changes in synaptic function. Our data showed that intra-PFC IGF1 administration restored heroin abstinence-induced decrease in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor- and N-methyl-D-aspartate (NMDA) receptor-mediated evoked excitatory postsynaptic currents ($n = 8$-20cells/3-5 mice/group, AMPAR: $F_{treatment}(2, 29) = 7.3$, $p < 0.01$; NMDAR: $F_{treatment}(2, 37) = 6.1$, $p < 0.01$; Two-way rmANOVA). In addition, IGF1 also recovered the elevated AMPA/NMDA ratio in response to heroin-associated cues in mice underwent heroin abstinence ($F2, 38 = 20.7$, $p < 0.001$, Two-way ANOVA). To explore the underlying molecular mechanisms, we used translating ribosome purification method coupled with RNA-sequencing. We found that Igf1 induces genome-wide restoration of genes involved in neural signaling in PFC pyramidal neurons from mice that underwent heroin abstinence.

Conclusions: These data indicate that IGF1/IGF1R system in the PFC play a key role in regulating heroin-induced behavioral and synaptic plasticity, which will provide a novel therapeutic target for the development of OUD treatment strategies.

Keywords: IGF-1, PFC, Heroin Self-Administration, Synaptic Function, RNA-seq

Disclosure: Nothing to disclose.

P618. Impact of THC Vape on the Proteomic Profile of Extracellular Vesicles in the Brain

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Background: With legalization of cannabis in the US, there is an urgent need to more clearly understand the drug's effects on central signaling mechanisms. Extracellular (EVs) vesicles have been identified as intercellular signaling mediators, which contain a variety of cargo, including proteins, enzymes, and RNA

transcripts. The focus of these studies was to examine whether the main psychoactive component in cannabis, Δ9-tetrahydrocannabinol (THC), alters EV cargo in the brain.

Methods: In vitro studies were first conducted to determine whether THC can act on primary epithelial cells derived from the dorsal third ventricle of rats. Next, to examine the impact of THC in vivo, male and female rats were exposed to aerosolized THC or vehicle in vapor chambers. The first cohort of rats ($n = 12$/group/sex) received a single session of exposure, and the second cohort ($n = 12$/group/sex) received 14 consecutive daily sessions of exposure. CSF was collected from the cisterna magna, and EVs were extracted with SBI SmartSEC and then processed for label free quantitative proteomics analyses via high resolution tandem mass spectrometry. Quantitative LFQ mass spectral data were analyzed using Progensis QI Proteomics software.

Results: Cannabinoid receptor (CB1R) expression was localized in the choroid plexus, and THC upregulated the expression of c-fos, CB1R mRNA, and mir-204, a transcript localized in EVs. In the THC vape exposed rats, multiple EV proteins were identified as being differentially expressed following either acute or chronic exposure. Interestingly, opposing effects were found with some proteins (such as APOE), in which acute exposure decreased, but chronic exposure increased, expression. These findings are paralleled by our prior data examining RNA transcripts packaged in CSF EVs.

Conclusions: Our findings reveal that cannabinoids can modulate intercellular signaling mechanisms in the brain, with differential effects following single or chronic exposure. Our data further support the contention that THC can act on CB1 receptors in the choroid plexus to mediate EV signaling, which may then integrate into different brain regions to modulate cellular function.

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Keywords: THC, Extracellular Vesicles, Proteomics

Disclosure: Nothing to disclose.

P619. Gene Expression Within Reward-Specific 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: The first step of the proposed experiments was to establish a protocol for tissue collection that would allow us to sort the cells tagged in different ensembles, i.e., small percentages of 1% or less of the NAcore cells, using FACS technology without damaging the mRNA and hamper sequencing. The presentation will include an in-depth description of the protocol with the key takeaways and practical advice to increase reproducibility and yield of good quality mRNA. We then identified genes differentially regulated within each neuronal ensemble tagged, the cocaine-, sucrose-, and overlapping ensembles, compared to non-ensemble cells.

Conclusions: Establishing differential gene expression profiles exclusively linked to drug-seeking raises the possibility to therapeutically targeting maladaptive drug seeking without affecting essential and biologically adaptive seeking of natural rewards.

Keywords: Cocaine, c-Fos-Expressing Ensembles, Reward Neural Network

Disclosure: Nothing to disclose.

P620. Delta-9-THC in Marijuana and the Adolescent Amygdala

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Background: Compared with adult onset, adolescent-onset cannabis use confers a higher risk for developing neuropsychiatric symptoms and disorders, some of which emerge during adulthood. The risks may be amplified currently as more youth are using cannabis daily and/or consuming higher potency cannabis (high THC: Δ9-tetrahydrocannabinol). These unprecedented trends present major gaps in our knowledge of age-dependent molecular adaptations engendered by exposure to frequent high THC exposure and of strategies to mitigate cannabis-induced maladaptive changes.

Methods: In adolescent nonhuman primates, we measured the effects of daily long-term THC (1 mg/kg Δ9-tetrahydrocannabinol; 4 months; $n = 4$) or THC + CBD (1 mg/kg: 3 mg/kg; $n = 4$) or vehicle ($n = 4$) on gene (RNA-Seq) or protein expression of amygdala, whether CBD (cannabidiol) antagonized THC effects, and if findings were associated with sleep disturbances.

Results: RNA-Seq analysis of left amygdala showed that THC dysregulated canonical pathways associated with sleep disorders (ranking first statistically), with dysregulated genes including TRPC7, IL1R, RBFOX1, and genes encoding hormone receptors. Other dysregulated canonical pathways were associated with cannabis use disorder, anxiety, emesis, dopamine neurodevelopment and psychotic disorders. THC + CBD produced overlapping but differently regulated genes than THC alone. An inflammatory marker (GFAP) was up-regulated in amygdala of THC-treated nonhuman primates which correlated with sleep fragmentation in the same subjects. Coadministration of THC with cannabidiol attenuated sleep fragmentation and GFAP up-regulation.

Conclusions: Maladaptive changes in amygdala elicited by THC may contribute to a range of cannabis-induced adverse behavioral and physiological consequences, including sleep disturbances that are associated with early onset, heavy marijuana use by adolescents. As CBD mitigated some effects of THC, CBD content of cannabis and THC:CBD ratios conceivably can modulate specific negative outcomes of high frequency, high potency cannabis.

Keywords: Marijuana, Adolescent, Sleep Disturbances, High Potency THC, Neurodevelopmental Disorders, Cannabidiol, CBDV, Translational Models, Clinical Trials

Disclosure: Kennedys Law Firm: Consultant (Self).

P621. Cocaine Use During Oxycodone Withdrawal Reduces Somatic Signs of Withdrawal and Exacerbates Oxycodone-Induced Glutamate Dyshomeostasis

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Background: Opioid use disorder (OUD) is a leading public health crisis in the United States, and commonly co-occurs with cocaine use. Mechanistically, glutamate signaling within cortico-striatal circuitry underlies use of both opioids and cocaine, such that self-administration (SA) of these substances alters synaptic plasticity measured as changes in AMPA-to-NMDA current ratios (A/N) within the nucleus accumbens core (NAcore). Specifically, cocaine potentiates A/N, whereas opioids induce long term depression (LTD) of NAcore medium spiny neurons (MSNs). The current study determined whether cocaine use during oxycodone withdrawal (1) reduces somatic withdrawal signs, and (2) reverses oxycodone-induced LTD as measured by A/N.

Methods: Long Evans male and female rats ($N = 34$) underwent oxycodone (0.03 mg/kg/infusion) or food and cocaine (0.25 mg/kg/infusion) SA in an A-B-A-B design. Rats underwent a minimum of 10 oxycodone SA ("A") sessions prior to the first cocaine SA ("B") phase, and following the second phase of oxycodone SA ("A"), rats underwent one cocaine SA session ("B") at the 24-hr oxycodone or food withdrawal timepoint. Somatic signs of withdrawal were measured at 0, 22, and 24 hrs post-oxycodone or food SA. NAcore A/N was then measured via electrophysiological recordings.

Results: Cocaine consumption significantly increased following oxycodone withdrawal, ($p < 0.01$, comparing cocaine consumption prior to and following 24 h oxycodone withdrawal), but not following food SA ($p > 0.05$). Further, cocaine SA significantly reduced somatic signs of oxycodone withdrawal ($p < 0.05$), but did not significantly alter somatic signs in the food control group ($p > 0.05$). Compared to food SA, we found that rats in withdrawal from oxycodone SA showed significantly lower A/N ($p < 0.01$). Further, A/N was significantly lower following cocaine SA during oxycodone withdrawal as compared to cocaine SA following food "withdrawal" ($p < 0.01$). Finally, compared to cocaine SA in a food control group, cocaine SA during oxycodone withdrawal decreased A/N.

Conclusions: Cocaine intake during oxycodone SA reverses oxycodone withdrawal, which on its own renders synapses in a de-potentiated state. Together, these data suggest that oxycodone use induces persistent changes in glutamatergic signaling, which may exacerbate motivation for cocaine co-use due to alleviation of withdrawal symptomatology.

Keywords: Co-Abuse, Opioid Withdrawal, Cocaine and Opioid Use Disorders, Glutamatergic Transmission, Glutamate Transporter (EAAT3)

Disclosure: Nothing to disclose.

P622. Sex Differences in Cortico-Striatal-Limbic Responses to Stress and Alcohol Cues Predict Future Heavy Drinking Outcomes

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Background: Growing research suggests that chronic alcohol use disrupts neural stress and reward pathways, and such dysfunction increases stress- and alcohol cue- related craving and underlies high alcohol relapse risk in alcohol use disorder (AUD). Previous research has shown that specific disruptions in the ventromedial prefrontal cortex (vmPFC) and striatal responses to stress and alcohol cues are associated with future heavy drinking days (HDD). While sex differences in neural stress and cue responses have been reported, their association with alcohol use outcomes has not been studied thus far. Therefore, the current study examined sex differences in the prospective association between neural stress and alcohol cue responses and future alcohol use outcomes during an 8-week behavioral AUD treatment.

Methods: Seventy-two treatment-entering men and women with AUD (ages 18-60, 43 males and 29 females) underwent functional Magnetic Resonance Imaging (fMRI) scanning during which they participated in a well-validated cue provocation task exposing them to standardized and matched visual cues for stress (S), alcohol (A) and neutral control (N) cues over six successive runs in a randomized block design per condition. Whole brain, voxel-based second-level fMRI analyses were conducted using linear mixed effects models (3dLME, AFNI) covarying for age and number of days of abstinence at treatment initiation assessing Condition (S, A, N) x Drinking Outcome (i.e., %HDD), and Condition x %HDD x Sex as predictors and Participant as a Random effect. Subjective alcohol craving was repeatedly assessed in each block per condition.

Results: Across all participants, S and A significantly increased alcohol craving with no sex differences ($p's < 0.01$). In fMRI, significant Condition x %HDD interaction showed that greater right amygdala, caudate, putamen, hippocampus, fusiform gyrus, left vmPFC, dorsomedial thalamus, dorsolateral prefrontal cortex (dlPFC) as well as bilateral anterior insula (AIC), orbitofrontal cortex (OFC) and dorsal anterior cingulate (dACC) activation was associated with subsequent higher %HDD during treatment. Post-hoc analyses revealed that increased activation in the cortico-striatal regions (e.g., vmPFC, caudate, putamen) during A-N as well as limbic regions (e.g., amygdala, hippocampus) during S-N predicted higher %HDD. Greater putamen activation was associated with higher provoked alcohol craving in the entire sample. Notably, a significant three-way interaction indicated that decreased responses of bilateral bed nucleus of stria terminalis (BNST) and hypothalamus to S-N and increased responses of right OFC, caudate and bilateral vmPFC and dACC to A-N were associated with greater future %HDD in female relative to male AUD participants (all fMRI analyses, at $p < 0.001$ threshold, and additional whole brain cluster correction at $\alpha < 0.05$).

Conclusions: Taken together, these findings suggest specific altered neural responses within a distributed cortico-striatal-limbic circuit during both stress and alcohol cues that predicts subsequent higher %HDD in both sexes, with additional limbic mid-brain alterations being specific to women. Identification of these shared and unique neural markers of poor alcohol use outcomes in men and women with AUD are noteworthy, given that women are markedly more susceptible than men to alcohol-related harms, and timely considering worrisome recent statistics showing significant increases in problem drinking in women, who

are catching up to men in levels of alcohol consumption and AUD risk. Our study has important implications for understanding sex-specific differences in neurobiology underlying response to stress and reward cues, vulnerability to relapse as well as development of novel, more effective AUD treatments tailored to each sex and improved prognostic predictions.

Keywords: Alcohol Use Disorder, Cue Reactivity, fMRI, Sex Differences

Disclosure: Nothing to disclose.

P624. Body Mass Index as a Predictor of Extended-Release Naltrexone Treatment Retention in Individuals With Opioid Use Disorder

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Background: Despite the proven efficacy of injectable extended-release naltrexone (XR-NTX) for opioid use disorder (OUD), its clinical use is constrained by poor retention in treatment. Growing evidence suggests that food and opioids interact at the brain reward circuits modulating each others' rewarding properties. However, implications of OUD patients' nutritional status on the treatment outcomes are unknown. In this secondary analysis, we used body mass index (BMI) to explore the role of nutritional status on XR-NTX treatment retention in individuals with OUD.

Methods: 61 OUD individuals who participated in XR-NTX treatment trials and had baseline BMI available, were included in this secondary analysis. Participants were offered up to three monthly injections of 380 mg XR-NTX. Treatment completion was defined as having received all three injections, whereas treatment noncompleters prematurely withdrew from study after recieving 1 or 2 doses. The association between pre-treatment BMI and probability of treatment completion was examined with univariate logistic regression. Moreover, a multivariate analysis was performed with BMI, opioid craving at baseline and age as factors and bootstrapped with 5000 samples.

Results: A total of 41 individuals completed the 3-months' study treatment protocol, whereas 20 dropped out prematurely. Univariate binary logistic regression [χ 2(1)=6.4, p = 0.01; Nagelkerke R2 = 0.14] demonstrated significant positive association between BMI and likelihood of treatment completion (OR = 1.2; 95% CI = 1.03- 1.4). BMI remains a significant predictor of treatment completion in the expanded multivariate logistic regression that controlled for covariates [χ2(3)=11.51, p = 0.009; Nagelkerke R2 = 0.25]. While opioid craving at baseline was not associated with BMI, both BMI and opioid craving demonstrated significant association with treatment completion in the multivariate analysis.

Conclusions: In OUD individuals undergoing XR-NTX treatment, increased BMI was associated with beneficial effects in retaining individuals on treatment. Further research is needed to understand the causality underlying the observed interrelationship between BMI and opioid antagonist treatment outcomes, and examine the implications of our study findings on long-term abstinence and relapse.

Keywords: Opioid Antagonist Treatment, Nutrition, Opioid Use Disorder, Treatment Outcome

Disclosure: Nothing to disclose.

P625. A Practice-Quit Model for Screening Medications for Alcohol Use Disorder

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Background: While developing novel medications to treat alcohol use disorder (AUD) remains a high priority research area, there are major opportunities to refine the process of screening novel compounds. The premise of this study is that screening novel AUD medications can be more efficient and clinically meaningful if early efficacy (phase 2) studies combine the internal validity of laboratory testing with the external validity of clinical trials. To that end, we developed and tested a novel laboratory model in which participants with AUD engage in a practice quit attempt lasting 1-week. In order to leverage the existing literature, we conducted a comparison of a placebo arm versus two established medications for AUD, namely naltrexone and varenicline. We hypothesize that both active medications would result in a (a) higher percentage of days abstinent (b) lower number of drinks per drinking day during the practice quit attempt, and (c) dampened cue-induced craving for alcohol, as compared to placebo.

Methods: Participants were non-treatment seeking individuals with current AUD (moderate-to-severe) who completed a randomized, double-blind, placebo-controlled, crossover study of naltrexone (50 mg), varenicline (2 mg bid), or matched placebo. Participants took the study medication for one week prior to starting the 1-week practice quit attempt. During the quit attempt, participants completed daily interviews with the research staff. All participants completed an alcohol cue-exposure paradigm pre and post-randomization. Analysis of Variance (ANOVAs) followed by planned comparisons across the three medication conditions were conducted to examine the drinking outcomes. Additional analyses are underway to examine medication effects on cue-reactivity. This clinical trials is registered in clinicaltrias.gov, NCT04249882.

Results: A total of 53 individuals with current AUD were randomized to one of three conditions: placebo ($n = 18$), naltrexone ($n = 15$) and varenicline ($n = 17$). Initial analyses, without covariates, found no overall differences across the three conditions on total number of drinks consumed during the practice week. Observed Means were 3.39 drinks for placebo, 7.79 drinks for naltrexone and 5.41 drinks for varenicline, [F(2,49)=1.26, p = 0.29]. Additionally, there was no medication main effect on the likelihood of remaining abstinent for the duration of the practice quit. The observed complete abstinence rates were 55.56% on placebo, 53.33% on naltrexone, and 41.18% on varenicline, [Chi square=0.82, p = 0.66]. Analyses are underway to consider multiple drinking outcomes as well as to test the effects of medication on cue-reactivity.

Conclusions: These initial results do not support the sensitivity of the practice quit paradigm to detect medication effects for AUD. Ongoing analyses will consider other drinking outcomes, include relevant covariates, and test cue-reactivity measured before and after medication treatment. Together, these results and further analyses will develop the practice quit model of medication screening in conjunction with two established pharmacotherapies (naltrexone and varenicline) and leveraging an established screening tool (alcohol cue-reactivity in the laboratory).

Keywords: Alcohol Use Disorder - Treatment, Clinical Trial, Clinical Trials Methodology

Disclosure: Nothing to disclose.

P626. Pharmacotherapy Effects on Alcohol Cue-Reactivity in the Human Laboratory: A Systematic Review

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Background: Alcohol Use Disorder (AUD) is a prevalent but severely under-treated condition. The need for the development of novel, effective pharmacotherapies is critical. Testing novel compounds in humans using experimental psychopharmacology paradigms is a method proposed to detect initial efficacy for AUD treatment. One such human laboratory method is cue-reactivity (CR), which assesses a medication's effect on individuals' behavioral and biological responses to alcohol cues (e.g. craving for alcohol, heart rate, salivation, and blood pressure) in a controlled context, consisting of exposure to images of or in vivo alcoholic beverages. This systematic review aims to describe and evaluate the literature on alcohol cue-reactivity in the laboratory as a screening tool for AUD pharmacotherapies.

Methods: Published reviews on AUD pharmacotherapies were used to identify 40 medications that have been screened using human laboratory paradigms. Searches were conducted in January 2022 with each of the 40 medications in combination with applicable search terms such as "alcohol cue-exposure", "cue-reactivity", "alcohol craving", as well as "cues" and "craving" MeSH terms. Inclusion criteria for the alcohol cue-reactivity studies were: (1) the administration of a pharmacological agent approved or being developed for the treatment of AUD, (2) placebo-controlled, (3) cue exposure in the laboratory, (4) self-reported cue-induced craving, and (5) reported in the English language, or translated to English. PubMed searches yielded 358 studies that were screened on title and abstract, of which 59 were retrieved for full-text screening. Thirty-six eligible studies were included in the final analysis following full-text screening. All studies were independently screened and coded by two reviewers.

Results: The 36 studies included in the final analysis comprised 19 medications, including two combination pharmacotherapy studies. Seventy-five percent of studies were conducted in populations with AUD, while 25% included heavy-drinking individuals. Studies were primarily mixed biological sex (males and females), on average 71% male; 8% of studies enrolled males only. At baseline, participants consumed an average of 190.89 drinks per month, with a range from 39.03 to 412.72 (information available in 72% of studies). Twenty-two percent of studies reported a within-subjects crossover design, while the remaining 78% used a between-subjects design. For cue-reactivity paradigms, 72% of studies exposed participants to in vivo cues, 25% to visual cues, 6% to auditory scripts, and 3% to odor cues. Thirty-six percent of studies also reported at least one biological outcome in addition to clinical assessments of craving.

Conclusions: Alcohol cue-reactivity paradigms are often used to detect an early efficacy signal in medication development for AUD. However, there is a need to standardize experimental procedures. This systematic review identified wide variability with regards to many key methodological considerations, including sample characteristics and quantity of alcohol use, type of cue presentation, crossover versus between-subject designs, and collection of biological outcomes. These results can inform efforts to standardize and leverage cue-reactivity in the human laboratory as a screening tool for novel AUD pharmacotherapies. Further, there is little quantitative data demonstrating that a medication signal in the alcohol cue-reactivity paradigm predicts medication efficacy. Future directions aim to test the predictive utility of cue-reactivity results toward randomized clinical trial outcomes. The results from this work may help streamline the process of screening novel compounds for AUD.

Keywords: Alcohol Use Disorder - Treatment, Cue-Reactivity, Human Laboratory, Alcohol

Disclosure: Nothing to disclose.

P627. Baseline C-Reactive Protein Levels are Predictive of Treatment Response to a Neuroimmune Modulator in Individuals With an Alcohol Use Disorder: A Preliminary Study

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Background: Inflammation has been implicated in the development and maintenance of alcohol use disorder (AUD). Ibudilast is a novel neuroimmune modulator that has shown promise for the treatment of AUD. AUD is a highly heterogeneous disorder, subtypes of which present distinct characteristics and may require distinct treatment strategies. High inflammation, as indicated by high circulating levels of c-reactive protein (CRP), represents a possible subtype of AUD which may identify individuals who are likely to be more responsive to anti-inflammatory AUD medications like ibudilast. The current study evaluated CRP as a predictor of treatment response to ibudilast; hypothesizing that ibudilast would be more effective at reducing drinking and alcohol cue-reactivity in individuals with higher CRP levels.

Methods: This is a secondary analysis of a clinical trial of ibudilast for AUD, which found that ibudilast reduced heavy drinking in individuals with AUD (ClinicalTrials.gov identifier: NCT03489850). Fifty-one individuals were randomized to receive ibudilast ($n = 24$ [16 M/8 F]) or placebo ($n = 27$ [18 M/9 F]) for two weeks. Participants provided blood samples at baseline to assess CRP levels, completed daily assessments of alcohol use, and an fMRI alcohol cue-reactivity task at study mid-point. General linear models were used to evaluate the effects of medication, CRP levels, and their interaction on drinks per drinking day and alcohol cue-reactivity. Tukey post-hoc tests were used to conduct pairwise comparisons to identify group differences.

Results: There was a significant interaction between medication and baseline inflammation ($F(1,44) = 3.80$, $p = 0.03$, $\eta\_p\text{^}2 = 0.11$).Tukey post hoc tests showed that individuals in the ibudilast high CRP group had significantly fewer drinks per drinking day compared to individuals in the ibudilast low CRP group ($p = 0.007$). There was a significant interaction between medication and baseline inflammation on alcohol cue-elicited brain activation in a large cluster extending from the left inferior frontal gyrus through the anterior cingulate cortex to the right caudate and right putamen ($Z = 4.55$, $p < 0.001$). Follow-up analyses identified several group differences, driven by attenuated alcohol cue-reactivity in the ibudilast high CRP group relative to the ibudilast low CRP group and placebo high CRP group ($Z's > 3.33$, $p's < 0.04$).

Conclusions: In this proof-of-concept study, baseline CRP levels significantly moderated the effect of ibudilast treatment on alcohol consumption during the two-week trial, suggesting that individuals with elevated CRP levels showed a beneficial treatment response compared with placebo. Neuroimaging results showed that baseline CRP levels were associated with neural alcohol cue-reactivity in mesocorticolimbic regions associated with incentive salience and motivation attributed to alcohol-related stimuli. This study serves as an initial investigation into predictors of clinical response to ibudilast treatment and suggests that a baseline proinflammatory profile may enhance clinical efficacy.

Keywords: Alcohol Use Disorder - Treatment, C-Reactive Protein, Ibudilast, Inflammation, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P628. Mifepristone as a Pharmacological Intervention for Stress-Induced Alcohol Craving: A Translational Randomized Trial

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Background: Preclinical and clinical work suggests that mifepristone, a glucocorticoids receptor antagonist, may represent a treatment for alcohol use disorder (AUD). We investigated the safety, tolerability, neuroendocrine variation, alcohol craving and consumption after oral administration of mifepristone in a human laboratory comprised of administration of yohimbine, a cue-reactivity procedure and alcohol self-administration.

Methods: This was an outpatient, cross-over, randomized, double-blind, placebo-controlled, human laboratory study with non-treatment-seeking individuals with AUD ($N = 32$). The design was within-subject, with two counterbalanced stages with mifepristone and/or placebo administered for a week. Outcomes were assessed using Generalized Estimating Equations and mediation and moderation analyses assessed mechanisms of action and precision medicine targets

Results: Safety outcomes were excellent and no statistically-significant difference between the mifepristone, compared to placebo, in the hemodynamic response, alcohol subjective effects and pharmacokinetics parameters. Mifepristone significantly reduced alcohol craving, urge and salivary output and increased cortisol level. Cortisol was not a mediator of the relationship between condition and alcohol craving indicators. Moderation analysis testing the interaction between family history density of AUD (FHDA) and mifepristone, suggested that reduced craving and urges were present in individuals with low, but not high FHDA.

Conclusions: The study confirms the safety and tolerability of mifepristone when co-administered with yohimbine and alcohol. Mifepristone reduced the cue-induced alcohol craving; however, this effect was not related to mifepristone-induced increase in cortisol. Moderation of FHDA suggested the importance of evaluating AUD subtypes in order to inform a personalized medicine approach in AUD.

Keywords: Alcohol Use Disorder - Treatment, Neuroendocrine Responses, Cortisol Response to Stress, Glucocorticoid Antagonists

Disclosure: Nothing to disclose.

P629. Substituted Benzothiazole Analogues as Dopamine D4 Receptor Selective Ligands to Treat Substance Use Disorder

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Background: The dopamine D4 receptor (D4R), a G protein-coupled receptor, is predominantly expressed in the prefrontal cortex in which it plays an important role in cognition, attention, and decision making. Studies have indicated that the D4R is a promising therapeutic target for the treatment of neuropsychiatric conditions such as substance use disorders (SUD). D4R ligands have been shown to alter cognition and behavior in animal models of drug addiction. A better understanding of D4R-mediated signaling is essential to treating D4R-associated disorders, including SUD. Despite its clinical importance, there are no currently approved medications that selectively target the D4R for cocaine use disorder. The present study focuses on the design of D4R ligands based on a benzo[d]thiazole scaffold template as the initial parent compound.

Methods: D4R ligands were designed and synthesized following computational modeling predicting favorable interactions. 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, or D4R). The ligands were also studied in β-arrestin recruitment and cAMP inhibition assays for their effects on D2R-like function. We calculated in silico brain penetration using central nervous system multiparameter optimization of chemical features (CNS MPO) and performed Caco-2 membrane permeability tests of selected D4R compounds. We further performed in vitro and in vivo pharmacokinetic analyses with selected compounds.

Results: We produced a library of eighteen compounds with varied substitutions on the pyrimidinylpiperidinyl (PP) ring and/or the benzothiazole moieties. Compounds were profiled using radioligand binding displacement assays, β-arrestin recruitment assays, cAMP inhibition assays, and computational modeling. 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 (>91-fold vs. D2R and D3R). Several compounds were selective D4R antagonists in functional β-arrestin recruitment and cAMP inhibition assays. Based on the analysis profiles, lead compounds were selected for in vitro metabolic stability in rats and human liver microsomes of which some displayed acceptable stability profiles. One of the lead candidates was selected for in vivo pharmacokinetics assessment in rats where it displayed excellent brain penetration with AUCbrain/plasma>3.

Conclusions: These novel ligands display high binding affinity and subtype selectivity for D4R with advantageous pharmacokinetic profiles. Further development of these compounds may provide insights to targeted drug discovery leading to a better understanding of the role of D4Rs in neuropsychiatric disorders such as SUD.

Keywords: Dopamine D4 receptor, Antagonist Ligands, benzo[d]thiazole, Substance Abuse Disorder

Disclosure: Nothing to disclose.

P630. A Peri-Ceorulear Neuropeptidergic Pathway for Modulating OFC-Mediated Reward Seeking

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Background: Research demonstrates that both acute and chronic stress can reduce as well as potentiate an animal's drive for seeking reward, depending on the modality. In humans, anxiety disorders are comorbid with depression and substance use disorders. Previous work indicates that a relatively unknown neuropeptide called neuropeptide S (NPS) acts to reduce anxiety-like behavior as well as drives reward-seeking through activation of its cognate Gq-coupled protein receptor NPSR1. We generated both NPS-Cre and NPSR1-Cre driver mouse lines for accessing and examining the neuromodulatory circuit components of NPS/NPSR1-mediated behaviors. We isolated a population of NPS-containing cells that reside partly within and surrounding the locus coeruleus (LC), known as the periLC. The LC's and peri-LC are known to be involved in stress and cue-processing throughout the brain. They also project to the cortex where there is dense

expression of NPSR1, particularly in the orbitofrontal cortex (OFC). Previous work has shown that NPS administered through intracerebroventricular infusions promotes cue-induced reinstatement of reward-seeking behavior, while intracranial infusion of NPSR1 antagonist to the lateral hypothalamus blocks drug-seeking. In addition to the observed involvement of NPS and NPSR1 in drug-seeking, intracranial infusion of NPS into the amygdala reduces conditioned fear responding and increases exploration. These prior studies indicate that NPS and its receptor, NPSR1 may regulate reward-seeking behavior and anxiety-like states and suggest this neuronal pathway may be sensitive to stress. In our preliminary studies, we found that the periLC sends excitatory monosynaptic projections onto OFC NPSR1-expressing neurons. Thus, the OFC is a strong candidate for NPS-regulation of avoidance and reward-like behavior. Here I tested the hypothesis that periLC-NPS neurons act to modulate reward-seeking behavior through positive gain control in OFC-NPSR1 neurons.

Methods: Whole cell patch clamp electrophysiology: NPSR1-Cre reporter mice ($n = 8$ [4 female/4 male]) expressing AAV-CaMKIIA-ChR2 in the periLC were recorded in NPSR1-positive cells from the OFC. OFC-NPSR1 neurons that receive input from the periLC were identified using ChR2-assisted circuit mapping. In vivo Fiber Photometry: Both NPSR1-Cre ($n = 16$ [8 female/8 male]) and NPS-Cre ($n = 16$ [8 female/8 male]) mice received unilateral infusions of AAVDJ-DIO-GCaMP6s-eYFP to either the OFC or periLC, respectively. In addition, all mice received unilateral fiber photometry implants to the OFC on the same side as the viral injection. This cohort underwent classical conditioning, a fixed ratio 1 (FR1) task, and a social interaction task. A separate cohort ($n = 16$ [8 female/8 male]) then underwent fentanyl' self administration in a FR1 task. 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 1 mm diameter GRIN lens positioned above. After recovery, mice underwent a head-fixed classical conditioning task where they were exposed to multiple cues that predicted delivery of water (neutral), 10% sucrose (appetitive), or 10% quinine solution (aversive) through individual lick spouts. 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, $p = 0.0001$) showing an interesting pattern of activity across cue and drug delivery. Finally, in our preliminary 2p experiments, we are observing that there is mixed heterogeneity in the responsivity of OFC-NPSR1 neurons during delivery of stimuli with varying modalities.

Conclusions: A better understanding of potential targets that are important to anxiety and substance use disorder are necessary for the development of more effective therapeutic strategies. Examining the mechanisms of less understood neuromodulators, such as NPS in driving reward-seeking behavior and anxiety-like phenotypes addresses this behavioral connection. Future work aims to combine Crispr with fiber photometry to gauge NPS transmission's role in the calcium dynamics observed in the OFC-NPSR1 neurons. These experiments serve as the groundwork for developing our understanding of the basic neurobiology of the NPS system in reward. This work was supported by NIH grants F32DA0055480 (Kasey Girven), DA007278, and MH112355.

Keywords: Neuropeptide S, Locus Coeruelus, Orbitofrontal Cortex, 2-Photon Techniques, Electrophysiology

Disclosure: Nothing to disclose.

P631. Neural Basis of Reward Pursuit and Threat Avoidance

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Background: Background: Reward pursuit and threat avoidance comprise fundamental building blocks of behavior that are impacted in a range of neuropsychiatric disorders. The orbitofrontal cortex (OFC), amygdala, and anterior cingulate cortex (ACC) have all been implicated in networks differentiating reward and threat, but these are rarely assessed in naturalistic tasks or using invasive recording in humans. The objective of our study was to determine whether activity in these areas differs between dynamic states of reward pursuit and threat avoidance in humans.

Methods: Electrophysiology data were recorded from electrodes implanted in the OFC, amygdala, and ACC of epilepsy patients while they performed a naturalistic pursuit/avoidance task based on the "Pacman" video game. In one condition they had to pursue "prey", and in another they had to both pursue "prey" and avoid "predators". For each condition, wavelet convolution was performed to obtain induced power at frequencies from 2 to 150 Hz over a one second window from 500 ms before the appearance of predators and prey to 1000 ms after this event. We then subjected each time-frequency (TF) point in this window to a linear mixed effects model with induced power as a function of predator/prey condition, and with subjects and channels as random effects. We corrected for multiple comparisons within areas using permutation testing and between areas with a Bonferroni correction. Clusters of adjacent TF points with a corrected p value of < 0.05 were then treated as significant differences between predator and prey conditions.

Results:. In OFC (5 subjects, 41 channels), theta power (4-8 Hz) was elevated in the predator condition compared to the prey condition at approximately 365-625 ms after the appearance of prey/predators, and again at 650-1000 ms; delta power (2-4 Hz) was elevated at 565-1000 ms, and beta (12-30 Hz) and gamma power (30-150 Hz) were reduced at 775-885 ms. In the amygdala (5 subjects, 32 channels), delta and beta power were elevated in the predator condition at approximately 625-895 ms and 685-800 ms, respectively. In ACC (4 subjects, 18 channels), alpha (8-12 Hz) and beta power were reduced in the predator condition at 200-270 ms, as were beta and gamma power at 330-450 ms, and gamma power again at 770-815 ms. Theta and delta power in ACC were increased in the predator condition at 585-810 ms and 845-1000 ms, respectively.

Conclusions: The multiple clusters of significantly different activity between the prey and predator conditions in the Pacman task suggest that the OFC, amygdala, and ACC all participate in differentiating between reward pursuit and threat avoidance under dynamic, naturalistic conditions. The naturalistic task and human intracranial data give these results an unprecedented level of ecological validity.

Keywords: Intracranial EEG, Approach/Avoidance, Prefrontal Cortex

Disclosure: Nothing to disclose.

P632. Divergent Relationships Between Excitatory and Inhibitory Responses in the Nucleus Accumbens and Sign Tracking / Goal Tracking Behavior

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Background: The ability of reward-associated cues to produce approach and/or interaction varies widely among individuals. For example, if a cue (e.g., extension of a lever) predicts a reward in a different location (e.g., a sugar pellet delivered to a food cup), some rats will preferentially approach and interact with the lever – a behavior known as sign tracking (ST) – and others will approach the site of reward delivery, a behavior known as goal tracking (GT). A propensity towards ST has been linked to susceptibility to drug-taking, relapse, and related behaviors, and it has been shown that sign trackers and goal trackers have distinct patterns of NAc dopamine release during the acquisition of reward-seeking behavior. Moreover, we and others have shown that ST is relatively resistant to extinction and reward devaluation.

We have previously shown (Gillis and Morrison, 2019) that cue-evoked excitations in the NAc encode the vigor of both ST and GT behavior. At the same time, among sign tracker individuals (but not goal tracker individuals), reward-related excitations showed a sharp decrease over the course of training, which may reflect a decreasing reward prediction error encoded by phasic dopamine release. However, a substantial subset of NAc neurons respond to reward-predictive cues with inhibitions that, similar to excitations, encode the vigor of approach during instrumental tasks (Morrison et al., 2017). Therefore, we set out to compare the patterns of excitatory and inhibitory cue- and reward-evoked responses during the acquisition of ST and GT in sign tracker and goal tracker individuals. In addition, we wished to test the hypothesis that cue-evoked responses, whether excitatory or inhibitory, would be relatively insensitive to reward devaluation in sign tracker individuals.

Methods: Using custom-constructed microelectrode arrays, we recorded the activity of individual neurons in the NAc core throughout the acquisition and expression of ST and/or GT behavior. We also recorded neural responses during extinction sessions following either reward devaluation or sham devaluation. Subjects were male and female Long-Evans rats.

Results: Similar to excitatory cue-evoked responses, we found that both the proportion of inhibited cells and the magnitude of inhibitory responses increase over the course of learning; this is true for both sign tracker and goal tracker individuals. Unlike cue-excited neurons, reward responses in cue-inhibited neurons, which are predominantly (but not exclusively) inhibitory, do not diverge between sign tracker and goal tracker individuals. During extinction sessions without reward devaluation, some cue-evoked excitations appear to "extinguish" their response, but others do not; we found that this is also the case for cue-evoked inhibitions. Following reward devaluation, on the other hand, the proportion of cue-excited neurons drastically decreased while the proportion of cue-inhibited neurons and non-responsive neurons increased. This difference was apparent even among the first few cue presentations following reward devaluation. Finally, we were surprised to find that some cells retained robust excitatory or inhibitory responses to omitted reward during extinction sessions, even following reward devaluation.

Conclusions: Inhibitory responses in the NAc, like excitations, evolve during learning; but our findings suggest that, unlike excitations, their activity does not directly reflect a dopaminergic reward prediction error signal. This is consistent with a previous report that cue-evoked inhibitions, unlike excitations, are not sensitive to dopamine receptor antagonists (du Hoffman and Nicola, 2014). Interestingly, some inhibitory responses can be "unmasked" by dopamine receptor blockade; our results suggest that a similar phenomenon may occur when approach behaviors are suppressed following extinction or reward devaluation. Finally, the preservation in some neurons of responses to cues and/or at the time of omitted reward might provide a substrate for the maintenance of cue-reward associations and recovery/relapse after extinction.

Keywords: Nucleus Accumbens, Single-Unit Electrophysiology in Vivo, Sign-Tracking, Reward Devaluation, Extinction

Disclosure: Nothing to disclose.

P633. Brain Mechanisms of Hypersensitivity to Distress in Substance Use Disorder

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Background: Negative valence systems, such as stress, depression and anxiety, are known to increase one's risk for the development of substance use disorder (SUD) and vulnerability for SUD relapse; hence, the high comorbidity between SUD and mood disorders. Based on the scientific premise that the lateral habenula (LHb) encodes negative motivational stimuli, thereby, playing a critical role in regulating mood, reward and valence-based learning, we tested a model that hypersensitivity within the brain's lateral habenula (LHb) is related to negative valence and substance use disorder (SUD). We hypothesized that increased functional connectivity between the LHb and areas reported during motivational processing (Filbey et al., 2013) will be associated with greater stress and negative mood symptoms as well as negative motivation. Furthermore, we tested that severity of substance use mediates the relationship between LHb connectivity and negative valence and motivation.

Methods: We used fMRI resting state data from 69 adults with self-reported substance use (age: $M = 27$, $SD = 9$; males$= 45$). Negative valence was measured using the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), the Perceived Stress Scale (PSS), and the Early Life Stress (ELS). The Monetary Incentive Delay (MID) task was used to measure negative motivation. LHb whole brain resting state functional connectivity (rsFC) was evaluated using left and right LHb seeds in CONN Toolbox. Regression analyses were conducted that included connectivity z-scores [right and left LHb seed connectivity with a priori ROIs - right frontal pole (rFP), right cingulate gyrus (rCg), right anterior cingulate gyrus (rACG), right fusiform gyrus (rFFG), right post-central gyrus (rPoCG), right putamen (rPU), left cerebellum (lCBM), left parahippocampus (lPHP), and left amygdala (lAMG)], BDI, BAI, PSS, and ELS as well as substance use. Mediation analyses were then conducted to determine whether LHb connectivity mediates the relationship between negative valence and symptoms of SUD from the Structural Clinical Interview for DSM-5 (SCID).

Results: The regression analyses found multiple significant correlations between LHb rsFC and measures of negative valence. BAI was significantly positively correlated with right LHb-right rFP rsFC ($r2 = 0.06$, $p = 0.05$). PSS scores showed a significant positive correlation with left LHb-rFP rsFC ($r2 = 0.09$, $p = 0.05$), as well left LHb-rFFG rsFC ($r2 = 0.10$, $p = 0.05$). ELS scores were significantly positively correlated with right LHb-lCBM rsFC ($r2 = 0.09$, $p = 0.04$) and left LHb-lCBM rsFC ($r2 = 0.08$, $p = 0.05$). BDI scores were not correlated with LHb rsFC. LHb rsFC was also positively correlated with negative motivation such that number of successful punishment trials during the MID task was significantly positively correlated with right LHb-lCBM ($r2 = 0.06$, $p = 0.05$), right LHb-left LHb ($r2 = 0.14$, $p = 0.01$), left LHb-rFP ($r2 = 0.07$, $p = 0.04$), and left LHb-lAMG rsFC($r2 = 0.08$, $p = 0.03$).

Mediation analyses of the above significant correlations found significant mediating effect of symptoms of SUD. Alcohol use disorder mediated the relationship between (1) PSS and left LHb-rFP rsFC ($r2 = 0.24$, $F(3,47) = 5.04$, $p = 0.004$) and (2) PSS and left LHb-rFFG rsFC ($r2 = 0.25$, $F(3,53) = 5.27$, $p = 0.003$). Symptoms of

hallucinogen use disorder mediated the relationship between ELS scores and right LHb-lCBM rsFC (r2 = 0.15, F(3,58)= 3.32, p = 0.03), and between ELS scores and right LHb-lCBM rsFC (r2 = 0.141, F(3,58)= 3.17, p = 0.03). Symptoms of other SUD mediated the relationship between PSS and left LHb-rFP rsFC (r2 = 0.14, F(3,53) = 2.75, p = 0.05), and PSS and left LHb-rFFG rsFC (r2 = 0.14, F(3,53) = 2.9, p = 0.04). SCID scores for alcohol use disorder (r2 = 0.21, F(3,53)= 4.62, p = 0.01), cannabis use disorder (r2 = 0.18, F(3,59) =4.17, p = 0.01), opioid use disorder (r2 = 0.17, F(3,58)= 4, p = 0.01), cocaine use disorder (r2 = 0.16, F(3,58)= 3.77, p = 0.02), hallucinogen use disorder (r2 = 0.16, F(3,58)= 4.17, p = 0.01), and other SUD (r2 = 0.1, F(3, 58)= 3.82, p = 0.01) mediated the relationship between negative motivation via response during punishment trials and left LHb-right LHb rsFC. Other SUD scores further mediated the relationship between left LHb and the rFP and response during negative motivation (r2 = 0.12, F(3,58)= 2.83, p = 0.05).

Conclusions: These results provide evidence for the relationship between LHb intrinsic network connectivity with brain motivational networks, negative valence, and SUDs. Our findings further suggest that SUDs exacerbate the disruptions in negative valence systems and LHb connectivity. Future studies should examine the direction of these effects.

Keywords: Alcohol and Substance Use Disorders, Negative Valence System, Resting State Functional Connectivity, Lateral Habenula

Disclosure: Nothing to disclose.

P634. Dopamine Release in the Nucleus Accumbens Core Controls Behavior in Response to Aversive Stimuli

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Background: Understanding how behavior is controlled by environmental stimuli is critical to understanding how and why behavior occurs, as well as how this is dysregulated in disease. The addiction field has focused on understanding how drugs drive future behavior (i.e. positive reinforcement), how repeated drug use results in deficits in these processes, and how associations between drugs and environmental stimuli (classical conditioning, conditioned reinforcement) underlie craving and relapse. These behavioral processes, as well as the interactions between them, are critical drivers of substance use disorders, making the understanding of neural circuits that underlie these behaviors a major goal of the field. Therefore, by manipulating the environment of subjects (i.e. mice) we can begin to understand what and how decisions are made and the deficits that arise with disease.

Methods: Here we utilized fear conditioning and negative reinforcement paradigms to investigate dopamine release patterns during passive and active behavioral responses while keeping stimulus valence constant (i.e. negatively valenced). The optical sensor dLight in conjunction with in-vivo fiber photometry in the NAc core was used to monitor real-time dopamine responses during each behavioral paradigm. We timestamp each component of the behavior task (cues, shocks, and conditioned responses). This allows us to obtain precise temporal information about when behavior occurred along with a custom pipeline for robust and reproducible analysis. Sample sizes range from 2-5 mice depending on the experiment, and included both males and females.

Results: In the first experiment, we modulated the duration of a discriminative cue (the cue that signaled that responses would be reinforced) in a negative reinforcement task and recorded dopamine responses in the NAc. When the cue was shorter, animals learned more slowly, and the dopamine signal to this cue was decreased. Once animals learned to respond in the task the dopamine response increased to the cue. We first hypothesized that this was due to learning about the escapable nature of the shock. Thus, we compared a fear conditioning task – where the shocks were inescapable – with animals that had learned negative reinforcement. We did find that dopamine was reduced following a fear cue (where the shock presentation was inescapable), and was increased to cues signaling negative reinforcement (where the shock was escapable). However, introducing novel and neutral stimuli into the fear conditioning context caused an increase in dopamine in response to the fear cue – even though the shock remained inescapable. Thus, the directionality of the dopamine response changed in the absence of a change in the contingencies or predictions. Additionally, the temporal dynamics of the dopamine response changed in the presence of novelty where dopamine levels remained elevated for more than 30 seconds.

Conclusions: Dopamine signaling is complex and more than a valence or prediction error detector as the direction of its response to aversive cues differed based on auxiliary information present in the environment (even when that information did not signal changes in predictions or changes in value). Not only does this dopamine signal appear to track novelty in a temporally defined window, but how long dopamine remains elevated is also modulated by the novelty of introduced stimuli. Critically, these cue-induced elevations in dopamine are well-known to modulate the speed of behavior acquisition which holds important implications for dopamine-based disease states such as addiction and how dopamine may be playing a role in observed deficits in cognition.

Keywords: Addiction, Dopamine, Nucleus Accumbens, Negative Reinforcement, Fiber Photometry

Disclosure: Nothing to disclose.

P635. Associations Between the Endocannabinoid System and Drug Craving in Chronic Cocaine Users

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Background: Stress has been proposed as a crucial risk factor for developing drug addiction and relapse, consequently maintaining the vicious circle of substance use disorder. Recent evidence from preclinical and clinical studies suggests that the endocannabinoid system (ECS), directly or indirectly activated by the endocannabinoids anandamide (AEA), 2-arachidonoyglycerol (2-AG), palmitoyl- ethanolamide (PEA), and oleoylethanolamide (OEA) binding at the type-1 cannabinoid (CB1) receptors, plays a key modulatory role in stress vulnerability and resilience. Accordingly, activation of the ECS has been suggested to be directly linked to stress-induced substance craving and reward in animals and humans due to its stress-buffering effects. Although recent preclinical studies indicate the role of the ECS in substance use disorder, human translational studies have been limited so far. Therefore, the present study aimed to assess potential alterations of the ECS in chronic cocaine users.

Methods: We used plasma samples of 195 participants (women and men) from Zurich Cocaine Cognition Study (ZuCo2St). We compared plasma concentrations of the endocannabinoids: 2-AG, AEA, OEA, and PEA between cocaine-naïve control group ($n = 92$) and chronic cocaine users ($n = 103$; 69 recreational cocaine users [RCU] and 34 dependent cocaine users [DCU]). Cocaine

dependence was diagnosed following the DSM-IV criteria, with DCU fulfilling and RCU not meeting these criteria. Further inclusion criteria for the chronic cocaine users were cocaine use of at least 1 g per month, cocaine as primary used illegal substance, and a current abstinence duration <6 months. Exclusion criteria for the user groups were past or current use of opioids, a polytoxic substance use pattern, and an axis-I DSM-IV adult psychiatric disorder with the exception of cocaine, cannabis, and alcohol use disorder as well as history of affective disorders and attention deficit hyperactivity disorder (ADHD). Exclusion criteria for the control group were acute or history of any axis-I DSM-IV psychiatric disorder.

Endocannabinoid plasma analysis was performed by using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Analyses of covariance (ANCOVAs) were used, with Group as the fixed factor, to control for well-known confounding variables sex, age, and acute cannabis use (THC and CBD plasma levels). For our secondary and exploratory analyses, we used stepwise linear regression analyses, forward and backward method, within chronic cocaine users to investigate the association between endocannabinoid plasma concentrations and specific subjective and objective cocaine use variables. For significant findings, we further checked if the results are driven by the cocaine user GROUP (DCU or RCU) by adding GROUP as an additional regressor into the linear regression analysis in a second step (model 2).

The statistical comparisons were carried out with a significance level of $p < 0.05$ (two-tailed).

Results: ANCOVAs showed a significant group effect for 2-AG ($F(2,188)=3.4$, $p = 0.035$, $eta2 = 0.04$) but no group differences were found for AEA, PEA, and OEA (p's > 0.176). More precisely, post-hoc pairwise comparisons identified the highest 2-AG plasma concentrations specifically in the DCU group compared to controls ($F(1,20)=6.6$, $p = 0.012$, $eta2 = 0.05$) and on a trend-level to RCU ($F(1,97)=3.7$, $p = 0.059$, $eta2 = 0.04$), whereas the control and RCU group did not differ in 2-AG plasma levels ($F(1,155)=1.3$, $p = 0.715$).

Within chronic cocaine users (RCU and DCU), 2-AG was a significant regressor for cocaine craving ($F(1, 101)=7.7$, $p = 0.006$, $R2 = 0.07$). Model 2 of the linear regression analysis including the cocaine user GROUP did not significantly explain more variance of the dependent variable ($F(1, 100)=1.6$, $p = 0.216$, $R2 = 0.09$) indicating model 1 including only 2-AG as a predictor for cocaine craving as the best fit.

Conclusions: Elevated 2-AG baseline levels in the DCU group might indicate higher reward response to cocaine in this group, which may lead to increased vulnerability of cocaine dependence. Moreover, our results suggest that elevated baseline 2-AG plasma levels might have stress-buffering effects in terms of cocaine craving, which was observed overall chronic cocaine users and not specific to cocaine dependence. Therefore, the ECS might be a promising pharmaco-therapeutic target for novel treatments of cocaine use disorder to prevent stress-induced drug relapse and improve abstinence in the context of cocaine craving.

Interestingly, our current preliminary findings of endocannabinoid plasma concentrations in non-medical prescription opioid users yielded elevated AEA, OEA, and PEA baseline levels in chronic opioid users ($n = 23$) compared to healthy controls ($n = 29$), but no differences in 2-AG levels. These results indicate distinct alterations of the ECS in cocaine and opioid users suggesting substance specific targets of the ECS in a pharmaco-therapeutic context. Our preliminary endocannabinoid findings in opioid users will be discussed in more detail at the ACNP meeting.

Keywords: Endocannabinoids, Cocaine Addiction, Addiction, Craving

Disclosure: Nothing to disclose.

P636. Astrocyte Activities in the External Globus Pallidus Regulate Action-Selection Strategies in Reward-Seeking Behaviors

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Background: In the striatopallidal circuits, the external globus pallidus (GPe) has been considered an integrative hub for behavioral flexibility in reward-related behaviors because it coordinates the top-down neurotransmission from the two distinctive dorsal striatum regions, the dorsomedial and dorsolateral striatum (DMS and DLS). These regions are the central neural substrates for goal-directed and habitual reward-seeking behaviors, respectively. Indeed, the GPe redistributes the dorsal striatal inhibitory GABAergic tone and signals through the fast-spiking prototypical and slow-spiking arkypallidal neurons in the GPe to the downstream brain region. Interestingly, recent studies demonstrate that the GPe contains abundant astrocytes, which are distinguishable to the neighboring brain regions, and its astrocytes directly modulate the neuronal activities in the GPe, leading to the consequent behavioral changes. However, the role of GPe astrocytes in goal-directed and habitual action strategies is poorly understood.

Methods: We utilized the two operant tasks upon effort- or time-based reward (10□□l of 20% sucrose solution) delivery to establish the dominance of goal-directed behavior or habit. Then, we employed in vivo calcium imaging to probe the temporal dynamics of GPe astrocytes during goal-directed and habitual learnings. Subsequently, using a support vector machine, we tested whether the type of operant tasks is predictable with GPe astrocytic calcium dynamics. Lastly, we determined whether enhancing the calcium signaling in GPe astrocytes and attentional stimulus increase behavioral flexibility.

Results: Using in vivo calcium imaging with fiber-photometry, we found significantly silenced GPe astrocytic activity during habitual learning compared to goal-directed learning. Moreover, the support vector machine (SVM) analysis predicted whether mice perform goal-directed or habitual behaviors. Interestingly, chemogenetic activation of the astrocytes dampened GPe neuronal firing and increased goal-directed reward-seeking behavior. Similarly, attentional stimuli shifted the habitual to goal-directed behaviors, indicating increased behavioral flexibility.

Conclusions: Our study demonstrated that GPe astrocytic dynamics are reduced during habit learning. Chemogenetic and attentional stimulation can prevent the dominance of habitual reward-seeking, at least partly through the activation of the Gq pathway in GPe astrocytes. This novel finding may help to treat maladaptive habit-related disorders such as addiction and obsessive-compulsive disorders.

Keywords: Astrocyte, GPe, Habit, Goal-Directed, Reward

Disclosure: Peptron Inc: Advisory Board (Self).

P637. Lifespan Development of Thalamic Nuclei and Differential Sensitivity to Alcohol Use

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Background: The thalamus is a heterogeneous structure composed of multiple nuclei with critical roles in sensorimotor and cognitive function, and guiding cortical development. Abnormal thalamic morphology has been implicated in the pathophysiology of multiple neurodevelopmental and aging disorders (e.g. Schizophrenia and Alzheimer's Disease). Substance use is highly comorbid and has been identified as a risk and maintenance factor, and emerging preclinical and clinical studies also indicate the thalamus may be especially sensitive to the deleterious effects of substance use, particularly in Alcohol Use Disorder (AUD). To inform the etiology of thalamic dysfunction in psychiatric and neurological disorders, and clarify the effects of alcohol use and thalamic nuclei, the current study 1) characterized age effects on thalamic nuclei volumes across the lifespan, 2) quantified associations between thalamic nuclei and cognitive functions and 3) determined whether thalamic nuclei were differentially affected in AUD.

Methods: The cross-sectional Human Connectome Project Lifespan dataset ($n = 1952$, age range 5-100, 1071 Female) was used to establish age effects on thalamic nuclei volumes. Linear and curvilinear models of age were compared for nine nuclei of the thalamus spanning higher-order and sensorimotor nuclei, controlling for sex, sibling relationship, and total intracranial volume. The association between thalamic nuclei volumes and cognitive function as measured by the NIH Toolbox were also examined. The Enhanced Nathan Kline Institute – Rockland Sample (NKI-RS), a large-scale, cross-sectional, community ascertained cohort was used to determine the effects of AUD on thalamic structure (AUD group: $n = 45$, mean age = 44.9, 27 Female, Healthy Controls: $n = 45$, mean age = 44.4, 31 Female).

Results: Thalamic nuclei volumes showed curvilinear effects of age characterized by three patterns. The first pattern, present in mediodorsal and centromedian nuclei showed decreasing volumes in youth that stabilized in young adulthood and further decreased in older adults. The second pattern, observed in the pulvinar and medial geniculate nuclei showed relatively steady decrease from youth to older adults. The final pattern observed in the ventral posterolateral, ventrolateral, ventral anterior and lateral geniculate nuclei showed stable volumes in youth and young adults followed by a prominent decrease in older adults. Mediodorsal and pulvinar nuclei volumes showed a positive association with executive function scores ($F = 9.365$, $p < 0.01$ and $F = 13.570$, $p < 0.001$ respectively) and this effect did not show any interactions with age. AUD was associated with smaller thalamus volumes compared to healthy controls ($F = 5.065$, $p < 0.05$, $\eta p2 = 0.057$), due to smaller right pulvinar volumes ($F = 8.152$, $p < 0.01$, $\eta p2 = 0.088$).

Conclusions: Our finding that thalamic nuclei have 1) different effects of age on volumes, 2) different associations with executive functions and 3) differential effects in AUD all highlight the importance of examining individual thalamic nuclei when studying thalamus abnormalities in psychopathology. The most prominent differences in age effects on thalamic nuclei volumes were observed in youth and young adults, suggesting that it is especially important to examine individual thalamic nuclei in neurodevelopmental disorders.

Keywords: Thalamus, Brain Development, Ageing, Subcortical Volume, Alcohol Use Disorder

Disclosure: Nothing to disclose.

P638. Social Navigation in Individuals With Cocaine Use Disorder and Childhood Trauma

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Background: Navigating social interactions depends on social mapping which represents the spatial-like organization and use of abstract social information, similar to that of physical space. It is hypothesized that interaction between the hippocampus and prefrontal cortex may guide social navigation similar to their role in physical navigation with the hippocampus building maps and the prefrontal cortex directing map-based decision-making. Cocaine use disorder (CUD) and childhood trauma both show concurrent social dysfunction and neural dysfunction in these regions with prefrontal dysfunction characteristic of CUD and hippocampal dysfunction common in childhood trauma. Here, we use a naturalistic social interaction task to test whether social relationships are tracked differently in navigation-related regions in the prefrontal cortex of individuals with CUD relative to healthy controls (HC), and differently in the hippocampus as a function of childhood trauma.

Methods: Demographically matched groups of CUD ($n = 30$) and HC ($n = 33$) completed the social interaction game during functional magnetic resonance imaging (fMRI). In the task, participants interact with fictional characters to accomplish social goals (e.g., find a job). Unbeknownst to participants, their decisions with each character were modeled as a series of locations through a social affiliation by power space – the "social navigation" of relationships. To probe the neural correlates of this navigation-like behavior, subject-level fMRI activity was regressed onto the angles from the point-of-view of the participant to the characters' locations (the characters' dynamic power to affiliation ratio, relative to the participant). We used regions-of-interest analyses to test two predictions. 1) We predicted that tracking of social locations would be reduced in prefrontal cortex in people with CUD - specifically in right inferior frontal gyrus, a region involved in processing navigational demands (Javadi et al., 2017). To test this, we compared the groups with a t-contrasts in a flexible factorial model, as well as with group classification (a Decision Tree with leave one out cross-validation and permutation-based significance testing). 2) We predicted that individuals with higher childhood trauma would have a reduced social location effect in left hippocampus, given the region's sensitivity to stress and its role in tracking the social angles in this task (Tavares et al., 2015). To test this, we modeled the interaction between childhood trauma (Childhood Trauma Questionnaire score) and group (CUD v. HC) in the same factorial model and used t-contrasts.

Results: As predicted, voxels in the right inferior frontal gyrus showed a smaller parametric modulation effect in CUD than HC (peak voxel FWE-corrected $P < 0.05$). Group classification was well above chance using this region (mean accuracy=66%, $P < 0.001$) but below chance using a control region (motor cortex). Also as predicted, childhood trauma negatively correlated with the activity of a left hippocampus region previously shown to track abstract social locations. Regardless of CUD status, there was negative hippocampus effect (peak voxel FWE-corrected $P < 0.01$).

Conclusions: Our findings suggest that both CUD-related prefrontal dysfunction and childhood trauma-related hippocampal dysfunction extend to the neural tracking of social relationships. Childhood trauma had similar reductions in left hippocampal effects in both CUD and HC participants, suggesting that the effect of trauma is separable from the effects of cocaine addiction in representing social relationships. CUD and childhood trauma may thus impact the representation of social navigation in different but potentially complementary ways. Further research is needed to fully characterize these effects – such as the interaction between these regions in both CUD and childhood trauma, as well as implications for real-world social behavior.

Keywords: Social Cognition, Cocaine Use Disorder, Functional MRI (fMRI), Childhood Trauma, Cognitive Neuroscience

Disclosure: Nothing to disclose.

P639. Metabotropic Glutamate Receptor Subtype 5 and Memory in Methamphetamine Use Disorder

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Background: The group-I metabotropic glutamate receptor subtype 5 (mGlu5) is implicated in methamphetamine (MA) intake and preference. Mice selectively bred for high methamphetamine oral consumption exhibit higher mGlu5 levels in the nucleus accumbens than those bred for low MA drinking (Szumlinski et al., 2017). Expression of mGlu5 receptors in the nucleus accumbens correlates with MA-conditioned place preference, and repeated daily doses of MA increase mGlu5 receptor function, indexed by agonist-stimulated glutamate release in the nucleus accumbens of mice (Szumlinski et al., 2017). In humans, mGlu5 receptor availability is lower in the striatum and orbitofrontal cortex (OFC) in individuals with cocaine dependence as compared to healthy control subjects (Martinez et al., 2014). Associations between cognitive function and mGlu5 have also been observed. A positive association between hippocampal mGlu5 binding and episodic memory was observed in a PET study comparing adults with Alzheimer's Disease and controls (Mecca et al., 2020). mGlu5 positive allosteric modulators also alleviated MA-induced memory deficits in rats (Reichel et al., 2011).

Associations between mGlu5 receptors, cognitive function, and MA use have not been studied in human subjects.

Methods: Twelve individuals with severe Methamphetamine Use Disorder (MUD; 11 men and 1 woman) and fourteen individuals with no history of drug use (6 men and 8 women) participated in this study in which mglu5 glutamate receptor volumes of distribution (VT) were measured in brain with the radiotracer [18 F]FPEB. Participants with MUD were in outpatient ($n = 2$) or residential ($n = 10$) treatment programs and were abstinent from substances of abuse for fewer than 70 days (mean 41 days). All participants completed a drug use questionnaire that assessed lifetime and recent drug use. Those with MUD self-reported their craving for methamphetamine on the Methamphetamine Craving Questionnaire – Brief (MCQ-Brief). Verbal memory was assessed on the Rey Auditory Verbal Learning Test (RAVLT; Rey, 1964), with the variable of interest being immediate memory (age-adjusted Z-score trials A1-A5 total score).

To measure mglu5 volumes of distribution (VT), [18 F]FPEB was administered intravenously (5 mCi bolus/infusion [kbol 205 min]). PET data were acquired on a Siemens Biograph mCT, combined into three images containing data averaged across 10 minutes and corrected for motion with FSL MCFLIRT. The images were co-registered with FSL FLIRT to T1-weighted structural MRI scans. Volumes of interest (VOIs) were derived from MPRAGE images using FSL FIRST (left and right hippocampus) and manually placed masks (ventral striatum, lateral OFC, medial OFC). Time-activity curves provided average tissue (brain) activity in each VOI. Venous blood samples collected at 10-minute intervals during PET data acquisition were used to calculate average decay-corrected plasma activity from 109 to 139 min after the start of the radiotracer, when radioactivity in the brain was at a steady state. VT was calculated as the ratio of activity in brain to plasma activity of the free tracer.

Group differences in mGlu5 VT in bilateral ventral striatum, lateral OFC and medial OFC VT and immediate memory were tested with unpaired t tests. Associations of MA use and craving with VT in the ventral striatum, lateral OFC, and medial OFC were tested with correlational analyses controlling for cigarettes smoked per day and age. The association of immediate memory and right and left hippocampal VT were tested with correlational analyses, controlling for cigarettes smoked per day. Significant sex differences were not observed in RAVLT scores, self-report measures, or VT; thus sex was not included as a covariate.

Results: Individuals with MUD exhibited lower ventral striatal ($t = 2.5$, $p = 0.02$), lateral OFC ($t = 2.3$, $p = 0.04$), and medial OFC ($t = 2.2$, $p = 0.04$) mGlu5 VT than control subjects. MUD VT in these regions was 17-19% lower than control values. Self-reported craving, duration of abstinence, and frequency of methamphetamine use before entering treatment (days per month) did not correlate with VT, though lifetime exposure (years of use X grams per day) approached significant association ($p = 0.07$) with ventral striatal VT.

Immediate memory scores were significantly lower in MUD versus control groups ($t = 2.1$, $p = 0.04$), and correlated with left hippocampal ($r = 0.60$, $p = 0.0019$) and right hippocampal ($r = 0.60$, $p = 0.0019$) mGlu5 VT. Secondary analyses by group found an association between hippocampal VT and immediate memory only in the controls (left hippocampus $r = 0.75$, $p = 0.0049$; right hippocampus $r = 0.70$, $p = 0.012$).

Conclusions: Severe MUD is associated with lower ventral striatal and OFC mGlu5 VT than controls, similar to the difference between participants with Cocaine Dependence and controls observed using a different mGlu5 receptor radiotracer (Martinez et al., 2014). A deficit in mGlu5 expression may impair cognitive function during early abstinence from chronic methamphetamine use, and thereby could interfere with cognitive-based treatments for MUD.

Keywords: Metabotropic Glutamate Receptor 5 (mglu5), Methamphetamine Use Disorder, Verbal Memory

Disclosure: Nothing to disclose.

P640. Ventral Striatal Activation During Anticipation of Smoking Rewards—But Not Monetary Rewards—Predicts Relapse During a Quit Attempt: Exploratory Analyses From a Randomized Clinical Trial

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Background: Cigarette smoking remains a leading cause of death, and the majority of quit attempts end in relapse. Growing evidence supports the role of a "hijacked" reward system, in which chronic smoking is associated with both hypersensitivity to smoking and related cues and hyposensitivity to alternative reinforcers. In our own previous work we found that withdrawal from smoking was associated with an increase in ventral striatal (VS) activation to smoking reward, and a parallel decrease in activation to monetary reward, suggesting a clear dissociation and supporting the hijacking model. However, a recent meta-analysis found that decreased activation to both smoking cues and non-smoking reward cues within the striatum among smokers was associated with greater severity of nicotine dependence, suggesting an overall deficit in reward-related functioning. Direct comparisons between smoking and non-smoking reward processing are challenging due to fundamentally different fMRI task paradigms typically used for each reward type (smoking cue reactivity versus monetary incentive delay tasks). As such, it is

unclear whether heightened sensitivity to smoking reward, deficits in sensitivity to non-drug rewards, or a global deficit in reward processing may be most critical to maintaining smoking behavior. In the present analysis, we leveraged existing data from a small randomized clinical trial with a baseline fMRI scan to examine VS activation during anticipation of both smoking and monetary rewards as a predictor of relapse during a quit attempt.

Methods: Thirty-five daily smokers completed an fMRI scan and were then randomized to one of two treatment conditions. During the fMRI scans, participants completed a previously validated rewarded guessing task designed to evaluate striatal BOLD response during anticipation of both smoking and monetary rewards (both delivered immediately after the scan). In order to mimic the early stages of the quit attempt, participants abstained from smoking for 24 hours prior to the scan, but were provided with a 21 or 14 mg nicotine patch. The primary purpose of the study was to examine the effects of a combined behavioral and pharmacological intervention on reward-related brain function and quit outcomes. Participants in both conditions were provided with very low nicotine content Spectrum cigarettes and nicotine patches for 4 weeks prior to their quit date. They also completed 8 behavioral treatment sessions (4 pre-quit and 4 post-quit) consisting of either behavioral activation or a health education control condition. Following the quit date, participants were provided with a standard 8-week course of nicotine replacement therapy and were followed for 10 weeks to assess quit outcomes. Present analyses examined signal change extracted from within right and left VS regions of interest for the money > neutral anticipation and smoking > neutral anticipation contrasts. Extracted values were entered in survival analyses to predict time to relapse, controlling for age, sex, treatment group, and baseline nicotine dependence.

Results: A total of 29 participants had useable fMRI data. Of these, 11 (38%) relapsed during the quit attempt, defined as smoking at least 1 cigarette for 7 consecutive days. An additional 4 participants (14%) withdrew prior to completion and were presumed to have relapsed. Neither money > neutral anticipation nor smoking > neutral anticipation for right or left VS was correlated with baseline smoking characteristics, including cigarettes smoked per day or nicotine dependence. However, smoking > neutral anticipation in the right VS was positively correlated with craving at the time of the scan ($r = 0.43$, $p < 0.05$) and with the number of cigarette puffs smoked immediately after the scan (out of a possible 12 puffs earned; $r = 0.40$ for the right VS and $r = 0.43$ for the left VS, both p's < 0.05). In survival analyses, greater right VS activation for smoking > neutral anticipation was associated with greater likelihood of relapse (Odds ratio = 63.4, $p < 0.05$). This association remained significant when analyses were restricted to those with complete quit outcome data, and when both contrasts (money > neutral and smoking > neutral) for right VS activation were included in the same model.

Conclusions: We found that a heightened incentive value of smoking reward reflected in right VS activation—rather than a deficit in non-drug reward processing—was predictive of smoking behavior both immediately after the scan and weeks later during a quit attempt. These findings partially support a reward hijacking model, but indicate a less critical role for non-drug reward processing than seen in our prior work. Given nicotine's reward-enhancing effects, it is possible that the use of the nicotine patch at the time of the scan and during the quit attempt served to normalize non-drug reward deficits that could otherwise have been present during withdrawal. As such, these preliminary findings could provide a better window into neural processes contributing to relapse when a quit attempt is aided by nicotine replacement therapy. Future work should further characterize reward-related changes in a larger sample and examine potential avenues for attenuating hypersensitivity to smoking reward.

Keywords: Reward Anticipation, Nicotine Addiction, Smoking Cessation

Disclosure: Nothing to disclose.

P641. Speech and Viewing Choice as Naturalistic Markers of Drug-Biased Salience Attribution in Individuals With Heroin Use Disorder

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Background: Drug addiction is characterized by excessive salience attribution to drug at the expense of nondrug cues. Previously, in a verbal fluency task, individuals with cocaine addiction generated more addiction-related words than healthy controls (HC), illustrating a drug-biased neuropsychological speech-based function. Similarly, in a picture-based choice task that served as an estimate of simulated drug seeking, compared to HC, individuals with cocaine addiction made more choices to view drug images, as predictive of actual drug use outside the lab 6 months later. We tested whether individuals with heroin use disorder (iHUD) exhibited a similar drug bias in speech fluency and behavioral choice. In an entirely novel computational natural language processing (NLP) approach, we further tracked drug bias in salience attribution using the participants' recounting of a drug-themed movie, assessing correlations with the other measures.

Methods: Thirty-two inpatient, medication-stabilized iHUD (age = 40.8 ± 9.0 years, 7 women) and 21 age and sex-matched HC (age = 39.8 ± 10.8 years, 8 women) participated in the study. In the fluency task, participants were instructed to generate as many drug words as possible in 1 min. In the choice task, participants made deterministic selections between two fully-visible heroin, pleasant, unpleasant, or neutral pictures presented side by side. In the movie task, subjects viewed the first 17 minutes of the heroin-themed movie, Trainspotting, during fMRI; their verbal recounting of the movie was recorded immediately after the scan. We transcribed recordings and a priori segmented the movie into drug and nondrug scenes. Independent raters analyzed the proportion of drug vs. nondrug scenes recalled and speech duration. NLP of the speech samples extracted the number of words used, identified frequency of drug-related words based on a word bank derived from all drug words generated in the fluency task, and frequency of first- and third-person pronouns to approximate participants' attribution of personal relevance.

Results: Similar to the patterns in individuals with cocaine addiction, iHUD generated significantly more drug-related words compared to the HC in the fluency task ($p = 0.020$; no group difference in nondrug words: $p = 0.100$). In the choice task, consistent with previous results, iHUD made more frequent choices to view drug pictures compared to the HC ($p = 0.007$). After watching the heroin-related movie, iHUD recalled proportionally more drug compared to nondrug scenes ($p = 0.045$), with a similar pattern for drug vs. non-drug scene related speech duration ($p = 0.024$). NLP measures indicated comparable use of number of words between the groups ($p = 0.365$), but higher drug word frequency ($p = 0.022$), and higher first-person pronoun use in iHUD compared to HC ($p = 0.004$), suggesting a more drug-biased and personal recounting of the movie in iHUD. Importantly, the more frequent use of drug words as indicated by NLP, the more the drug vs. pleasant picture choices in iHUD ($p = 0.004$), suggesting a link between naturalistic measures of drug-biased functioning as potentially predictive of risk for future drug use outside the lab.

Conclusions: We demonstrate pronounced drug bias in fluency and choice measures in iHUD, as previously documented in cocaine addiction. Using machine learning, we show that the semantic content of speech samples from matched iHUD and HC indicates a more personal and drug-biased recounting of the movie in the former, despite comparable speech volume. Importantly, these verbal/semantic and choice features were interrelated, potentially of use for identifying at-risk individuals for early prevention efforts. The neural substrates of this drug biased, multi-dimensional behavioral profile remain to be explored.

Keywords: Heroin, Computational Neuroscience, Natural Language Processing (NLP), Decision Making, Incentive Salience

Disclosure: Nothing to disclose.

P642. Leveraging Daily Diary Methods in Alcohol Use Disorder: Influence of Real-World Cue Exposure on Drinking

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Background: Alcohol cue reactivity paradigms are frequently used in laboratory settings to capture the effect of alcohol stimuli on measures of craving, mood, physiological response, and future drinking behavior. Yet, a paucity of research has assessed the impact of exposure to alcohol cues in the natural environment, such as through ecological momentary assessment (EMA) or daily diary methods, in clinical samples of alcohol use disorder (AUD). As such, the current study sought to assess, across a two-week period, the daily impact of alcohol-relevant cues in the natural environment on quantity of alcohol intake among non-treatment seeking adults with AUD. Our primary hypothesis for this exploratory analysis is that greater daily exposure to alcohol-relevant cues in the natural environment would predict same-day increases in the number of drinks consumed. We further tested whether one's degree of exposure to alcohol-relevant cues during the two-week period would predict an increase in average number of drinks consumed.

Methods: This study utilized data collected during a two-week randomized clinical trial (NCT03489850) of ibudilast that enrolled 52 non-treatment seeking participants with AUD. During the trial, participants completed three in-person visits and morning daily diary assessments (DDAs). For the 14 days of the trial, participants were asked to complete DDAs each morning to report on their previous day's experiences in terms of alcohol intake, craving, and exposure to alcohol-relevant cues. Of primary interest, participants reported on their exposure to any of eight alcohol cues in their natural environment. If participants reported alcohol intake, only daily cue exposure before drinking initiation was included, to capture the effect of cues on subsequent alcohol intake. Participants who completed at least one DDA during the study ($n = 50$: females $= 17$, males $= 33$) were included in these analyses. A daily total cue exposure variable was created (range 0 – 8) and served as the main predictor. Important level-2 covariates included in the model were biological sex, AUD severity (DSM-5 AUD symptom count), and medication condition. As this data comes from a micro-longitudinal trial with repeated measures, a multilevel model with random intercepts and random effect of daily cue exposure was conducted to account for clustering of observations within participants. Model-based multiple imputation was used to treat missing data prior to fitting the regression model, as this procedure readily accommodates mixtures of numerical and categorical missing values and reduces potential bias from random effects. In Blimp 3, we created 50 imputed data sets from 50 MCMC chains that included two additional auxiliary variables: baseline heavy drinking days and daily urge. We then used MPlus 8.6 to fit the multilevel model with an unstructured covariance matrix and pool the resulting parameters estimates and standard errors.

Results: Across all participants, 653 DDAs were completed (92.6% completion rate) with participants missing between 0 to 4 days of diaries and reporting an average of 7.92 drinking days and 3.52 drinks per day during the two-week period. For the cue exposure predictor variable, 53 observations were missing and for the outcome variable, number of drinks, 54 observations were missing. The analysis results from model-based Multiple Imputation show us that, after accounting for relevant covariates, one's daily degree of alcohol cue exposure was significantly associated with same-day number of drinks ($p = 0.008$). The slope coefficient for cue exposure, $\beta 1 = 0.27$ (SE 0.10) tells us that we would expect a relative 0.27-point increase in same-day number of drinks for each additional cue seen. However, average cue exposure over the course of the two-week period, $\beta 2 = 0.20$ (SE 0.26) was not significantly associated with number of drinks consumed ($p > 0.05$). In terms of covariates, only AUD symptom severity, but not biological sex nor medication condition, was significantly associated with average number of drinks ($p = 0.004$), such that greater AUD severity at baseline predicted greater drinking over the two-week period.

Conclusions: This investigation furthers the field's understanding on the impact of naturalistic alcohol cues on drinking in adult clinical samples of AUD. Results showed that daily fluctuations in one's exposure to a range of alcohol cues, such as a bar, alcohol advertisements, other people drinking alcohol, and places alcohol is typically consumed, predicted relative changes in daily drinking behaviors. These findings are in line with those from laboratory cue-reactivity paradigms and speak to the potential impact of real-world exposure to alcohol cues on relapse and heavy drinking. Additional planned analyses with this dataset include examining moderators of cue-reactivity in the natural environment, to potentially identify behavioral phenotypes of AUD that display enhanced incentive sensitization. Future research should extend these findings to treatment-seeking and higher severity samples of AUD and explore sex differences by utilizing ecologically valid reports.

Keywords: Alcohol Use Disorder, Cue Reactivity, Naturalistic Drug Cues, Statistical Methods

Disclosure: Nothing to disclose.

P643. Neural and Hormonal Factors Underlying Sex Differences in Poor Inhibitory Control and IV Alcohol Self-Administration

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Background: Poor inhibitory control is a well-established risk factor for AUD, and recent evidence suggests this is especially true for women. Female drinkers display poorer inhibitory control than male drinkers, and poor inhibitory control is a stronger prospective predictor of escalation of binge drinking in women. Further, we have preliminary data showing less brain activity during response inhibition among heavy drinking women compared to men, particularly in the early follicular phase of the menstrual cycle, when sex hormones (estradiol and progesterone) are low. However, little is known regarding the degree to which neural and hormonal factors underlying sex differences in inhibitory control influence current drinking behavior.

Methods: Female and male heavy drinkers, matched on demographic and alcohol consumption measures, performed the stop signal task to assess inhibitory control while undergoing fMRI. Women were tested at three phases of the menstrual cycle:

early follicular phase (low estradiol and progesterone), late follicular phase (high estradiol, low progesterone), and mid-luteal (moderate estradiol, high progesterone), and men were tested three times at matched intervals. Blood samples were taken to assess serum hormone levels at each session. Immediately following the fMRI scan, participants completed a 60-minute free-access IV alcohol self-administration period.

Results: Data collection is currently ongoing and to date 9 women and 5 men have completed the study. Analyses of estradiol and progesterone levels confirmed that we successfully achieved the targeted menstrual cycle phases. Preliminary analyses show less brain activity in right frontal regions implicated in response inhibition (i.e., right supplementary motor area and inferior frontal gyrus/insula) in women compared to men across sessions. Further, women showed less brain activity during the early follicular phase (low hormones) compared to the late follicular and mid-luteal phases (high hormones). Finally, activity in the IFG was negatively related to IV alcohol administration in women, such that women who had less brain activity during inhibition self-administered greater amounts of alcohol.

Conclusions: These findings replicate our previous findings showing that female drinkers display less neural inhibitory function than males, and that women display less neural inhibitory function when sex hormones are low. Importantly, these results extend previous findings to show an inverse relationship between less neural inhibitory function in women and increased alcohol self-administration. Taken together, these preliminary findings suggest that the early follicular phase, when hormones are low, could be a time of pronounced risk for excessive alcohol consumption in women due to impairments in neural mechanisms of inhibitory control.

Keywords: Alcohol, Women, Hormones, fMRI, Inhibitory Control

Disclosure: Nothing to disclose.

P644. A Neural Circuit Selective for Fast Drug Reward in Humans

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Background: The faster an addictive drug enters the brain, the greater its rewarding effects. This can have powerful implications. When a drug like methylphenidate (MP) is taken orally, resulting in slow brain delivery and slow increases in the neurotransmitter dopamine, it is therapeutically beneficial for attention deficit-hyperactivity disorder. However, when MP is administered intravenously (IV) it enters the brain quickly and like cocaine is highly rewarding. Yet we lack basic knowledge about what underlies this phenomenon in the human brain: which circuits are sensitive to the speed at which a drug enters the brain? This information would be invaluable for development of new therapies to combat addiction.

Methods: We used simultaneous PET-fMRI imaging to triangulate dynamic changes in brain dopamine signaling, functional brain activity, and the self-reported experience of 'high' as healthy adults received doses of MP at different speeds. Twenty individuals received MP in slow (oral 60 mg) and fast (IV 0.25 mg/kg) doses on different sessions in a double-blind, counterbalanced, placebo-controlled study. We first developed a novel PET method to assess dynamic dopamine increases, which we validated in microdialysis recordings of extracellular dopamine in five rats given IV MP, and in two monkeys given oral MP.

Results: Applying this model to our human data, we found that the speed of dopamine increases was higher for IV than oral MP ($p < 0.0001$, paired t-test), as hypothesized. We then tested where brain activity significantly correlated with speed of dopamine increases to oral and IV MP, using multiple regression. This analysis yielded two distinct circuits. The first involved the orbitofrontal cortex, significantly decreasing its activity with both slow (oral) and fast (IV) dopamine increases. However, it's activity did not significantly correlate with 'high' ratings. A second circuit involved the dorsal anterior cingulate cortex (ACC) and its connections with the striatum, which was activated and responded only to fast (IV) MP. Remarkably, ACC activity significantly correlated with IV dopamine increases in all 20 subjects, and it was the only brain region whose activity significantly correlated with 'high' ratings ($p < 0.05$ FDR-corrected).

Conclusions: These data provide first evidence in humans for a network selective for fast drug-induced dopamine increases. This work identifies the ACC as a key region for the subjective perception of drug reward.

Keywords: Substance Use Disorder, Dopamine, Pharmacokinetics, positron Emission Tomography (PET), fMRI

Disclosure: Nothing to disclose.

P645. Behavioral and Brain Reactivity to Uncertain Stress Prospectively Predicts Binge Drinking in Youth

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Background: Alcohol abuse is associated with tremendous burden and there is an urgent need to understand who is vulnerable, and why/how, to facilitate accurate detection and prevention. Prior studies indicate that one potential source of risk for problematic alcohol use is exaggerated reactivity to uncertain threat (U-threat) – or threat that is unpredictable in timing, duration, or intensity. Our lab and others have shown that compared with controls, individuals with alcohol use disorder (AUD) reliably demonstrate greater behavioral reactivity (i.e., startle eyeblink potentiation) to U-threat, but not predictable threat (P-threat). Individuals with AUD also exhibit heightened neural reactivity to U-threat, reflected in increased anterior insula (aINS) and dorsal anterior cingulate cortex (dACC) reactivity and connectivity. Magnitude of startle and brain U-threat reactivity correlates with AUD symptom severity and coping-oriented motives for alcohol use. Taken together, robust cross-sectional evidence indicates that AUD is characterized by exaggerated behavioral-brain reactivity to U-threat. It is theorized that this brain-based individual difference factor emerges early in life and contributes to the onset and escalation of alcohol problems. No study to date, however, has tested this theory using a within-subjects longitudinal design. It is therefore unclear to what extent increased reactivity to U-threat reflects a risk phenotype that can be targeted in early detection and prevention strategies.

Methods: The current study was a multi-session laboratory paradigm with a 1-year tracking period. Given that onset and escalation of alcohol problems typically occurs in late adolescence and early adulthood, male and female participants were required to be 17-19 years old at enrollment. Participants were young adults from the community ($n = 95$; M age $= 18.4 \pm 0.7$; 67.4% Caucasian) with minimal initial alcohol exposure (consuming > 1 but <100 lifetime alcoholic beverages). Individuals were selected to be at-risk for onset of alcohol abuse by virtue of affiliating with risky peers and self-reporting easy access to alcohol. Participants completed two laboratory sessions, approximately 7 days apart. Both laboratory sessions included administration of a well-validated No-Predictable-Unpredictable (NPU) threat-of-shock task designed to probe reactivity during anticipation of U-threat and P-threat – once during collection of startle eyeblink potentiation

as a behavioral index of aversive reactivity and once during functional magnetic resonance imaging (fMRI). Standardized residual scores were calculated to quantify startle eyeblink potentiation during U-threat relative to no-threat and P-threat relative to no-threat. Extracted parameter estimates from anatomical bilateral aINS and dACC masks were used to capture brain reactivity during U-threat > No-threat and P-threat > No-threat. At baseline and 1-year later, participants reported their drinking behavior using a 90-day time-line follow-back procedure. Binging drinking at the 12-month follow-up was the primary dependent variable. To test our hypotheses, we fit a series of multilevel hurdle models which allowed us to model the binary outcome of whether binge drinking occurred (yes or no) and the continuous outcome of number of binge drinking episodes.

Results: We used the zero-inflated submodels to estimate the probability of binge drinking versus not binge drinking at the 12-month follow-up. These submodels revealed that greater baseline startle reactivity to U-threat ($b = -0.64$, $p = 0.009$), greater bilateral aINS reactivity to U-threat ($b = -0.67$, $p = 0.030$), and greater dACC reactivity to U-threat ($b = -0.69$, $p = 0.035$) were associated with increased probability of binge drinking one-year later. There were no associations between behavioral or brain reactivity to P-threat and probability of binge drinking. We used the conditional submodels to estimate the positive count process, or frequency of binge drinking at the 12-month follow-up. No significant associations emerged between any brain or behavioral threat measure and binge drinking in these conditional submodels. All results were adjusted for biological sex and baseline drinking behavior.

Conclusions: Results indicate that behavioral-brain reactivity to U-threat prospectively predicts binge drinking one-year later in a cohort of at-risk youth. These results demonstrate that exaggerated reactivity to U-threat is not just an AUD disease marker; it is a brain-based individual difference factor that connotes risk for problem drinking. These findings also add to a growing literature implicating aINS and dACC (together labeled the 'salience network') dysfunction in the pathophysiology of alcohol abuse. Taken together, exaggerated reactivity to U-threat is a relatively novel objective AUD prevention target, anchored in the brain's salience network.

Keywords: Alcohol Abuse, Anxiety and Stress, Salience Network, Acoustic Startle Response

Disclosure: Nothing to disclose.

P646. Methamphetamine Reduces Striatal Neural Activation to Monetary Reward

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Background: Stimulant drugs like methamphetamine (MA) activate brain reward circuitry, which has been linked to subjective drug reward and the development of drug abuse. However, it is not clear how drugs like MA alter immediate neural responses to a non-drug reward. In this study, we examined if an acute dose of MA altered neural response to receipt of a monetary reward. We hypothesized that MA (vs. placebo) would increase mesolimbic neural activation to reward.

Methods: In a within-subject, randomized, double-blind, placebo-controlled design, 41 healthy adults completed the Doors monetary reward task during fMRI approximately 70 minutes after ingestion of placebo or 20 mg MA. We examined effects of the drug on neural response to reward receipt using a priori anatomical striatal regions of interest (nucleus accumbens (NAcc), caudate, putamen). We also ran exploratory whole-brain analysis to examine drug effects on neural response to receipt of monetary reward.

Results: MA resulted in less NAcc BOLD activation during Win>Loss compared to placebo ($p = 0.007$). The drug did not significantly alter caudate or putamen BOLD activation during Win>Loss (p-values > 0.05). Exploratory whole brain analysis revealed that MA decreased BOLD activation during Win>Loss in the striatum, including the NAcc, caudate, and putamen, compared to placebo ($p < 0.05$, corrected).

Conclusions: Our findings suggest that stimulant drugs like MA that are known to activate brain reward circuitry may acutely decrease mesolimbic neural response to the receipt of non-drug, monetary rewards. It is possible that MA administration masked the expected neural response to reward by increasing basal dopaminergic tone.

Keywords: fMRI, Mesolimbic Reward Circuitry, Methamphetamine, Healthy Individuals

Disclosure: Nothing to disclose.

P647. Anterior Insula to Nucleus Accumbens Brain Tract Structure but Not Functional Connectivity Predicts Relapse to Alcohol Use

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Background: Great strides have been made recently in identifying the biopsychosocial factors that contribute to and are associated with alcohol use disorders (AUD). Recent findings indicate that structural and functional connectivity between the anterior insula (AIns) and the nucleus accumbens (NAcc) may play a critical role in our understanding of addiction. Specifically, white matter integrity is reduced among individuals with AUD relative to healthy controls, while functional connectivity is elevated while viewing alcohol cues. Yet little is known about whether these metrics within the AIns-NAcc circuit can predict treatment outcomes. Therefore, we sought to determine whether structural and functional qualities of tracts projecting from the AIns to the NAcc were associated with relapse to alcohol use six months after treatment. Lastly, we tested whether the relationship between structural connectivity and relapse was mediated by functional connectivity.

Methods: A total of $N = 74$ Veterans (18 women) in AUD treatment were scanned with 3 T functional MRI during an alcohol cue-reactivity task, diffusion tensor imaging, and were followed for 6 months post-treatment. DTI data were processed using mrDiffusion and fMRI data were analyzed using CONN. DTI measures included right and left functional anisotropy (FA), radial diffusivity (RD), medial diffusivity (MD) and axial diffusivity (AD). Functional connectivity was defined as correlated BOLD signal between the AIns and NAcc while viewing alcohol images and neutral images. Binary logistic regression models with backward conditional parameter estimates were used to determine structural and functional predictors of relapse, with bootstrapped validation, followed by a mediation analysis.

Results: Following treatment, 73% of the sample had relapsed by six months. Structural connectivity metrics between the AIns and NAcc were significantly predictive of relapse. Specifically lower right FA ($\beta = -.713$, $p = 0.032$) and higher left MD ($\beta = 1.176$, $p = 0.029$) suggested that lower white matter integrity in the AIns-NAcc tract predicted relapse in this sample. Classification accuracy of a model including these two variables was 83.8% and bootstrap results were significant (right FA CI: -1.448 to -.099, $p = 0.015$; left MD CI: .285 to 2.577, $p = 0.029$). Functional

connectivity metrics during the alcohol cue-reactivity task did not predict relapse ($p > 0.05$), thus the mediation test was not performed. Gender was included as a covariate in all models and results remain unchanged.

Conclusions: These findings highlight a structural target for predicting relapse to alcohol use following standard treatment. In addition, functional connectivity during an alcohol-cue reactivity task was not predictive of relapse in this sample, nor was functional connectivity associated with structural metrics. There has been recent interest in transcranial magnetic stimulation (TMS) to deeper cortical targets, as well as preliminary evidence that repetitive TMS may increase white matter integrity (specifically FA). Thus, future studies targeting the AIns for AUD treatment may be warranted.

Keywords: Alcohol Use Disorder, Addiction Circuitry, Structural and Functional Connectivity, Relapse Biomarkers

Disclosure: Nothing to disclose.

P648. Hydrocortisone Alters the Formation of Alcohol-Related Episodic Memories

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Background: Memory plays a crucial role in alcohol use disorder (AUD), including by promoting relapse in contexts where patients used to drink. We recently showed that, relative to social drinkers, patients with AUD remember alcohol-related episodes differently, with preferentially enhanced memories for alcohol-paired contexts (Goldfarb, Fogelman and Sinha 2020 Neuropsychopharm). One possible mechanism driving this bias is related to the stress response: acute stress also enhances memory for emotionally salient contexts. The memory-altering effects of stress are often driven by glucocorticoids, which are dysregulated in AUD and acutely elevated in response to alcohol. However, the impact of glucocorticoids on the formation of alcohol-related memories, and the consequences of this exposure for later drinking behavior, are unknown. Here we used a pharmacological-fMRI approach to investigate the cognitive and neural mechanisms by which glucocorticoids influence memory formation for alcohol-related episodes.

Methods: We used a double-blind, placebo-controlled, crossover design. Participants ($N = 27$ social drinkers) each performed two rounds of encoding and delayed retrieval (24 h after encoding). In one round, they received 20 mg of hydrocortisone ~1 hour prior to encoding; in the other round, they received a visually identical placebo tablet. fMRI data was acquired during encoding.

During encoding, participants formed associations between 80 photographs of objects and neutral scenes and rated their emotional responses to each object/scene pair. Of these 80 objects, 50% were neutral (handheld household objects) and 50% were alcoholic beverages, previously validated to be comparable in perceptual features. During retrieval, participants were asked to remember different components of these episodes, including item-level (object recognition) and context (recognition of scene associated with object) representations. Immediately following these retrieval tests, participants had an opportunity to freely consume alcohol in the laboratory as part of a previously validated alcohol taste test.

Results: We found that glucocorticoid administration prior to encoding amplified the perceived emotional salience of object/scene pairs ($b = 0.08$ [.04], $p = 0.03$). This change was adaptive for later memory. Specifically, following hydrocortisone, higher emotional salience was associated with enhanced memory for items and contexts. However, this association was flipped following placebo, with higher emotional salience impairing both forms of memory (arousal x drug; item: $F1,71 = 11.34$, $p = 0.001$; context: $F1,71 = 10.2$, $p = 0.002$).

We also identified key associations between glucocorticoid effects and risky drinking behavior. First, we found that glucocorticoids induced a memory bias similar to one that we previously observed among patients with AUD. Following placebo, participants had worse memory for salient alcohol-paired contexts (replicating our past findings in social drinkers; $b = 0.09$ [.03], $p = 0.02$). However, these same individuals showed enhanced context memory for these salient alcohol/scene pairs following hydrocortisone ($b = 0.09$ [.03], $p = 0.009$), a similar bias to that shown previously by participants with AUD. Second, we found that individuals who engaged in riskier drinking behavior (higher AUDIT scores) achieved higher breath alcohol levels in the laboratory, demonstrating the ecological validity of the alcohol taste test ($F1,25 = 11.53$, $p = 0.002$). Critically, these risky drinking individuals also consumed more alcohol after retrieving memories that had been encoded under hydrocortisone (AUDIT x drug: $F1,72 = 9.36$, $p = 0.003$), suggesting that glucocorticoid-induced effects at encoding had consequences for later drinking behavior.

Preliminary analyses of brain responses during encoding indicate that glucocorticoids modulated memory-related processes in the hippocampus (altering connectivity between subfields) and amygdala (influencing representational similarity). Specifically, hydrocortisone increased intrahippocampal connectivity, and changed the relationship between connectivity and subsequent memory performance. Hydrocortisone also promoted pattern separation in the amygdala for alcohol-related episodes, which was in turn associated with less "gisty" alcohol-related context memories.

Conclusions: These results highlight the importance of glucocorticoids in the formation of biased alcohol-related memories. Consistent with past studies of emotional memory, we found impaired recall of contexts paired with emotionally salient objects following placebo. However, we found that hydrocortisone flipped this pattern, selectively amplifying context memory for these emotionally salient experiences. Intriguingly, elevated glucocorticoids at encoding promoted both a bias toward remembering alcohol experiences similarly to patients with AUD and, among riskier drinkers, led to more drinking following retrieval of those experiences. Together, these findings begin to elucidate a novel neurocognitive mechanism in which elevated glucocorticoids bias memory formation and promote later drinking.

Keywords: Cortisol, Episodic Memory, Alcohol, Emotional Arousal, Task fMRI

Disclosure: Nothing to disclose.

P649. The Neural Processes Underlying Avoidance Learning Dysfunction in Problem Drinking

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Background: Drinking as a pain-avoidance coping behavior plays a central role in the maintenance of problem drinking and escalation to alcohol use disorders. Specifically, individuals often seek alcohol to alleviate or avoid painful physical and emotional states. As drinking escalates, consumption is progressively driven by individuals' heightened sensitivity to the painful consequences of alcohol intake cessation. Paradoxically, chronic alcohol use heightens pain reactivity which further motivates drinking as an avoidance coping strategy. Over time, this maladaptive behavior becomes increasingly less amenable to cognitive control, trapping

drinkers in a spiraling cycle of drinking and distress. Yet, the underlying circuits of avoidance learning in problem drinking are poorly understood.

Methods: We acquired fMRI and behavioral data that assessed avoidance learning in 30 problem drinkers and 32 social drinkers who 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. We hypothesized that relative to social drinkers, problem drinkers would exhibit (1) poorer avoidance learning, (2) weakened prefrontal cortical activation to avoidance learning; and (3) greater pain circuit activity in responses to pain.

Results: Our findings confirmed the hypotheses. Specifically, problem drinkers showed lower learning rates during pain avoidance conditions, coupled with reduced dorsolateral prefrontal cortical activity. Brain regions implicated pain reactivity including the insula, dorsal anterior cingulate cortex, amygdala, and periaqueductal gray showed hyperactivation during shock feedback and this activity was positively correlated with the drinking-to-cope measure.

Conclusions: The current work sheds light on the relationships between problem drinking and the neural as well as cognitive processes underlying avoidance learning dysfunction. The characterization of these relationships offers further understanding of negative reinforcement drinking in humans, thus bridging the gap with preclinical research.

Keywords: Alcohol Abuse, Pain, Approach/Avoidance, Cognitive Control, Brain Imaging, fMRI

Disclosure: Nothing to disclose.

P650. Mapping Social Behavior in Non-Treatment-Seeking Heavy Drinkers

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Background: Individuals with alcohol use disorder (AUD) present with social and environmental difficulties that severely impact their quality of life and mental well-being. Progressive impairment of social function is both a consequence and a cause of relapse, creating a vicious circle for people suffering from AUD. Therefore, understanding what factors shape social processing in AUD becomes critical. Using a simulated social media environment, we previously showed that female adolescents with nonsuicidal self-injury, a clinical group with high levels of social stress and at high risk of developing addiction, presented with a negative social bias. Compared to controls, individuals with nonsuicidal self-injury felt rejected significantly more often than controls, liked to see their face less, and were more sensitive to being rejected by other players. Importantly, this negative social bias was associated with engaging in self-harm, and with a distinct brain pattern during anticipation of social feedback. Here, using the same task, we examined social bias and social behavior in a group of young adults that engage in heavy versus light social drinking.

Methods: After screening and upon inclusion, we tested $N = 30$ light social drinkers ($N = 15$ men drinking < 15 drinks/week and $N = 15$ women drinking < 10 drinks/week) and $N = 30$ heavy, non-treatment-seeking social drinkers ($N = 15$ females). We used a task developed by our group, which simulates an online interaction, where participants like and dislike photos of other putative players. Similarly, they anticipate and receive feedback from other players. During and after the game, participants were presented with questions aimed at addressing a potential social bias. In addition, to measure social behavior, the percentage of negative feedback towards other players was collected. The Alcohol Use Disorders Identification Test (AUDIT) and Drug Use Disorders Identification Test (DUDIT) were collected to measure alcohol and drug use severity. Personality traits and impulsivity were collected using the NEO-FFI questionnaire and the Baratt Impulsiveness Scale (BIS-11) respectively. We used a 2×2 ANOVA with group and sex as between factors for all measures. In addition, to identify potential predictors of social behavior, we ran stepwise regressions using percentage of negative social feedback as a dependent variable.

Results: In the heavy (but not in the light) drinkers, AUDIT and conscientiousness scores were significant predictors of negative feedback towards others, explaining about 42% of variance ($F(1, 28) = 9.78$, $p < 0.001$, $R2 = 0.42$). No significant predictors were identified for the light drinkers ($p > 0.5$). There was no between-group difference in self-reports during and after the online game, indicating no social bias in the heavy drinkers ($p > 0.05$). And there was no significant between-group difference in percentage of negative feedback towards others ($p > 0.6$). AUDIT scores were higher in the heavy drinkers, indicating good group categorization ($p < 0.001$). In addition, the heavy drinkers had lower conscientiousness scores ($p = 0.002$) and marginally higher extraversion scores ($p = 0.064$). We identified some sex effects. Females liked to see their face significantly less than males ($p = 0.003$). In addition, females had higher neuroticism ($p = 0.009$), openness ($p = 0.029$), and agreeableness ($p < 0.001$) scores. All participants were free from psychiatric diagnoses.

Conclusions: The negative social bias we initially observed in the clinical population of female adolescents with nonsuicidal self-injury was not evident in our sample of young adults that engage in heavy drinking. This suggests that social bias might emerge only after long-term exposure to harmful behaviors, that is, after drug use has had a clear impact on social function. However, social behavior seems to be influenced by heavy drinking already early stages, as severity of alcohol use predicted negative feedback towards others in the heavy drinking group. In addition, low conscientiousness predicted negative feedback towards others. Altogether, this study identified factors that might influence social processing in people engaging in harmful drinking and that are both dependent and independent on the onset of problematic alcohol use.

Keywords: Alcohol Use, Social Processing, Social Behavior, Big Five Personality Factors, Sex Differences

Disclosure: Nothing to disclose.

P651. Sex Differences in Corticolimbic Microglia Levels in Individuals With Alcohol Use Disorder Compared to Healthy Controls

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Background: Alcohol use is one of the leading causes of disability in the United States and female drinkers are more vulnerable than male drinkers to many of the consequences of alcohol use (NIAAA, 2017). Alcohol stimulates microglia, the resident immune cells of the brain, triggering signaling pathways that activate microglia to carry out repair functions. However, excessive activation releases substances such as inflammatory cytokines and reactive oxygen species that contribute to neuronal dysfunction and death. These pathways may contribute to alcohol-induced neurodegeneration. Neurodegeneration occurs over years of alcohol use and impairs neurocognition, further precipitating alcohol drinking and driving the addiction cycle. Women with alcohol use disorder (AUD) have greater neurocognitive impairments than men with AUD. Importantly, neurocognitive deficits predict poorer treatment outcomes.

The goal of this study was to investigate sex differences in the neuroimmune system that may underlie sex differences in neurocognition in AUD. While our group and others (Hillmer, Mol Psychiatry, 2017; Kalk, Transl Psychiatry, 2017) previously reported that individuals with AUD have lower levels of TSPO, a microglia marker, than controls, few women were included. We hypothesized that women with AUD would show lower levels of microglia relative to sex-matched healthy controls in hippocampus, frontal cortex, and cerebellum and that lower microglia levels would be related to poorer cognitive performance.

Methods: To date, twenty-four individuals with AUD (mean 13 drinks/day, 20 drinking years; 8 females) and 24 age-, sex-, smoking status- and rs6971 single-nucleotide polymorphism genotype-matched healthy control subjects have undergone positron emission tomography (PET) scans with [11 C]PBR28. [11 C]PBR28 measures levels of 18-kDa translocator protein (TSPO), a marker of microglia. PET scans were acquired for 120 min after bolus injection of 541 ± 177 mBq. [11 C]PBR28 on a High Resolution Research Tomograph with arterial blood sampling acquired throughout to measure the metabolite-corrected input function. The outcome measure, volume of distribution (VT), was estimated regionally with multilinear analysis (t*=30 min) as a measure of TSPO availability. Univariate analysis of variance models (one per brain region of interest – hippocampus, frontal cortex, and cerebellum) 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. 'moderate' affinity binders) as a fixed-factor. A priori post hoc analyses were conducted to compare VT values between women with AUD women and sex-matched controls. A subset ($n = 35$; $n = 6$ AUD women, $n = 3$ healthy control women) of subjects completed a cognitive battery. Performance on three cognitive tasks related to verbal learning and memory, executive function, and motor function was compared between the AUD and healthy control group using independent-samples t-tests. Exploratory analyses of relationships between VT and relevant cognitive task performance were conducted using linear regressions.

Results: We found a main effect of diagnostic group such that individuals with AUD had significantly (or trending) lower levels of TSPO availability than their healthy control counterparts in the three brain regions of interest ($0.073 \leq p \geq 0.017$, effect sizes: $0.073 \leq \eta p2 \geq 0.125$). We found preliminary evidence of AUD-related sex differences in TSPO availability. Interactions of diagnostic group and sex were trending ($p \leq 0.010$, $\eta p2 \geq 0.051$) for all three brain regions. A priori pairwise comparison between groups within each sex revealed that women with AUD had significantly lower VT in all three regions (hippocampus $p = 0.034$, $\eta p2 = 0.100$; frontal cortex $p = 0.030$, $\eta p2 = 0.104$; cerebellum $p = 0.018$; $\eta p2 = 0.123$) compared to sex-matched controls. Preliminary analyses revealed that the AUD group performed worse than the healthy control group on the executive function ($p = 0.003$, Cohen's $d = 0.993$), and trended in the same direction on the verbal learning and memory ($p = 0.061$) and motor function tasks ($p = 0.108$). Preliminary analyses revealed that VT values were not related to cognitive performance ($p \geq 0.125$).

Conclusions: Consistent with our prior work (Hillmer, 2017), TSPO availability was significantly (or trending) lower in individuals with AUD compared to healthy control subjects in hippocampus, frontal cortex, and cerebellum. Preliminary analyses revealed that this group difference may have been driven by differences between AUD women and their sex-matched controls. Consistent with our hypotheses, TSPO availability was lower in women with AUD than healthy control women in all three brain regions. While individuals with AUD performed worse on cognitive tasks than their healthy control counterparts, this was not related to TSPO availability. These data suggest that neuroimmune suppression may be more pronounced in women with AUD compared to sex-matched controls and that this could underlie reports of greater neurodegeneration in women. Future analyses will explore brain-behavior neurocognition relationships in an expanded sample with more women.

Keywords: Alcohol Use Disorder, Positron Emission Tomography, Sex Differences, Microglia, Translocator Protein (TSPO)

Disclosure: Nothing to disclose.

P652. Resisting Tobacco Smoking is Linked to Faster Evidence Accumulation During Decision-Making

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Background: Tobacco smoking remains a primary cause of mortality. Continued substance use despite such well-known consequences may reflect disrupted neurocognition, resulting in impaired decision-making. To test this hypothesis, we applied the Hierarchical Drift Diffusion Model (HDDM) to probabilistic reward task (PRT) data; the data were acquired from adult smokers who also completed a relapse analog task (RAT). PRT performance typically measures response bias, which is used to index reward sensitivity. The HDDM offers a richer set of measurements. It conceptualizes decisions in the PRT as a process of evidence accumulation towards response boundaries, and thus generates quantitative estimates of three key parameters: threshold (the distance between the response boundaries, indicating how much evidence must accumulate before a decision is made), starting point bias (whether the evidence accumulation process is shifted towards one response boundary vs. the other at the outset of each trial), and drift rate (the speed of evidence accumulation). A fourth parameter, non-decision time, captures the time needed to perceive stimuli and execute a motor response once a decision has been reached. By relating these four parameters to successful vs. unsuccessful abstinence on the RAT, we attempted to identify specific cognitive mechanisms (threshold, starting point bias, or drift rate) that might relate to the ability to forgo smoking. Drift rate was of key interest, as this parameter has shown sensitivity to psychopathology in several prior studies.

Methods: 122 otherwise healthy nicotine dependent individuals performed the PRT following smoking as usual. During the PRT, a cartoon face was shown onto which a mouth was quickly flashed (mouth duration: 100 ms). Participants were asked to identify whether the mouth was short (11.5 mm) or long (13.0 mm). For each participant, correct responses to one mouth length (e.g., short, the "rich" stimulus) resulted in a monetary reward three times more frequently than correct responses to the other length (e.g., long, the "lean" stimulus). This asymmetric reinforcement rate was used to induce a response bias (RB), such that participants were more likely to respond "rich" than "lean". The ability to discriminate between the two stimuli was also measured. Prior to a second, independent visit, participants abstained from smoking for a minimum of 16 hours. Abstinence was verified by a breath carbon monoxide (CO) level of <10 ppm. Participants then performed a RAT, where they could earn money by delaying smoking for the 50-minute session. At the beginning of the RAT, participants were given 8 cigarettes and told that every 5 minutes of abstinence would result in $0.20 reward. Most participants waited either 0-minutes ($n = 36$) or the full 50-minutes ($n = 44$) before smoking a cigarette, thus the PRT data was compared between these two groups.

Results: No differences between the 0-min and 50-min groups were noted for age (0- min: 43.69 ± 10.59 yr; 50-min: 40.64 ± 10.88, $p = 0.209$), sex (Fisher's exact = 0.228), or education ($p = 0.250$). Nor were there group differences in nicotine dependence level as

defined by the Fagrestrom test of nicotine dependence (0-min: 5.83 ± 1.94; 50-min: 4.93 ± 2.33, p = 0.067) or average number of cigarettes per day (0-min: 17.79 ± 5.44; 50-min: 16.5 ± 7.52, p = 0.398). In the PRT, a response bias developed over the 3 blocks ($p < 0.01$) and "rich" responses were faster than "lean" responses ($p < 0.001$). There was no group difference in RB. However, the 50-min group had better discriminability than the 0-min group (p = 0.002). Moreover, the HDDM revealed that drift rate was higher in the 50-min group (1.21 ± 0.08) vs. the 0-min group (0.79 ± 0.09; q < 0.001). There was no group difference in threshold, non-decision time, or starting bias.

Conclusions: Compared to adults who smoked immediately, those who abstained for the full 50-minutes showed better discriminability and higher drift rates in the PRT. This indicates that the speed of evidence accumulation—which is captured by drift rate and critical for discriminability—was higher in the abstainers. This is important because evidence accumulation is implicated in a wide range of decisions, not just in laboratory tasks like the PRT. Consequently, the data suggest that adults who fail to abstain from substance use may show decision-making deficits on a range of tasks due to slow evidence accumulation, although this remains to be confirmed. By contrast, no group difference was found for response bias. This suggests that individual differences in reward sensitivity were not a critical determinant of abstinence in this study.

Keywords: Smoking, Reward Sensitivity, Computational Models of Decision-Making

Disclosure: Nothing to disclose.

P653. Electrophysiological Markers of Aberrant Cue-Specific Exploration in Heavy Drinkers

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Background: To optimize behavior in an uncertain world, it is often necessary to test novel and unfamiliar actions at the expense of the foregone value of familiar actions. Managing these competing demands is known as the 'explore/exploit' tradeoff in decision-making. We recently demonstrated that the neurocomputational mechanisms underlying explore/exploit decisions are conserved across human and nonhuman primate species (Hogeveen et al., 2022, Neuron), suggesting this represents a powerful translational paradigm for basic and translational science studies on patients with pathological decision-making. To wit—heavy drinking is associated with pathological alcohol-related decision-making, but little is known about the mechanisms that drive heavy drinkers to explore novel alcohol cues in the first place. Here, we merge behavior, computational modeling, and electroencephalography (EEG) to elucidate the electrophysiological correlates of aberrant alcohol-related exploration in heavy drinkers.

Methods: We used computational model-based decomposition of explore/exploit decision-making during a 3-armed bandit reinforcement learning paradigm in heavy drinkers ($N = 27$; $N = 24$ of whom met criteria for a lifetime diagnosis of alcohol use disorder) and matched controls with a current alcohol use disorder identification test (AUDIT) score less than or equal to 3 ($N = 27$). Participants made speeded choices between three neutral images which were rewarded at a low (p = 0.2), medium (p = 0.5), or high (p = 0.8) probability. Occasionally, a novel stimulus insertion trial occurred: a novel image with a randomly assigned reward probability was inserted in lieu of one of the existing options. Half of the novel insertion trials contained an alcohol cue, whereas the other half of the insertion trials contained a non-alcohol beverage cue. In the immediate trials after each novel insertion, participants faced the difficult decision to either exploit the most valuable familiar option, or explore the novel option to learn its reward probability. An optimal decision policy was derived using a Partially-Observed Markov Decision Process model (POMDP), which derived separate estimates of each participants' tendency to make decisions weighted by the relative future value of exploring novel options, versus their tendency to more heavily weight the immediate value of exploiting familiar options. Separate estimates were generated for the relative future valuation of alcohol cues (i.e., explore-alc-value), relative future valuation of non-alcohol cues (i.e., explore-nalc-value), immediate valuation of alcohol cues (i.e., exploit-alc-value), and immediate valuation of non-alcohol cues (i.e., exploit-nalc-value). Critically, continuous EEG data were collected across the scalp during the bandit task. We specifically focused on the P3a event-related potential over mid-frontal electrodes. The P3a is known to respond and habituate rapidly to novel stimuli, and is thought to reflect a top-down executive orienting response that might be essential for driving novelty-driven exploration on our task.

Results: Observed explore/exploit decision-making behavior was modeled well by the POMDP (r = 0.74, 95% CI = 0.60-0.84). Participants tended to exploit the best alternative more often than the novel stimulus (p = 0.008), but in turn they also explored the novel stimulus more often than they chose the worst stimulus ($p < 0.001$). The POMDP explore-alc-value parameter demonstrated a two-way interaction between group and novel stimulus condition (p = 0.038). This was specifically driven by individuals in the heavy drinking group demonstrating greater weighting of the relative future value of exploring novel alcohol cues compared to control participants (p = 0.031). Groups did not differ in their weighting of the explore-nalc-value parameter, and the tendency to weight both the exploit-alc-value and exploit-nalc-value parameters did not differ between groups. This heighted motivation to explore novel alcohol stimuli based on the POMDP derived parameters was associated with a heightened P3a response to novel alcohol cues in the heavy drinking group. Explore-alc-value estimates were predicted by a two-way interaction between chosen stimulus type and P3a amplitude (p = 0.005), and paired comparisons showed a significantly larger relationship between exploration value and P3a amplitudes (p = 0.005), and separated by group, this cue-specific effect was found only in the heavy drinking group (p = 0.004).

Conclusions: The current data assembles behavioral, electrophysiological, and computational evidence to examine the heightened tendency for heavy drinkers to explore novel alcohol stimuli. Our POMDP-derived latent value estimates suggest that individuals with clinically-significant drinking behaviors tend to overvalue the potential future reward value of exploring novel alcohol stimuli. Critically, evoked P3a responses to novel alcohol cues may represent a neurocomputational marker of heightened exploration tendencies in response to alcohol cues in heavy drinkers. Whereas the preponderance of existing studies of value-based decision-making in the alcohol field have focused on tendencies to learn to exploit alcohol cues based on prior reinforcement, this represents the first evidence for a potential biomarker that may help to explain why some individuals—but not others—engage in problem drinking behavior when the stimuli are novel and their reinforcement value is not known.

Keywords: Value-Based Decision-Making, Computational Models of Decision-Making, Alcohol, Drinking Disorders, EEG Biomarkers

Disclosure: Nothing to disclose.

P654. Identification and External Validation of a Problem Cannabis Use Brain Network

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Background: Cannabis is the most commonly used illicit drug [1], and substantial evidence indicates that its use is associated with clinically-significant harms for a subset of users [2]. In the context of recent decriminalization/legalization of cannabis—in tandem with significant increases in potency [3]—further work is urgently needed to (i) identify who is most vulnerable to cannabis-related harms and (ii) elucidate neurobiological mechanisms of risk. Existing studies examining neural correlates of cannabis use in adolescence yield inconsistent results [4], and provide insufficient information to distinguish neural risk factors of problem-level vs. recreational use. Connectome-based predictive modeling (CPM) is a novel, data-driven, whole-brain, machine learning approach to identify neural networks related to specific behaviors of interest [5]. Therefore, the aims of the current study were to 1) apply CPM to identify a neuromarker of problem-level cannabis use in a non-clinical sample of adolescents/emerging adults, and 2) validate the identified network in an independent clinical sample of individuals entering treatment for cannabis use.

Methods: Data were drawn from the NIAAA-funded Brain and Alcohol Research in College Students (BARCS) Study, a 2-year longitudinal study of alcohol and substance use behavior. The current analyses include $N = 191$ participants for whom usable baseline neuroimaging data were available. Problem cannabis use was defined as self-reported use ≥10 times/month [6] at any clinical follow-up. Reward task (Monetary Incentive Delay Task) fMRI data were used to compute functional connectivity matrices that were entered into connectome-based predictive models (CPMs). CPM identifies features that are positively and negatively associated with problem-level use and to fit a linear model that can be applied to novel data using internal cross validation. Notably, this method allows for the identification of neural features underlying successful models, facilitating a better understanding of the neurobiological mechanisms underlying risk for problem cannabis use. Clinical relevance of the identified network was assessed in an independent clinical sample of $N = 33$ individuals entering cannabis use treatment who were enrolled in randomized clinical trials of cognitive behavioral treatments for substance use.

Results: CPM of reward task data successfully identified a network predictive of problem-level cannabis use in college-age adolescents/emerging adults ($r = 0.20$, $p = 0.005$). Consistent with other CPM work, identified networks were complex and included both cortical and subcortical connections. Despite this complexity, the spatial extent included only 1175 edges (618 positive, 557 severity), or less than 3.3% of possible connections. Network anatomy indicated that increased connectivity between the motor sensory network and medial frontal and frontoparietal networks, and decreased connectivity between the cerebellar network and medial frontal and frontoparietal networks was linked to problem-level cannabis use. Out-of-sample analyses supported the clinical relevance of the identified network: increased problem cannabis use network strength was positively associated with addiction severity at treatment entry ($rho = 395$, $p = 0.023$) and negatively associated with cannabis abstinence during treatment ($r = -.393$, $p = 0.024$) in an independent sample of individuals seeking treatment for cannabis use.

Conclusions: These data for the first time identify a neural network predictive of problem-level cannabis use in adolescence/emerging adulthood. Furthermore, follow-up analyses in an independent clinical sample validate the clinical relevance of the identified problem cannabis use network. Elucidating neural mechanisms of risk for problem use is crucial to facilitate the development of targeted prevention/intervention approaches to mitigate cannabis-related problems for persons at risk.

Keywords: Predictive Models, Marijuana, Problem-Level Use

Disclosure: Nothing to disclose.

P655. Where is the Main Target of the Most Commonly Used TDCS Electrode Montages for the Treatment of Neuropsychiatric Disorders?

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Background: Background: Transcranial electrical stimulation (tES) over the dorsolateral prefrontal cortex (DLPFC) has been widely used to modulate neurocognitive functions in both healthy and clinical populations. Most clinical trials place stimulating electrodes over F3/F4 location in EEG standard system based on the assumption that the active electrode will mainly stimulate the underlying brain region, DLPFC, as the main target. However, it has been shown that the maximal electric field (EF) during tES can fall outside the area under the active electrodes as commonly assumed. Here, we computationally assessed the spatial distribution and strength of the stimulation dosage during tES over DLPFC at both group and individual levels with the aim of determining the prefrontal regions that are targeted by DLPFC montages. We present our results for a subset of healthy subjects in the Human Connectome Project (HCP) database. To consider an independent clinical population, analyses were replicated with structural MRI of participants with methamphetamine use disorder (MUD) from our pre-registered trial data (NCT03382379). Here, we propose that the frontopolar cortex would receive the highest EF intensity in the DLPFC montages.

Methods: Methods: To generate individualized head models, unprocessed T1 and T2-weighted MRIs from 80 (44 female) randomly selected healthy adults (age (year) between 22-35) were obtained from the freely available HCP database. For each model, two common montages for modulating DLPFC (asymmetric: F4/Fp1, symmetric: F4/F3) were simulated. We extracted the averaged EF strength in (1) the center of stimulating electrode (F4), and (2) the top 1% of voxels in individualized EF maps by using a whole brain 99th percentile threshold. Paired sample t-tests with FDR corrections were used to compare EFs between the center of the electrode and hotspots. Inter-individual variabilities were quantified with standard deviation (SD) in terms of (1) hotspot location, and (2) hotspot value. Atlas-based parcellation of head models was also used to determine averaged EFs in main subregions of PFC using the Brainnetome atlas. All steps were replicated with 66 participants with MUD as an independent clinical population (age (year) 18-60, all male). The overlap between hotspot locations in healthy subjects and MUDs was calculated using Euclidean distance.

Results: Results: Results related to healthy subjects showed that, despite inter-individual variability in strength and location, hotspots were found in the medial frontopolar area; a region occupying the anterior portion of the brain's frontal lobe (corresponding to Brodmann's area 10) which is distinct from DLPFC (which is located on the lateral and dorsal part of the medial convexity of the frontal lobe) where the stimulating electrode was placed (comprises Brodmann's areas 9, 46). EF hotspot was significantly higher than the EF "under" the stimulating electrode (F4) in both symmetric (hotspot: 0.41 ± 0.06, F4: 0.22 ± 0.04; mean ± SD in V/m) and asymmetric

(hotspot: 0.38 ± 0.04, center of electrode: 0.2 ± 0.04) montages with large effect sizes (Hedges'g for: symmetric = 0.86, 95% CI (0.53,1.19), asymmetric = 0.76, 95% CI (0.43,1.09)). Inter-individual variabilities showed that, in symmetric montage, group-level location for hotspots in MNI space was [-1.21, 57.66, 18.67] with [6.43, 4.37, 8.08] as SD such that all hotspots were placed in a cube with a volume of 29 cm3 (X = 3.1, Y = 1.9, Z = 4.9). EF strength ranged from 0.31 to 0.68 V/m. In the asymmetric montage, group-level location for the hotspots in MNI space was [-2.62, 49.00, -4.54] with [7.05, 7.71, 8.94] as SD and all hotspots were placed in a cube with a volume of 46 cm3 (X = 3.3, Y = 3.4, Z = 4.1). EF strength ranged from 0.28 to 0.59 V/m across the population. Using atlas-based parcellation of head models, cumulative EF strength within the right frontopolar (A10m + A10l) was significantly higher than right DLPFC (A9/46 v + A9/46d) in both montages ($P < 0.01$ with Hedges' g = 0.34). Results for the MUDs were in line with healthy subjects and EF strength in the medial frontopolar (symmetric: 0.31 ± 0.07, asymmetric: 0.32 ± 0.07) where EF hotspots were located was significantly ($P < 0.001$) higher than DLPFC (symmetric: 0.18 ± 0.05, asymmetric: 0.19 ± 0.05) underneath the stimulating electrode with large effect sizes (Hedges'g for symmetric = 1.6190 with 95% CI (1.19, 2.04) and asymmetric = 1.3599 with 95% CI (0.95, 1.77)). Averaged peak coordinates in symmetric [1.88, 60.34, 19.12], and asymmetric montages [-3.14, 56.9, 6.33] were also located within the frontopolar cortex in both montages. However, there were inter-group variations between healthy participants and MUDs (maximum 4.3 cm3 distance between the averaged location of hotspots).

Conclusions: Conclusions: Here, we discussed that in common tES montages which are frequently used with the goal of modulating DLPFC, DLPFC was not maximally targeted. Considering inter-individual and inter-groups variability, our results highlighted the frontopolar area as the area receiving the highest electrical field in DLPFC montages. Optimizing targets in electrical stimulation at both group and individual levels is recommended for future trials with the hope of ultimately maximizing clinical benefits.

Keywords: transcranial direct current stimulation (tDCS), bipolar electrode montage, dorsolateral prefrontal cortex (DLPFC), frontopolar cortex, individualized head models

Disclosure: Nothing to disclose.

P656. Chronic Cocaine Use is Associated With Impairment in Utility Prediction Error Signal in Dopaminoceptive Regions in Humans

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Background: People with drug addiction struggle to adapt to changing reinforcement contingencies, a tendency often attributed to impaired computation of dopaminergic prediction error (PE) signals. PEs, the discrepancy between actual and expected reward value, are broadcast by dopamine neurons to downstream targets like striatum to guide value updating. Prominent theories of the acquisition of cocaine addiction posit PE alterations caused by dopamine oversupply, leading to increased expectation of reward that cannot match actual reward and thus continued drug pursuit. However, further preclinical work has shown chronic cocaine disrupts this system, causing globally degraded phasic dopamine firing and release and reduced PEs. Thus, a primary deficit in the computation of PE may explain how dopamine dysfunction drives addictive behavior as an inability to appropriately update the value of the drug, or the value of healthier alternatives to the drug. While preclinical models support this notion, it is unclear if PE deficits translate to humans. There are surprisingly few studies that have formally assessed mesolimbic PE encoding in people with addiction, and the data do not firmly support or refute PE differences from health. Thus, while alterations in dopamine receptors and transmission in cocaine addiction in humans are well documented, we do not know if these changes result in functional dysregulation of PE signals needed for value updating. The question is important because it is these teaching PE signals that have been proposed as a functional mechanism mediating and maintaining addictive behavior and that may provide a bridge for clinical translation. We aimed to address this gap using a detailed computational fMRI-based assessment of PEs in human cocaine addiction based on contemporary theories of dopamine function.

Methods: Participants were $n = 39$ individuals with cocaine use disorders who had used cocaine for 18 years on average and $n = 35$ matched controls (74% males). We adapted for fMRI a decision-making task previously used to probe single cell responses to a risk-sensitive form of PE ("utility PE") in nonhuman primates. Like sensory systems that encode stimulus intensity nonlinearly (typically as a saturating function), emerging data show phasic dopamine firing and release encode a nonlinear PE signal whose shape can be captured by rescaling reward size by individual preference for risk. Therefore, to obtain a robust estimate of individual risk preferences, participants completed the task three times: 3 weeks before an fMRI scan, on the day of the scan outside of the scanner, and during fMRI acquisition. Behavioral data were fit with an economic expected utility model to derive participant-specific risk preference parameters which were compared between groups with nonparametric tests. The multi-band fMRI data were preprocessed and quality checked using robust pipelines (fMRIPrep) and model-based fMRI analyses were used to isolate (nonlinear) utility PE signals based on individual risk preferences, focusing on unbiased regions of interest in bilateral ventral striatum. Competing neural models were tested via Bayesian model comparison: models that assumed objective (i.e., completely linear) encoding of reward vs. utility, as well as those that decomposed the PE signal into its expected reward and received reward terms.

Results: Risk preference parameters had moderate to high test-retest reliability across sessions, with '1-k' ICCs ranging from 0.69 [0.47, 0.83] (95% CI) in cocaine users to 0.77 [0.60, 0.88] in controls. They were invariant to current clinical state in the cocaine group, but cocaine users had higher risk tolerance in aggregate than controls (Z = 3.01, P = 0.003) especially those with earlier age of onset (rho = −0.36, P = 0.02), possibly reflective of longer-term vulnerability for substance use. Across the entire sample, utility PEs were robustly encoded in bilateral ventral striatum as observed whole-brain (FWE-corr $P < 0.05$) and in region of interest analyses (t = 4.12, P = 9.81×10-05). This striatal PE signal was better explained by the (nonlinear) utility PE model than the linear PE model (exceedance prob=1). Consistent with theoretical PE accounts, ventral striatum activity scaled positively with utility of received reward (P = 1.25×10-05) and negatively with its expected utility (P = 0.02). Focusing on this computationally defined utility PE signal, we found strong evidence for impairment in cocaine addiction: cocaine users had markedly reduced PE encoding in ventral striatum (t = −3.10, P = 0.003) and this was driven by reduced response to received reward (t = −3.02 P = 0.003), with no differences in expectancy encoding (t = 0.08, P = 0.94). Exploratory analyses showed impaired PEs across the mesolimbic circuit, notably orbitofrontal cortex.

Conclusions: These findings provide unambiguous evidence for impaired PE signals in dopaminoceptive regions of human cocaine users, specifically utility PEs such as those encoded by dopamine cells. Our data underscore a relationship between human drug

addiction and this core teaching signal that has so far eluded translational neuroscience despite its central role in addiction theories. An important future direction will be to evaluate the time course of PE deficits in relation to vulnerability and development of addiction. This would be a major advance in our understanding of how early dopamine disruption culminates in the chronic drug pursuit that characterizes addiction.

Keywords: Reward Prediction Error, Risk, Cocaine Use Disorder, Dopamine, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P657. Explainable Machine Learning Analysis Reveals Sex and Gender Differences in the Phenotypic and Neurobiological Markers of Cannabis Use Disorder

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Background: Cannabis Use Disorder (CUD) has been linked to a complex set of risk factors, including neurobiological, individual-level (e.g., personality, cognitive), and environmental factors. These factors have, until now, often only been investigated in a fragmented way, with researchers focusing on a small number of factors in each study or focusing on a single domain of interest. Therefore, while many studies have revealed sex/gender differences for single risk factors, sex/gender differences in the relative importance of a complex set of risk factors has not been described.

Methods: We evaluated the relative importance of a wide variety of risk factors associated with high cannabis use levels (lifetime uses) and cannabis dependence (semi-structured interview), as well as potential sex/gender differences in a well-described community sample [Human Connectome Project (HCP); $N = 1204$, aged 22-35; 54% female; 72% White, 13% African American, 15% Other]. The sample included 9% individuals with cannabis dependence ($N = 109$, 26% female). We included a wide array of self-report, diagnostic, and behavioral measures, as well as structural (FreeSurfer) and functional (both resting-state and all fMRI tasks) indices of brain function as potential risk factors, in total about ~2000 variables. We employed state-of-the-art machine learning methods [XGBoost (eXtreme Gradient Boosting)], a tree-based ensemble machine learning algorithm in combination with a feature ranking tool [SHapley's Additive exPlanations (SHAP)] to assign relative importance (i.e., SHAP values) to each of the associated risk factors.

Results: We confirmed that among a very large number of input factors (~2000), a small number of previously identified environmental (education level, social support), personality (openness), mental health (e.g., externalizing symptoms, childhood conduct disorder), neurocognitive (e.g., working memory), and brain factors (e.g., hippocampal volume) were consistently highly ranked (in the top 20 variables) for their contribution to the classification of cannabis use levels and diagnostic status. When comparing the average Area Under the receiver operating characteristic Curve (AUC) for different data modalities (phenotypic, FreeSurfer, resting-state fMRI, task fMRI), the phenotypic models had the highest AUC (0.74), with bimodal models slightly outperforming unimodal models (e.g., phenotypic + FreeSurfer: 0.80). Sex/gender was consistently ranked in the top 5 factors in all models that contained phenotypic data. Further inspection of sex/gender interactions revealed systematic sex/gender interaction effects. Risk factors with a larger contribution to the classification in men included personality (high openness), mental health (high externalizing, high childhood conduct disorder, high fear somaticism), neurocognitive (impulsive delay discounting, slow working memory performance) and associated brain factors (low hippocampal volume, increased postcentral thickness). Conversely, risk factors with a larger contribution to the classification in women included environmental factors (low education level, low instrumental support) and associated brain factors (smaller super temporal area).

Conclusions: In summary, environmental factors contributed more strongly to CUD in women, whereas individual factors had a larger importance in men. Results confirm the utility of machine learning approaches in describing sex/gender differences and suggest the importance of understanding how these differences may inform the development of sex/gender-specific treatment approaches for addiction medicine.

Keywords: Cannabis Use Disorder, Gender Differences, Addiction Phenotypes, Big Data Analysis, Machine Learning Classification

Disclosure: Nothing to disclose.

P658. Adolescent Decision-Making Trajectories are Altered in Environmental and Genetic Models of Addiction Susceptibility

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Background: Adolescence is a critical neurodevelopmental period associated with robust biobehavioral changes. These age-related changes include improvements in decision-making functions that we have found to predict drug use in adulthood. Deviations in these neurodevelopmental mechanisms may the mechanism by which addiction susceptibility emerges in individuals. We hypothesized that if adolescent decision-making trajectories are critical for regulating drug-taking behaviors, that adolescent decision-making would be altered in established models of addiction susceptibility.

Methods: To test this hypothesis, we assessed decision-making throughout adolescence and adulthood in rats that were 1) selectively bred to prefer or not prefer alcohol (e.g., P [N = 15] and NP [N = 15] rats; genetic model of addiction susceptibility) and in rats that were 2) either socially housed ($N = 11$) or isolated during adolescence ($N = 10$) or adulthood ($N = 9$; environmental model of addiction susceptibility). Rats were trained to acquire and reverse three-choice, spatial discrimination problems using a reversal-learning task and performance repeatedly assessed at P30, P60, P90, and P120. Trial-by-trial choice data was fitted with a reinforcement-learning model to obtain an estimate of reward-based decision-making.

Results: We found that the performance of P rats in the PRL task was significantly poorer than that of NP rats at each of the ages examined. The reinforcement-learning model indicated that the difference in performance was due to an attenuation in reward-based decision-making functions in P rats. Age-related changes in PRL performance were also attenuated in isolated rats compared to socially housed rats and rats that were only isolated during adulthood. The impairment in isolated rats was also found to be due to disruptions in reward-based decision-making functions.

Conclusions: Our data demonstrate that adolescent decision-making trajectories are altered in both the genetic and environmental model of addiction susceptibility. Collectively, these data suggest that decision-making disruptions that are predictive of drug use susceptibility emerge during adolescence and studies investigating adolescent neurodevelopment could provide critical insights into addiction susceptibility. Our ongoing studies are integrating proteomic and genomic approaches to investigate the

neurodevelopmental mechanisms mediating decision-making trajectories and drug use susceptibility.

Keywords: Reversal Learning, Reinforcement Learning, Juvenile Social Isolation, Alcohol-Preferring (P) Rats, Orbitofrontal Cortex (OFC)

Disclosure: Nothing to disclose.

P659. Prenatal THC Exposure Promotes Aberrant Reward Seeking, Exacerbated Dopaminergic Encoding of Reward-Predictive Cues and Transcriptomic Alterations in the Midbrain of Adult Rats

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Background: Marijuana is the most common illicit drug used by pregnant women and is associated with offspring's attention and learning deficits from early childhood until later in life. Previous research has identified midbrain dopaminergic neuronal alterations related to in utero exposure to THC. However, the effect of prenatal cannabis exposure (PCE) on future impairments of motivational processing in adulthood has not been specifically addressed.

Methods: In order to address this, we conducted a behavioral characterization of addition-like endophenotypes in PCE rats trained to obtain food (palatable pellets) and opioid (remifentanil) rewards.

Results: In utero cannabis exposure increased total food and opioid consumption, breaking points in a progressive ratio task as well as the intrinsic motivational value of both rewards under an economical demand task. Inhibitory motor control measures reported that PCE rats present higher impulsivity. In vivo fiber photometry dopamine recordings unveiled increased dopaminergic encoding of reward-predictive cues in the NAc, which was accompanied by a facilitation of cue-induced reinstatement of remifentanil-seeking behavior. Furthermore, the addition of sex as a co-variate revealed that male rats were more vulnerable to the effects of prenatal cannabis exposure. To explore the origin of such maladaptations, we conducted transcriptomic analyses (RNA-seq) in VTA, NAc and mPFC brain samples of PCE adult rats. Weighted Gene Co-expression Network (WGCNA), Differential Gene Expression (DGE) and Gene Ontology (GO) analyses all identified single-gene and network-wide alterations of VTA mitochondrial function related to PCE, reporting similar sex-dependent effects than the behavioral experiments.

Conclusions: Altogether, our results suggests that prenatal cannabis exposure is inducing, in a sex-dependent manner, an aberrant reward-seeking phenotype with excessive encoding of opioid and natural rewards that is accompanied by long-lasting, transcriptomic alterations in midbrain's mitochondrial function.

Keywords: Cannabis, Dopamine, Pregnancy, Opioid Abuse, Transcriptomics

Disclosure: Nothing to disclose.

P660. Brain Circuits for Maternal Sensitivity and Pain Involving Anterior Cingulate Cortex Functional Connectivity in Mothers Treated With Buprenorphine for Opioid Use Disorder

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Background: Opioid-induced deficits in maternal behaviors are well-characterized in preclinical rodent models. The prevalence of opioid use disorder (OUD) among pregnant women has risen sharply on a background of epidemic OUD. Despite gold standard buprenorphine treatment (BT) for withdrawal, related public health issues of postpartum depression, polysubstance use, relapse, and developmental risks to the infant plague affected mothers. We know that an evolutionary conserved Maternal Behavior Neurocircuit (MBN) governs maternal caring and aggressive behaviors, critical to healthy child outcomes. In the MBN, the anterior cingulate gyrus (ACC) participates in mother-child emotional bonding and attachment. However, ACC is also part of a "pain matrix" that processes the affective dimension and is extremely sensitive to opioid modulations. We know that prescription opioids affect resting-state functional connectivity (rs-FC) between the dorsal ACC, rostral ACC, and other regions related to MBN in healthy participants without OUD. Preliminary evidence suggests that prescription opioids may produce physical and emotional 'analgesic' effects through disruption of specific ACC-insula and ACC-putamen connectivity. However, even if acute use of opioids can reduce functional connectivity between these MBN-related regions, the effects of BT in mothers with OUD, who have chronically used prescription opioids remain unknown. We conducted a pilot longitudinal study of mothers receiving BT for OUD to examine ACC rs-FC.

Methods: In this exploratory study, we studied 32 mothers who completed fMRI scans at 1-month (T1) and 4-months postpartum (T2), including 7 mothers receiving BT for OUD (BT/OUD, years of age: $M = 29.86$, $SD = 6.62$) and 25 non-OUD mothers as a comparison group (CG, years of age: $M = 27.62$, $SD = 8.46$). The participants underwent a 6-minute resting-state fMRI scan at each time point. According to the literature, region-of-interest spheres (10 mm) in the DACC and RACC centered on MNI coordinates [0, 6, 40] and [0, 46, 2]. Thus, we restricted our analyses to insula and putamen, regions modulated by acute prescription opioid administration.

Results: We examined the Time by Group interaction effects on the rs-FC between the two seeds (RACC and DACC), and the regions insula and putamen. We found that the time-by-group interaction effects were significant in the RACC-dependent rs-FC with the left insula (MNI: [-40, 22, 2], 48 voxels, $Z = 4.62$, $p = 0.003$, family-wise error corrected) and the DACC-dependent rs-FC with the right putamen (MNI: [24, 16, -8], 169 voxels, $Z = 5.17$, $p < 0.001$, family-wise error corrected). BT mothers, as compared to CG mothers, showed lesser left insula-RACC rs-FC but greater right putamen-DACC rs-FC at T1, with these between-group differences reversed at T2.

Conclusions: Though preliminary, the current study pioneers the study of buprenorphine effects on mothers affected by OUD. Given that BT is currently the best practice for pregnant women suffering OUD, due to the potential adverse effects of any exogenous opioids on maternal behavior, it is important to examine whether buprenorphine, as a partial μ-opioid agonist and κ-opioid antagonist, exert beneficial or harmful effects on maternal brain and behavior during the postpartum. Previously, we have established that BT mothers differed from CG in periaqueductal grey-dependent rs-FC with the hypothalamus, amygdala, insular cortex, and other brain regions at T1 and many of these differences disappeared at T2, suggesting potential therapeutic effects of continuing buprenorphine treatment, i.e., normalizing the dysregulation of OUD. Similarly, in the current pilot study, we found time-by-treatment interaction effects on the DACC and RACC-dependent rs-FC. Our work may have identified a brain mechanism for the potential benefits of BT on reversing dysfunction of maternal brain and behavior: the initial group differences in rs-FC flipped by four months postpartum. This work anticipates studies to ascertain how BT affects maternal behaviors, mother-child bonding, and

intersubjective function, involving rs-FC in the pain matrix and ACC, as targets for novel interventions.

Keywords: Opioid Use Disorder, Maternal Brain, Affective Components of Pain, Resting State Functional Connectivity, Anterior Cingulate Cortex (ACC)

Disclosure: Nothing to disclose.

P661. Pathways of Risk for Opioid Misuse: Traumatic Stress and Neural Connectivity

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Background: In addition to impaired executive functioning, altered connectivity of large-scale brain networks (e.g., as measured by EEG functional connectivity) has been observed separately among individuals exposed to trauma and among those who misuse opioids. While there have been previous studies demonstrating alpha, beta, and theta band EEG coherence differences in OUD, they have had small and heterogeneous study populations (Ns: 15-36; mixed ages, genders, polysubstance abuse) and examined a limited number of frequency bands and electrodes), making the mixed findings difficult to interpret. Relatively larger studies (Ns~150) of EEG coherence differences in individuals exposed to trauma have found atypical left hemispheric alpha, beta, and theta coherence, and that decreases in alpha coherence mediated the effects of trauma on psychiatric impairment. It remains unknown what aspects of EEG coherence differ among those with OUD and how this varies as a function of trauma exposure. Further, the influence of sex and other common co-substance use/disorders (alcohol and cannabis) on EEG connectivity are unknown.

Methods: Drawing on data from the Collaborative Study on the Genetics of Alcoholism (COGA), a large family sample enriched for substance use disorders (N: 16, 866; 53% female, 24% Black; 18% of whom ever used opioids; 6% of whom with a DSM-5 Opioid Use Disorder; 70% trauma exposed), we examined the association of OUD with inter- and intra-hemispheric EEG coherence measures (27 inter- and intra-hemispheric bipolar pairs across theta, alpha, and beta frequencies) in males and females. Assessments of opioid misuse and DSM-5 substance use disorders were ascertained using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interviews. Assessments of traumatic exposures were also derived from the SSAGA, which includes information on 21 potentially traumatic events. Based on evidence that traumatic events cluster together, and our preliminary finding that sexual assaultive trauma has the greatest impact on neurodevelopment, in addition to an 'all' trauma composite variable (sum score ranging from 0-21), three non-mutually exclusive, composite variables were also examined, representing report of one or more physical assaultive traumas (sum score ranging from 0-7: stabbed, shot, mugged, threatened with a weapon, robbed, kidnapped, held captive), sexual assaultive traumas (sum score ranging from 0-2: rape or molestation), and non-assaultive traumas (sum score ranging from 0-5: life-threatening accident, disaster, witnessing someone seriously injured or killed, unexpectedly finding a dead body). EEG Recording protocols have been detailed previously. Bipolar electrode pairs were derived to reduce volume conduction effects, and the 27 coherence pairs used in this study were selected based on previous studies on EEG coherence. Conventional Fourier transform methods were used to calculate EEG interhemispheric and intrahemispheric coherence at the following frequency bands: theta (3-7 Hz), alpha (8-12 Hz), and beta (13-20 Hz). Utilizing Mplus v8.1, we examined the association of opioid use and OUD, four trauma exposure variables, and their interactions, with topological patterns of inter- and intra-hemispheric EEG connectivity. Initial models were conducted on all individuals and included the following covariates: sex, self-reported "race/ethnicity", age, birth cohort, familial relatedness. Secondary models were conducted stratified by sex.

Results: 3,086 (18.3%) participants responded affirmatively as having ever used an opioid not as prescribed, or as directed, by their doctor. 962 (5.7%) participants had a diagnosis of DSM-5 OUD. Among the 962 individuals with an OUD, 21.7% (N: 209) have an OUD only (without a co-morbid SUD), 78.3% also have an AUD, and 56.1% have a CUD. Opioid use, OUD, AUD and CUD were more common among males as compared with females, and rates of opioid use (but not OUD), and AUD were greater among White participants as compared with Black participants. 69.5% of the sample experienced at least one trauma. 31.6% of the sample experienced physical assaultive trauma (males: 43.6%, females: 20.2%), 13.4% sexual assaultive trauma (males: 4.3%, females: 21.5%), and 53.8% non-assaultive trauma (males: 59.0%, females: 48.8%). Rates of physically assaultive and non-assaultive trauma (but not sexually assaultive trauma,) were greater among Black participants (41.9%, 63.8%) as compared with Whites (26.2%, 49.0%). The most prominent effects (i.e., surviving Bonferroni test correction, $p < 0.001$) for OUD among those who are trauma exposed in the COGA data were observed with inter-hemispheric frontal alpha coherence among females.

Conclusions: Given the findings of altered neural network connectivity in OUD most prominently in those who are trauma exposed, the important neurodevelopmental changes observed across the lifespan, and the recent success of clinical interventions using neuroplasticity-based approaches, EEG coherence research can inform the development of therapies involving neurofeedback, cognitive remediation, and brain stimulation methods which draw on modifying neural network connectivity.

Keywords: Trauma Exposure, Opioid Addiction, EEG Electrophysiology

Disclosure: Nothing to disclose.

P663. Trends in Characteristics of Individuals Seeking Inpatient Treatment for Alcohol Use Disorder, 2005-2021: Sex Differences, Disorder Severity, and Psychiatric Comorbidity

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Background: Nationally representative survey studies have identified long-term changes in alcohol use and misuse in the United States during the past 15-20 years. Among the trends highlighted in these studies are a more pronounced increase in alcohol use and binge drinking among women compared to men, and a narrowing of the difference between men and women in the prevalence of past-year alcohol use disorder (AUD). These findings are informally referred to as "closing the gap". Irrespective of these findings, alcohol treatment utilization has steadily remained much lower among women compared to men, and several studies have shown this disparity is not accounted for by sex differences in severity of alcohol-related problems. The current study examined more than 15 years of data collected from individuals seeking inpatient treatment at the National Institute on Alcohol Abuse and Alcoholism (NIAAA) intramural clinical research program. The aims of this study were to 1) examine sex disparities over time among individuals seeking inpatient treatment for AUD, and 2) evaluate trends in severity of AUD, severity of withdrawal,

alcohol consumption levels, and psychiatric comorbidity among treatment-seeking men and women.

Methods: Data were drawn from 1,711 inpatient admissions (1182 M, 529 F) occurring across a 17-year period (2005-2021) at the NIH Clinical Center. All individuals were seeking treatment for AUD, diagnosed using the Structured Clinical Interview for DSM-IV (SCID IV) or DSM-5 (SCID 5), and were enrolled in NIAAA natural history protocols. Individuals were assessed for AUD severity (Alcohol Dependence Scale, ADS), withdrawal severity (Clinical Institute Withdrawal Assessment for Alcohol-Revised, CIWA-Ar), recent alcohol consumption (Timeline Follow-back, TLFB), psychiatric comorbidity (SCID IV or SCID 5), and anxiety and depression symptom ratings (Brief Scale for Anxiety, BSA; Montgomery-Asberg Depression Rating Scale, MADRS). Analyses included descriptive statistics, data visualization, and chi-square, Fisher's exact, and t-tests to compare outcomes between men and women overall and by year.

Results: Overall, 69.1% of treatment-seekers were men while 30.9% were women. This disparity remained more or less consistent across the years studied (all p-values <0.05 except for 2011). On average, women had higher AUD severity ($p < 0.0001$) and withdrawal severity ($p < 0.0001$), but this was not consistent across years. The number of heavy drinking days was not different between men and women seeking treatment for AUD, although men generally consumed more drinks per drinking day ($p < 0.0001$) and more drinks per week ($p < 0.0001$). A trend for increasing prevalence of comorbid mood disorders (current and lifetime) was observed for both men and women, with treatment-seeking women having a higher prevalence than men on average ($p < 0.001$ for both current and lifetime). Current and lifetime anxiety disorder prevalence was also higher among women compared to men ($p < 0.0001$ for both current and lifetime). Similarly, anxiety and depression symptom ratings were on average higher for women than men, but again this was not consistent across years. Differences between men and women in prevalence of comorbid substance use disorders were nominal.

Conclusions: On average and by year, men outnumbered women 2 to 1 among individuals seeking inpatient treatment for AUD. This is generally consistent with national statistics on prevalence of AUD in the population as a whole, although this gap between men and women has narrowed in recent years. On average, women seeking inpatient treatment for AUD showed higher AUD severity and psychiatric comorbidity, despite overall alcohol consumption levels being lower than that of men. Studies suggest that while the number of barriers to any form of treatment for AUD does not differ between men and women, the perceived need for treatment may differ – for example, women may be more likely to believe that they can get better on their own. Furthermore, increased severity of illness and comorbid depression and anxiety are associated with a higher probability of alcohol treatment utilization. These observations suggest that among women with AUD, those with greater disorder severity and/or psychiatric comorbidity may be more likely to seek inpatient treatment. Further research is needed on the motivation for, and barriers to, inpatient alcohol treatment in men and women.

Keywords: Alcohol Use Disorder - Treatment, Sex Differences, Psychiatric Comorbidity

Disclosure: Nothing to disclose.

P664. Lifetime Alcohol and Cannabis Use Among the U.S. Adolescents Across Age Groups: Differential Patterns by Sex and Race/Ethnicity

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Background: Alcohol and cannabis are among the most commonly used drugs in the U.S. and worldwide, leading to high morbidity, mortality, and economic/societal burden. Earlier age of onset of substance use, including alcohol and cannabis, is associated with poor long-term outcomes, such as increased likelihood of developing a substance use disorder in adulthood. The aim of this study was to examine the prevalence of alcohol and cannabis use among adolescents of different age groups, and to investigate differential patterns by sex and by race/ethnicity.

Methods: Data from the 2019 National Survey on Drug Use and Health (NSDUH), a cross-sectional nationally-representative survey, were used and the analyses were limited to 12-17-year-olds who reported being non-Hispanic White, non-Hispanic Black, or Hispanic/Latino ($n = 11{,}830$). Deming regression was applied to find the line of best fit between age groups and percent of lifetime alcohol/cannabis use, and the slopes of the regression lines were compared between the groups.

Results: In the full sample, lifetime prevalence of alcohol and cannabis use was 28.4% and 17.4%, respectively. At age 12, 6.4% of the respondents reported lifetime alcohol use and 1.3% reported lifetime cannabis use. At age 17, 53.2% of the respondents reported lifetime alcohol use and 35.9% reported lifetime cannabis use. Girls had a steeper increase for lifetime alcohol use than boys [$F(1, 8) = 3.40$, $p = 0.09$], while there was not a sex difference for cannabis. The increase in lifetime alcohol use across age was similar among non-Hispanic White and Hispanic/Latino respondents, whereas the rate of increase was significantly flatter among non-Hispanic Black respondents [$F(2, 12) = 21.26$, $p = 0.0001$]. For cannabis, non-Hispanic White and non-Hispanic Black respondents had similar increases in lifetime use by age, while Hispanic/Latino respondents had a comparatively higher rate of increase [$F(2, 12) = 3.17$, $p = 0.07$].

Conclusions: The prevalence of lifetime use of alcohol and cannabis increased substantially from age 12 to age 17 in the NSDUH survey. Increases in alcohol use were steeper for girls (vs. boys), and among White and Hispanic/Latino (vs. Black) respondents. There were no sex differences in increases in cannabis use, although Hispanic/Latino respondents had steeper increases than White and Black youth. The quick increase in the prevalence of substance use from 12-17 points to the need for early interventions in adolescents to prevent the development of substance use disorders. Furthermore, the sex and race/ethnicity differences here described highlight the need for evidence-based preventive interventions tailored for specific demographic groups.

Keywords: Alcohol, Cannabis, Adolescence, Sex Differences, Racial/Ethnic Differences

Disclosure: Nothing to disclose.

P665. Initiation and Retention of Buprenorphine and Methadone in Pregnant Individuals With Opioid Use Disorder: An Analysis of Insurance Claims in the United States (2006-2016)

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Background: The USA has experienced escalating rates of overdoses and adverse birth outcomes among women of childbearing age with opioid use disorder (OUD). The initiation and retention rates for buprenorphine and methadone have been seldom characterized using national data in underserved populations, such as among people using public insurance and minoritized individuals. The objectives of the current study were to (1) calculate the initiation and retention rates for buprenorphine and methadone in reproductive-aged people with OUD

using national data, and (2) characterize disparities in initiation and retention by insurance status and race.

Methods: In this retrospective cohort study, we analyzed data from female-identifying persons, ages 16-45 years, in the MarketScan databases (2006-2016). OUD and pregnancy status were identified based on inpatient or outpatient claims for established ICD-9/10 diagnoses and procedure codes. The main outcomes were buprenorphine and methadone initiation and retention, determined using pharmacy claims. Adjusting for age and cooccurring psychiatric and substance use disorders, we used logistic regression to estimate buprenorphine and methadone initiation, and Cox regression to estimate buprenorphine and methadone retention, stratifying analyses by insurance status and race.

Results: Our sample included 103,038 reproductive-aged women in the USA with OUD (mean age 30.7 years, 64.6% Medicaid, 84% White), of whom 2,982 (2.9%) were pregnant. 67,521(65.5%) were initiated on psychosocial treatment without MOUD, in comparison to 29,435 (28.6%) and 6,082 (5.9%) initiated on buprenorphine and methadone respectively. 52% of buprenorphine and 41% of methadone episodes were discontinued at 180 days. Medicaid status was associated with a significant decrease in buprenorphine initiation (OR = 0.26 [0.26-0.27]) in comparison to commercial insurance. In contrast, Medicaid status was associated with a nearly 5-fold increase in methadone initiation (OR = 4.92[4.47-5.43]). Decreased buprenorphine initiation was observed for treatment episodes among BIPOC individuals (OR = 0.72[0.65-0.80]) relative to White peers; in contrast, BIPOC individuals were more likely to be initiated on methadone (OR = 1.52[1.37-1.68]). Relative to commercial insurance, Medicaid status was associated with a nearly 20% increase in buprenorphine discontinuation (HR = 1.19[1.17-1.21]), mirrored by a nearly 20% decrease in methadone discontinuation (HR = 0.81[0.75-0.87]). We observed an increase in both buprenorphine (HR = 1.13[1.04-1.23]) and methadone (HR = 1.18[1.09-1.28]) discontinuation in association with treatment episodes among BIPOC people relative to White individuals.

Conclusions: Most reproductive-aged women with OUD in the USA do not receive buprenorphine or methadone. Large insurance-based disparities were observed such that Medicaid enrollees are less likely to be initiated and retained on buprenorphine as commercial insurance enrollees, and more likely to be initiated and retained on methadone. BIPOC individuals were significantly less likely to be initiated and retained in buprenorphine treatment; even though BIPOC individuals were more likely to be initiated on methadone, methadone retention rates among BIPOC patients were lower than White counterparts.

Keywords: Pharmacoepidemiology, Health Disparities, Opioid Agonist Treatment, Health Services, Women's Mental Health

Disclosure: Nothing to disclose.

P666. Probing Disparities in Exposure to Smoking Contexts Using Computer Vision

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Background: Substantive efforts by healthcare providers, scientists and policymakers have helped dramatically reduce cigarette smoking rates over the past 70 years. Yet, high rates of smoking and poor cessation outcomes persist among many minoritized and underserved groups (e.g., women, low SES, racial and sexual minority groups). Numerous potential explanations for tobacco use inequities have been posited, including targeted tobacco industry marketing, social minority stressors, differences in nicotine metabolism, treatment access and compliance, social norms, and the types of tobacco products (e.g., menthol flavor) most commonly used. However, less attention has been paid to the environments that these groups are exposed to in everyday life, how daily environments differ between groups, and the potential influence specific environments may have on smoking behavior. For example, two recent studies indicate the effects of SES on smoking behavior are mediated by increased exposure to environments where smoking is allowed. This topic has received substantially less attention to date as a potential cause for tobacco use inequities and existing research has been limited by exclusive reliance on self-report for assessing exposure to smoking and non-smoking contexts. In the present analysis, we used a novel machine learning algorithm developed and validated in our prior work (Engelhard et al., 2021) to examine differences in exposure to environments where smoking is allowed or the risk of smoking is high.

Methods: Daily cigarettes smokers residing in Durham, NC ($N = 48$) attended a baseline visit that included a detailed assessment of demographic characteristics and smoking behavior. They then completed a 14-day ecological momentary assessment (EMA) protocol during which they were asked to photograph their current environment each time they smoked and following six random prompts throughout the day. Each prompt also assessed the time since last cigarette, time of day and current location (e.g., home, work). An out-of-sample convolutional neural network model based on MobileNetV2 was pretrained on the ImageNet database and then trained to predict: (1) The probability that smoking was permitted; and (2) The probability of actual smoking based on the photograph and other contextual information. Mixed effect models were then used to examine overall differences in algorithm-predicted probabilities as a function of nicotine dependence, gender identity, race, sexual orientation and SES, as well as whether these effects differed across smoking and non-smoking contexts.

Results: Participants were diverse with respect nicotine dependence level (27% low, 42% moderate, 31% high), gender identity (65% women), race (48% non-white), sexual orientation (19% non-heterosexual) and SES (46% "do not" or "just" meet basic expenses). A total of 7,843 images were included in analyses. The only main effect of demographic characteristics was a relatively weak effect indicating higher algorithm-predicted smoking risk for men relative to women ($F = 4.3$, $p = 0.046$). However, interactions with context type (i.e. smoking versus non-smoking) revealed stark differences in both outcomes as a function of nicotine dependence (Permitted: $F = 43.0$, $p < 0.001$; Smoking: $F = 46.2$, $p < 0.001$), gender identity (Permitted: $F = 35.0$, $p < 0.001$; Smoking: $F = 20.1$, $p < 0.001$), race (Permitted: $F = 68.8$, $p < 0.001$; Smoking: $F = 51.9$, $p < 0.001$), and sexual orientation (Permitted: $F = 89.7$, $p < 0.001$; Smoking: $F = 56.0$, $p < 0.001$). In all cases, effects were driven by smaller differences in algorithm-predicted probability values between smoking and non-smoking contexts among participants with greater nicotine dependence or who were members of minoritized groups. The only effect of SES was relatively weak ($F = 4.4$, $p = 0.036$) and indicated higher algorithm-predicted probability that smoking was permitted in actual smoking contexts among low SES participants.

Conclusions: Findings indicate that individuals with higher nicotine dependence and those who are members of minoritized and underserved groups are exposed to substantially different environments with regards to smoking risk and the permissiveness of smoking than individuals with lower dependence and belonging to more privileged groups. Surprisingly, these differences were not driven by higher predicted risk for smoking or smoking permissiveness across all environments. Instead, effects were driven by a reduced difference in algorithm-predicted risk

between smoking and non-smoking environments. This lack of differentiation between smoking and non-smoking environments offers a potential new insight into smoking disparities. Interestingly, these effects were not accounted for by SES and indeed the effects of SES were quite limited when adjusting for other demographic characteristics. While speculative, clearer differentiation between contexts where smoking does and does not occur may have a protective effect and improve odds of successful cessation. Greater understanding of the specific environmental characteristics that drive these effects may be informative. Future work applying computer vision approaches may help yield answers to these questions and provide fresh lines of inquiry for identifying environmental drivers of tobacco-related inequities and informing treatment and policy interventions.

Keywords: Smoking, Health Disparities, Machine Learning

Disclosure: Nothing to disclose.

P667. A Single-Cell Atlas of Gene Expression and Chromatin Accessibility Changes Associated With Cocaine Addiction in the Rat Amygdala

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Background: The amygdala plays a key role in the negative emotional states associated with drug withdrawal and leading to relapse. Neuroanatomical and functional observations have uncovered the role of discrete amygdala subregions in different aspects of these negative affective states. However, the underlying transcriptional regulatory programs driving the function of distinct amygdala cell types remain unknown.

Methods: Here we generated an atlas of single nucleus gene expression and chromatin accessibility in the amygdala of male rats with low and high cocaine addiction-like behaviors that were subjected to prolonged abstinence from extended access to cocaine intravenous self-administration. We e utilize single-nuclei multiomic methods to measure gene expression and chromatin accessibility in the amygdala of rats with low or high addiction indexes. We perform validation experiments by combining pharmacological inhibition with electrophysiological recordings in tissue slices and cue-induced cocaine-seeking behavior in rats.

Results: Between rats of different addiction indexes, there are thousands of cell type-specific differentially expressed genes that are enriched for molecular pathways, including energy metabolism and GABAergic synapses in excitatory and somatostatin neurons. We demonstrate that higher addiction severity is linked to excessive GABAergic inhibition and, using pharmacological inhibition, we find that addiction-like phenotypes are regulated by the metabolite methylglyoxal. By analyzing differences in chromatin accessibility, we predict upstream transcriptional regulators associated with addiction-like behavior and find discordant regulation of key transcription factors among distinct cell populations.

Conclusions: Overall, we provide a comprehensive characterization of cell type-specific transcriptional changes in the amygdala and the regulatory mechanisms that shape them in the development of addictive behaviors.

Keywords: Transcriptomics, Epigenomics, Cocaine Addiction, GABA-A Receptors

Disclosure: Nothing to disclose.

P668. Integration of snRNA Sequencing and Spatial Transcriptomics Data Identifies Brain Region and Cell-Type Specific Expression Changes in Mice After Chronic Alcohol Exposure

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Background: Alcohol use disorders (AUDs) affected 8.6% of men and 1.7% of women globally and attributed to 5.3% of all global deaths in 2016. Transcriptomic studies identified dysregulated signatures in post-mortem brain samples from alcohol-dependent individuals as well as animal models of alcohol consumption and dependence. Using AUDs brain transcriptomic signature to identify potential therapeutics showed promise in animal models to reverse the escalation of alcohol drinking. Identification of cell-type specific transcriptomic changes in chronic alcohol use will improve our understanding of mechanisms mediating escalation of alcohol use and consequently refine targetable mechanisms to which therapeutics can be developed. In this work we aim to identify cell-type brain region specific changes mediating escalation of alcohol use. We utilized chronic intermittent exposure treatment paradigm (CIE), an alcohol dependence model that produces escalated drinking in mice, and neurobiological and behavioral adaptations in mice that mimic those found in humans with AUD.

Methods: C57Bl6/J mice were exposed to CIE paradigm. Briefly, animals were exposed to 16 hours of ethanol vapor/day (or air as control) for 4 days followed by 72 hours of forced abstinence then with 2 bottle choice for 2 hours; this paradigm was repeated for four cycles followed by animal sacrificing and brain harvesting. We performed single nuclei RNA sequencing (snRNA-seq; 10x Genomics) on micro-punches obtained from pre-frontal cortex (PFC) of CIE and control mice, to determine cell-type-specific alcohol-induced gene expression changes. To understand the spatial context of CIE transcriptomic changes, we generated 10x Genomics Visium Spatial transcriptomics data from coronal PFC sections (10 µm) from CIE mice and controls. We utilized an 'anchor'-based integration workflow introduced in Seurat on snRNA-seq data obtained from PFC micro-punches (reference) and the spatial RNA sequencing data (query), this pipeline outputs, for each spatial capture spot, a prediction score for each of the snRNA-seq derived cell types.

Results: We utilized transcriptomic data to identify cell types and subtypes of each cell. We identified differentially expressed (DE) genes and their enriched pathways in each subtype. Oligodendrocytes, astrocytes, and a subtype of inhibitory cells were the most susceptible to CIE. Integration pipelines identified the anatomical location of each subtype, we show regional specificity of each of the excitatory and inhibitory cell types identified in our snRNA seq data. Most susceptible inhibitory cell subtype was defined to specific layers across the cortical area of the section. We clustered the spatial loci based on their gene expression similarities, followed by differential expression in each cluster. We show that the clusters with highest number of differentially expressed genes are located within regions of myelin-dense fiber tracts, consistent with our results from snRNA sequencing showing the oligodendrocyte as a highly susceptible cell type, other highly affected spatial cluster is located in the olfactory tubercle within the ventral region of the section, this region is enriched in tyrosine hydroxylase genes, suggesting involvement of dopamine signaling pathways. To understand transcriptomic regulation mechanisms, we utilized the snRNA sequencing data and constructed gene co-expression networks in each cell type. Oligodendrocytes and inhibitory cells subtype C, two highly dysregulated cell types, contained gene co-expression modules with Cpa6 as a hub gene, most of gene members of

those co-expression modules were dysregulated in CIE samples. Cpa6, a metallocarboxypeptidase is involved in the regulation of neuropeptides and previously linked to high alcohol consumption in human GWAS studies. Additionally, we identified Pde4b as a hub gene of highly dysregulated module, and as an upregulated gene in oligodendrocytes. Pde4b gene has been previously shown to be a potential therapeutic target to reduce binge drinking. In the microglia we identified Plxdc2, a microglial enriched gene previously shown to be upregulated by alcohol in rat microglial culture, to be upregulated in CIE samples and to be a hub gene of a dysregulated module involved with cell migration and motility. Our data provide new cell-type specific insight into the molecular mechanisms underlying AUD.

Conclusions: Our data identifies (1) spatially defined cell types highly susceptible in chronic alcohol exposure models and dysregulated pathways in each cell type, (2) gene co-expression networks that link previously identified alcohol target to their highly dysregulated downstream network allowing for targeting those networks to reverse escalation of alcohol use. (3) glial cells involvement in chronic alcohol use

Keywords: Single Cell RNA-Seq, Spatial Transcriptomics, Alcohol Use Disorder, Alcohol Dependence, Gene Expression

Disclosure: Nothing to disclose.

P669. Genetic Relationships Among Seven Models of Addiction-Related Traits in Heterogeneous Stock Rats

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Background: Both environmental and genetic factors place certain individuals at greater risk for developing substance use disorder. Furthermore, this vulnerability is associated with non-drug traits such as impulsivity, sensation seeking, response to novelty, attentional control, social interaction, and cue-responsivity. Rat models of these intermediate phenotypes allow the combination of forward genetic approaches with detailed behavioral analysis to identify biological pathways associated with substance use disorder.

Methods: To characterize the genomic regions associated with these behaviors, we performed a genome-wide association study (GWAS) using up to 1645 male and female heterogeneous stock (HS) rats tested in several behavioral paradigms, including locomotor response to novelty, choice reaction time, social reinforcement, visual (light) reinforcement, delay-discounting, Pavlovian conditioned approach (PavCA), and cocaine-conditioned cue preference. Further, genetic correlations with other GWAS projects, including nicotine self-administration, were also calculated.

Results: Single nucleotide polymorphism (SNP) heritability for the key measures acquired for each of these traits ranged from 0.12 (goal-tracking during PavCA) to 0.30 (distance travelled during the locomotion test). Further, some traits were genetically correlated with nicotine self-administration, including measures of delay-discounting (0.86, $p = 0.006$) and average reaction time during the choice reaction time task (0.65, $p = 0.009$). Finally, many loci and candidate genes were identified. For example, four measures of reaction time reached genome-wide significant associations on chromosome 20 which contains Abcfl and also has a strong cis-acting expression quantitative trait locus (eQTL) that maps to this interval. Another region reaching significance for delay discounting on chromosome 15 includes the gene Otx2, a gene involved in the formation of dopaminergic neurons and already implicated in this trait.

Conclusions: These studies demonstrate the utility of using HS rats for GWAS: many of the identified candidate genes have been implicated by human GWAS, and others are novel candidates that will identify new avenues of research and potentially new treatment approaches for human psychiatric diseases.

Keywords: Substance Use Disorder, Genome-Wide Association Study, Cue-Reactivity, Impulsivity, Cocaine Sensitivity

Disclosure: Nothing to disclose.

P670. Using Whole Blood for Transcriptome-Based Drug Repurposing in a Model of Alcohol Dependence

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Background: Alcohol Use Disorder (AUD) is a chronic, relapsing condition and a major public health problem with few pharmaceutical treatments available. Recent evidence in rodents suggests that brain gene expression profiles can discriminate between alcohol dependent and non-dependent subjects and predict pharmaceuticals that decrease drinking. However, this methodology has limited clinical potential because it is not possible to obtain brain specimens from patients. We hypothesized that whole blood could be a more accessible transcriptome for diagnostic and therapeutic applications.

Methods: We used whole blood transcriptome profiles from mice exposed to chronic intermittent ethanol vapor (CIE) or air ($N = 40$ total; 10/sex/group) to define a blood CIE signature as the top DEGs based on p-value and fold change (the top 2% of each). To identify pharmaceutical candidates that might decrease ethanol consumption, we compared the blood CIE signature to those of pharmaceuticals in the NIH Connectivity Map database (CMap). Working under the hypothesis that medications with opposing effects on gene expression as the blood CIE signature will reduce voluntary alcohol consumption, we prioritized the compounds with negative connectivity scores (i.e., reversers). Additional prioritization included FDA-approval status, whether multiple drugs from the same category were predicted, and whether the same drug (or drug category) was predicted for both sexes. A proof-of-principal study was conducted with the top candidate compound to determine its effects on voluntary alcohol consumption in C57BL/6 J mice ($N = 20$ total; 5/sex/group) using the same CIE drinking protocol from which the transcriptome data were derived. Statistical significance was assessed using a three-way ANOVA with sex (male and female) and treatment (drug and vehicle) as between-subjects factors and time as a repeated measure. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: The CMap analysis identified 39 reversers for females and 29 reversers for males (permutation $p < 0.05$). Of these, several cardiac glycosides were predicted to be reversers for both sexes. Digoxin is relatively safe and is the most prescribed cardiac glycoside for the treatment of heart failure, so it could be repurposed if proven to be effective for AUD. We administered digoxin (1 mg/kg; i.p.) or vehicle (5% DMSO in saline; i.p.) to male and female mice once daily beginning after the fourth CIE vapor session and continuing throughout the 5-day voluntary drinking test and one more CIE vapor and 5-day voluntary drinking test. The time x treatment interaction was highly significant, and digoxin decreased alcohol intake compared with vehicle in both sexes (by ~50% in males and ~70% in females).

Conclusions: CMap analysis can predict pharmaceuticals that reverse the CIE transcriptional signature in blood. One predicted reverser, digoxin, decreased drinking in male and female C57BL/6 J mice. These results indicate that blood is an accessible tissue that can be used for transcriptome-based drug repurposing through the application of advanced computational approaches to identify therapeutics for AUD and potentially personalize AUD treatment.

Keywords: Gene Expression, Systems Pharmacology, Alcoholism, Alcohol Use Disorder - Treatment

Disclosure: Nothing to disclose.

P671. Cell Adhesion Molecule 2 Deletion Reduces Impulsivity and Voluntary Cannabinoid Intake, and Impairs Physiological Response to Δ9-Tetrahydrocannabinol in Mice

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Background: It is estimated that 3.9% of the global population uses cannabis annually and that approximately 9% of those who use cannabis within their lifetime develop cannabis use disorder. Consequently, there is a growing need to identify risk factors of cannabis use initiation and problematic use. Polymorphisms in Cell Adhesion Molecule 2 (CADM2), a gene associated with externalizing behaviours, were recently correlated with cannabis use initiation by genome-wide association studies. We hypothesized that these human findings would be recapitulated in a Cadm2 knockout (Cadm2-/-) mouse line, which was used to investigate the causal relationship of Cadm2 with voluntary cannabinoid intake, pharmacological response to Δ9-tetrahydrocannabinol (THC), and cognitive phenotypes associated with substance use.

Methods: Adult male and female Cadm2 + /+, Cadm2 + /-, and Cadm2-/- mice ($n = 11$-18/genotype) were used for each experiment. To assess cannabinoid self-administration and preference, a two-edible choice preference test was employed with use of either pure THC or THC-dominant cannabis oil in cookie dough. Drug consumption and preference across escalating THC doses were calculated based on consumption of drug- and vehicle-containing dough. In a separate cohort, the role of Cadm2 genotype in pharmacological response to THC was determined using the cannabinoid tetrad assay (i.e., assessment of THC-induced hypolocomotion, hypothermia, catalepsy, and analgesia) after single or repeated intraperitoneal injection of moderate or high (3 or 10 mg/kg) THC doses. Finally, a range of executive functions were assessed in a drug-naïve group of animals using the 5-choice serial reaction time task (5CSRTT) under drug-free and THC challenge conditions. Parametric data were analyzed using one-way or repeated measures ANOVA where appropriate; nonparametric data were analyzed using Kruskal-Wallis H test or aligned rank transformed ANOVA where appropriate.

Results: Significant differences in THC dough preference ($F(2,32)=4.85$, $p < 0.05$), cannabis oil dough preference ($F(2,34)=4.65$, $p < 0.05$), and cannabis oil dough consumption ($F(2,36)=3.57$, $p < 0.05$) were observed according to Cadm2 genotype. Cadm2-/- mice showed lower preference and consumption ($p < 0.05$) of drug dough compared to Cadm2 + /+ and Cadm2 + /- littermates. Cannabinoid tetrad assessment revealed an effect of genotype on baseline locomotor activity ($F(2,76)=37.90$, $p < 0.001$) and thermal pain tolerance ($F(2,77)=4.64$, $p < 0.05$), as well as THC-induced locomotor (moderate THC: $F(2,34)=50.92$, $p < 0.001$; high THC: $F(2,35)=63.69$, $p < 0.001$), body temperature (high THC: $F(2,36)=13.52$, $p < 0.001$), and analgesic (moderate THC: $F(2,32)=6.23$, $p < 0.01$) responses. Under drug-free conditions, Cadm2-/- mice were hyperlocomotive ($p < 0.01$) and exhibited greater thermal pain tolerance ($p < 0.01$) than littermates. Moderate ($p < 0.01$) and high ($p < 0.05$) THC doses exacerbated hyperlocomotion in Cadm2-/- mice, whereas characteristic hypolocomotion to high THC administration was observed in littermates ($p < 0.05$). In addition, acute injection of high-dose THC produced hypothermia in Cadm2 + /+ and Cadm2 + /- mice only ($p < 0.001$), and analgesic sensitization following repeated administration of moderate dose THC in Cadm2-/- mice ($p < 0.01$). Finally, the 5CSRTT revealed that Cadm2 expression affected performance accuracy ($F(2,32)=3.58$, $p < 0.05$) and premature responding ($H(2)=16.10$, $p < 0.001$) that was suggestive of attentional deficits ($p < 0.05$) and lower impulsivity ($p < 0.01$) by Cadm2 deletion. Acute administration of 2 mg/kg of THC did not affect impulsivity or accuracy in the 5CSRTT within any genotype.

Conclusions: These data indicate a role for Cadm2 in cannabinoid preference and consumption, pharmacological response to THC, and executive functions relevant to substance use disorders. Notably, these results suggest that Cadm2 expression is a risk factor in voluntary cannabinoid intake, possibly by affecting pharmacological response to THC and promoting impulsivity. These findings provide support for human correlational studies that propose CADM2 polymorphisms affect externalizing phenotypes, including impulsivity and substance use.

Keywords: Cannabis Use, Heritability of Substance Use Disorder, Drug Self-Administration, Executive Function, Translational Animal Models

Disclosure: Nothing to disclose.

P672. Metabolic-Epigenetic Exchange During Voluntary Alcohol Intake

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Background: Alcohol metabolites contribute to brain histone acetylation by the direct deposition of alcohol-derived acetate on histones. This reaction is dependent on the metabolic enzyme acetyl-CoA synthetase 2 (ACSS2), which is nuclear and chromatin-bound in neurons. Mice lacking ACSS2 do not deposit acetate onto histones in the brain and show no conditioned place preference for ethanol reward. Here, we explore the role of this pathway during voluntary alcohol intake.

Methods: We used the drinking-in-the-dark (DiD) paradigm to assess alcohol consumption in ACSS2 knock-out (KO) mice and wild-type littermates. We characterized histone acetylation (chromatin immunoprecipitation coupled with high throughput sequencing) and gene expression (RNA sequencing) to test the effect of alcohol-derived acetate during voluntary drinking.

Results: We found that ACSS2 KO mice consume significantly less alcohol while undergoing DiD and, surprisingly, show significantly elevated blood alcohol concentrations. Histone acetylation and gene expression changes in the hippocampus, the nucleus accumbens and other brain regions point to impaired expression of genes that have previously been linked to alcohol and other substance use disorders.

Conclusions: Direct incorporation of alcohol metabolites into histone acetylation has profound effects on gene expression in the brain, and potentially drives behaviors related to alcohol use disorder. Since inhibiting ACSS2 leads to decreased voluntary alcohol intake in mice, targeting this pathway could be a promising new therapeutic avenue.

Keywords: Alcohol, Epigenetics, Drug Metabolism

Disclosure: Nothing to disclose.

P673. Behavioral and Brainstem Transcriptome Analysis of FVB/N Substrains in a Mouse Model for Neonatal Opioid Withdrawal Syndrome (NOWS)

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Background: Concomitant with the opioid epidemic, there has been a rise in pregnant women diagnosed with opioid use disorder and infants born with neonatal opioid withdrawal syndrome (NOWS). NOWS refers to a heritable set of symptoms following cessation of prenatal opioid exposure that comprise low body weight and hyperirritability. However, the genetic factors contributing to differences in withdrawal symptom severity remain poorly understood. We aim to use mouse models to identify genetic variants contributing to NOWS severity and translate these findings to humans.

Methods: All mouse experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. First, we phenotyped genetically similar inbred substrains of the FVB/N strain (NJ, NCrl, NHsd, and NTac) to identify behavioral differences in NOWS model traits, including delayed growth, hyperalgesia, and hyperirritability. Male and female neonatal pups were injected twice daily from postnatal day 1 (P1) to P15 with morphine (10 mg/kg, s.c.; MOR) or saline (SAL). This early life exposure model represents the approximate third trimester in humans and is characterized by developmental milestones in the brain, including synaptogenesis and myelination. This exposure period is necessary and sufficient for inducing NOWS traits. Importantly, this model avoids dam exposure that could affect maternal care and permits control of MOR dosing across pups. We assessed several phenotypes during spontaneous MOR withdrawal (16 h) on P7 and P14, including ultrasonic vocalizations (USVs) followed by nociceptive testing (hotplate latency (52.5 C). On P8 and P15, we administered MOR or SAL, and recorded USVs to determine the effect of MOR treatment on USVs. In pups, USVs indicate emotional states and are emitted in response to thermal change, distress, and maternal separation. We explored the composition of USVs to determine if certain syllables were associated with opioid withdrawal, an aversive state. We also looked for divergence among FVB substrains in USV composition that would implicate genetic differences in USV model traits for NOWS. We used the machine learning software, DeepSqueak, to classify USV syllables, and developed a supervised classifier to distinguish USVs based on spectrotemporal characteristics. On P16 during withdrawal, brainstem tissue was collected for RNA-sequencing, which was conducted at the Microarray and Sequencing Resource Core Facility at Boston University using a 200 cycle P2 flow cell and Illumina NextSeq 2000 (100×100 bp paired-end reads).

Results: All MOR pups vs. SAL pups displayed lower body weights and showed thermal hyperalgesia (reduced nociceptive latency) on the hot plate assay (SAL, $n =$ NCrl: 11-17, NJ: 13, NHsd: 19-25, NTac: 10; MOR = NCrl: 11-15, NJ: 8-10, NHsd: 14-23, NTac: 8-9). During withdrawal on P14, MOR pups emitted more USVs, consistent with the excessive crying in NOWS infants (t-test, ****$p < 0.0001$). Additionally, there was an increase in the percentage of complex 3 syllables in MOR ($n = 40$) vs. SAL ($n = 51$) pups (t-test, ****$p < 0.0001$). Interestingly, in the NJ substrain, female MOR pups ($n = 5$) emitted more USVs (Two-way ANOVA F(1,18)=10, **p = 0.00540. Bonferroni correction ***padjusted=0.0002) and a greater proportion of complex 3 syllables compared to male MOR pups ($n = 5$) (Two-way ANOVA F(1,18)=2.9, p = 0.101. Bonferroni correction **padjusted=0.00980). On P15, we administered a relief dose of MOR or SAL. MOR reduced the total number of USVs emitted (Two-way ANOVA F(1,134)=36.37, ****$p < 0.0001$) and reduced the proportion of complex 3 syllables emitted by MOR pups toward a control-like level (Two-way ANOVA F(1,134)=36.4, ****$p < 0.0001$), suggesting that complex 3 syllables could be a unique marker associated with the opioid withdrawal state. RNA-seq analysis of NJ brainstem tissue (SAL,$n = 4$; MOR, $n = 4$) revealed downregulation of myelin basic protein, Mbp (log FC = −0.410, **padjusted=0.00589), and proteolipid protein 1, Plp1 (log FC = −0.387, *padjusted=0.0104), and upregulation of the μ-opioid receptor, Oprm1 (log2FC = 0.977, *padjusted=0.0454), k-opioid receptor, Oprk1 (log2FC = 0.339, padjusted=0.00553), in addition to upregulation of the dopamine transporter, Slc6a3 (log2FC = 3.62, padjusted=0.0859), that was driven by the females.

Conclusions: We expanded the third trimester-approximate model for NOWS to FVB/N substrains to show decreased weight gain and increased nociceptive sensitivity during withdrawal. MOR pups emitted more USVs than controls and a greater proportion of complex 3 syllables that were alleviated by a subsequent MOR maintenance dose. Based on the connection between USVs and emotional states in pups, our data suggest that complex 3 syllables could indicate negative emotional states associated with opioid withdrawal. Additionally, several addiction-relevant genes were upregulated in NJ MOR pups, while genes associated with myelination and development were downregulated. These results highlight sex and substrain differences in physiological and emotional withdrawal-associated phenotypes and provide insight into the biological mechanisms contributing to differences in NOWS severity. A future direction will involve quantitative genetic analysis and gene discovery underlying substrain variance in NOWS model traits in a reduced complexity cross.

Keywords: Behavioral Pharmacology, Neonatal Opioid Withdrawal, Addiction Genetics, Genetic Mapping

Disclosure: Nothing to disclose.

P674. The Neuron -Navigator-1 (Nav1) Gene Regulates Self-Administration of Cocaine and Palatable Food in Mice

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Background: Intravenous self-administration (IVSA) of potentially addictive drugs in laboratory animals models the volitional initiation and progression of drug use in humans and provides opportunities to discover the neurogenetic influences on this behavior. Cocaine IVSA is a complexly determined trait, and a substantial proportion of individual differences in cocaine use in both humans and animals is determined by genetic variation, though the specific causal genes and alleles remain mostly unknown. Cocaine IVSA procedures can be used in genetically diverse laboratory animal populations to rigorously and rapidly identify relevant genetic influences. We have utilized the Hybrid Mouse Diversity Panel (HMDP) to characterize cocaine IVSA and perform haplotype-based computational genetic mapping for this trait. These efforts nominated Neuron-Navigator1 (Nav1) gene on chromosome 1 as a cocaine IVSA candidate gene. Nav1 is known to be expressed in post-mitotic neurons and to affect neuritogenesis; however, relatively little is known about the effects of altered Nav1 expression on the nervous system function or behavior, particularly potential effects on addiction-relevant functions or behavioral traits. In order to validate the role of this gene in cocaine IVSA, we developed a genetically engineered Nav1 knockout (KO) model to test in cocaine IVSA.

Methods: CRISPR was utilized to induce a null allele at the Nav1 gene on the C57BL/6 J background and gene expression was measured to confirm that the mutation eliminates expression of functional Nav1. Mice were bred with male and female heterozygous mice to produce litters that include all 3 genotypes (homozygous wilde-type (wt), homozygous mutant (mutant) and heterozygous (het)). Some subjects were surgically catheterized, providing access to the jugular vein. Mice were tested in a between-subjects dose-response (0.1, 0.5 and 1.0 mg/kg) cocaine IVSA procedure ($n = 14$-15 per genotype/dose, sex approximately balanced across groups). Testing occurred in operant boxes under an FR1 schedule, in which a press of the active lever caused an infusion, presentation of a conditioned cue (flashing house light for 20 sec) and a 20 sec timeout. Sessions were terminated after 2 hours or when 65 infusions were earned. An additional cohort of naïve (no surgery) mice ($n = 10$-11 per genotype, sex approximately balanced across groups) were tested in a similar IVSA procedure with a palatable food as a reinforcer.

Results: Cocaine IVSA testing across three doses revealed that mutant mice in the Nav1 KO line self-administered more cocaine [main effect of genotype $F(2,113)=6.4$, $p = 0.002$] relative to wild-type and heterozygous mice. We did not find evidence that this genotype effect varied across cocaine dose [genotype by dose interaction $F(4,113)=0.9$, $p = 0.496$]. Thus, this data suggests that KO of the Nav1 gene produces an upward shift of the dose-response curve. In testing the effects of genotype on operant responding for a food reinforcer, we found that homozygous mutant mice again earned more reinforcers [main effect of genotype $F(2,26)=6.5$, $p = 0.005$].

Conclusions: Our forward genetic approach for cocaine IVSA in the HMDP nominated Nav1 as a candidate gene that may moderate intake of cocaine, and the present data has validated this role of Nav1. We found that KO of Nav1 increased intake of cocaine and palatable food in an operant self-administration procedure. These data suggest that the effects of Nav1 generalize across drug and non-drug reinforcers. Previous study of this KO line revealed additional phenotypes associated with homozygous mutant mice including altered response to opioid reward and analgesia, lower than expected production of mutant mice, locomotor hyperactivity and a potential higher incidence of seizures. Future research will seek to determine the effects of Nav1 on key neurobiological mechanisms that may mediate Nav1 effects on reward-seeking behaviors and other associated phenotypes.

Keywords: Addiction, Genetics, Cocaine, Self-Administration, Genomics

Disclosure: Nothing to disclose.

P675. Heritable Differences in Impulsive Behavior Associate With Altered Indices of Dopaminergic Transmission in the Orbitofrontal Cortex and Nucleus Accumbens in BXD Recombinant Inbred Mice

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Background: Impulsivity is a heritable phenotype in humans that is elevated in chronic drug and alcohol users, in part because it is a risk factor for the development of addiction. One hypothesis explaining the strong relationship between impulsivity and addiction is that genetic regulation of neuronal signaling within the orbitofrontal cortex (OFC), an area of the frontal cortex that exerts control over reward-guided behavior and plays a key role in addiction neurobiology, is involved.

Methods: Here, we measured dopaminergic function within the OFC and its efferent target, the nucleus accumbens (NAc), in six recombinant BXD strains selected for a high or low impulsive behavioral phenotype in a reversal learning task. Adult mice underwent reversal learning or were euthanized and the OFC and NAc regions were collected. HPLC or rtPCR was conducted on tissue for quantification of dopamine, serotonin, and their metabolites or relative expression levels of Drd1 and Drd2, respectively.

Results: Our measure of impulsive behavior and each of the measures of dopamine turnover and receptor expression were significantly heritable, with broad sense heritability estimates ranging from .076 to .180. HPLC analysis revealed increased dopamine turnover in the NAc of high impulsive strains ($F[1,82]=5.620$, $p < 0.05$, $N = 86$), lower DOPAC levels in the OFC of high impulsive strains ($F[1,61]=5.011$, $p < 0.05$, $N = 65$). rtPCR identified lower expression of Drd1 ($F[1,86]=8.033$, $p < 0.01$, $N = 90$) and Drd2 ($F[1,86]=7.254$, $p < 0.01$, $N = 90$) in the NAc of high impulsive strains.

Conclusions: These results thus far would indicate heritable differences in the dopaminergic system of the NAc, and potentially OFC, of high impulsive mouse strains, most notably elevated dopamine metabolism and lower Drd1/Drd2 expression in the NAc. Data from this study facilitates understanding of dopaminergic dysfunction in the cortico-striato-thalamo-cortical pathway that may underlie impulsive behaviors and associated susceptibility for substance use and use disorder.

Keywords: Impulsive Behavior, Dopamine, Nucleus Accumbens (NAA), Orbitofrontal Cortex (OFC)

Disclosure: Nothing to disclose.

P676. Cross-Ancestry Meta-Analysis of Tobacco Use Disorders Based on Electronic Health Record Data Uncovers Novel Loci and Reveals Associations With Numerous Health Outcomes

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Background: Tobacco use disorders (TUD) are the most prevalent substance use disorder in the US, with a high proportion of smokers meeting criteria for dependence. These individuals often have difficulty quitting, experience withdrawal symptoms when they stop, and continue smoking despite negative mental, social, and medical consequences. Genetic factors influence smoking behaviors and strides have been made in understanding aspects of tobacco initiation and use via genome-wide association studies (GWAS). However, due to limited sample sizes, GWAS of TUD still account for only a small amount of the variance in these traits. In addition, nicotine-related GWAS have primarily focused on individuals of European ancestry.

Methods: Here we leverage access to multiple biobanks (Vanderbilt University Medical Center, Mass General Brigham, Million Veterans Program, UK Biobank) to perform a cross-ancestral meta-analysis of TUD (derived via electronic health records, EHR) in 740,361 individuals of European, African American, and Latin American ancestries.

Results: The cross-ancestral meta-analysis identified 31 independent loci, including the nicotinic acetylcholine receptor CHRNA3/A4 gene cluster, which has been consistently associated with smoking behaviors. Other promising candidate genes include PDE4B, previously associated with smoking initiation and problematic alcohol use, and PTPRF, recently implicated in opioid

addiction and impulsive behaviors. TUD-EHR was also genetically correlated with traits derived from traditionally ascertained cohorts, including nicotine dependence and smoking cessation. Surprisingly, the genetic correlation between cigarettes per day and TUD-EHR, although significant and positive, was moderate in magnitude (rg < 0.5), suggesting that the genetic architecture of consumption and misuse is distinct. Lastly, we evaluated the use of TUD-EHR polygenic scores as genomic predictors of 1,335 psychiatric and medical traits, and revealed hundreds of associations, including HIV infection, heart disease, and pain.

Conclusions: This work furthers our biological understanding of TUD and its shared genetic risk with other mental and physical traits, and establishes that EHR are useful sources of phenotypic information for studying the genetics of TUD.

Keywords: Tobacco, GWAS, Substance Use Disorders, Electronic Health Record (EHR), Addiction Comorbidity

Disclosure: Nothing to disclose.

P677. Elevated DNA Damage in Specific Cell Types Associated With Opioid Use Disorder in Human Postmortem Brain

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Background: In the United States, rates of opioid use disorder (OUD) and deaths from overdose are unprecedented. Despite the enormous public health impact of OUD, we have a limited understanding of the changes in the brain in patients with OUD. Few studies have directly examined the cellular and molecular changes in the human brain associated with OUD. A recent study from our group utilized bulk RNA sequencing in human postmortem brain in the dorsolateral prefrontal cortex and nucleus accumbens in OUD and control subjects. This study identified significant alterations in transcripts enriched for neuroinflammatory and extracellular matrix signaling in the brains of subjects with OUD. Additionally, cell type specific enrichment of microglia markers in demonstrates a potential primary role for microglia in OUD. Collectively, this study suggests that OUD may induce the upregulation of neuroinflammatory markers, particularly in microglia. However, a limitation to using bulk RNA sequencing is the lack of cellular resolution. To address this limitation, we are utlizing single nucleus RNA sequencing in human postmortem brain. Human neuroimaging studies investigating OUD have implicated functional changes in the dorsal striatum, an area integral in reward processing, habitual drug-seeking, craving, and relapse. The dorsal striatum is comprised of the caudate nucleus and putamen. In the current study, we conducted single nuclei RNA sequencing on human postmortem caudate nucleus and putamen in subjects diagnosed with OUD and comparison control subjects in order to identify cell-type specific transcriptional changes associated with opioid use disorder. This study is the first of its kind to identify key changes in transcriptional signatures associated with opioid use disorder at the single nucleus level in human postmortem dorsal striatum.

Methods: In the current study, we conducted single nuclei RNA sequencing on postmortem brain tissue from male and female subjects diagnosed with opioid use disorder ($N = 6$; 3 females and 3 males; average age: 41.17) or comparison subjects ($N = 6$; 3 females and 3 males; average age: 44.67). For each subject, both the caudate and putamen were processed. Nuclei were isolated from 24 fresh frozen post-mortem human brain samples. Briefly, ~50 mg of tissue was dounce homogenized with 1,000 µl nuclei lysis buffer containing dapi using a glass douncer with 9 pestle strokes. Homogenate was filtered using a 40um cell filter. Samples were sorted on a BD FACS Aria II Flow Cytometer at the Boston University Flow Cytometry Core using Fluorescent Activated Cell Sorting (FACS) for dapi. Nuclei were sorted at a flow rate of 1.5-2.0 with gating criteria set to hierarchically select whole, single nuclei. For each sample, approximately 150,000 nuclei were collected into a tube containing 11.40 µl PBS with 0.04% BSA. Samples were evaluated for nuclei concentration and viability on a hemocytometer. 7,000 nuclei were targeted using the 10x single cell dual index 3' gene expression protocol to prepare dual index next generation sequencing libraries. Finally, libraries are pooled and sequenced on the Next-seq 2000 (illumina) at the Boston University Single Cell Sequencing Core. Sequencing targeted 7,000 nuclei per sample and 50,000 reads per nuclei.

Following completion of sequencing, data was pre-processed. Pre-processing included aligning the reads (STARsolo), ambient mRNA detection and correction, empty droplet and doublet filtering, and QC analysis. Next, data was labeled and verified using a dorsal striatum macaque dataset (He and Kleymen et al., 2021). Finally, full analysis was conducted using analyses for differential expression, DNA damage signatures, and differential abundance.

Results: We identified transcriptional alterations associated with OUD in specific cell types of the striatum, including significant shifts in the expression of dopamine receptor subtypes in GABAergic medium spiny neurons. When examining differentially expressed genes (DEGs) by cell types comparing OUD and control subjects, DEGs were found in the following cell types: astrocytes, endothelial, microglia, oligodendrocytes, D1-matrix, D1-striosome, D1/D2 hybrid, D2-matrix, D2-striosome, interneuron. Next, we analyzed DNA damage signatures. DNA damage signature analyses revealed increased DNA damage specific to OUD subjects compared to control subjects ($p = 0.002$). When looking at cell type specific DNA damage, mural cells have a higher proportion of DNA damage ($p = 0.025$) in OUD subjects. Additionally, looking at average DNA damage scores across cell types revealed increased DNA damage in OUD subjects in microglia ($p = 0.004$), endothelial ($p = 0.006$), oligodendrocytes ($p = 0.021$), and interneurons ($p = 0.023$). Additional exploratory approaches are currently underway to reveal cell-specific alterations in gene expression and compare different cell types across striatal subregions and sex.

Conclusions: Our results are the first to demonstrate significant alterations in gene expression across different cell types of the striatum in the human brain associated with OUD using single nuclei RNA-seq. Additionally, our results suggest that OUD is associated with higher rates of DNA damage, particularly in microglia subtypes. Uncovering molecular mechanisms of opioid use will propel the identification of new therapeutic targets and the development of successful treatment strategies for OUD.

Keywords: Opioid Addiction, Human Genetics, Single-Nucleus RNA Sequencing, Postmortem Human Brain Tissue, Cell-Type Specific Transcription

Disclosure: Nothing to disclose.

P678. Sex-Specific Associations of Genetic Variation With Treatment Outcomes in Men and Women With Alcohol Use Disorder

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Background: Sex and gender-related differences are known to be associated with alcohol consumption and risk for development of alcohol use disorder (AUD). Studies also indicate potential male/female differences in tendency to enter treatment programs and treatment response, including time to return to heavy drinking as well as vulnerability to risk factors, including negative affect or social pressure (Erol and Karpyak, 2015). So far, no differences have been found in treatment response to acamprosate, naltrexone or brief intervention between men and women with alcohol dependence. We recently completed a genome-wide association study (GWAS) of AUD treatment outcomes and demonstrated that genetic variation may have a polygenic effect on AUD treatment response, and that some genetic variants may be associated with medication-specific effects (Biernacka et al., 2021). Here we explored potential sex differences in genetic markers associated with treatment response to acamprosate, naltrexone and placebo.

Methods: We used genetic and clinical data from three studies of acamprosate and/or naltrexone treatment of AUD (COMBINE $N = 498$, PREDICT $N = 266$, and CITA $N = 252$) reported in our original GWAS of AUD treatment outcomes (Biernacka et al., 2021). This dataset includes a total of 1016 participants of European ancestry (24% female), including 484 patients treated with acamprosate, 200 with naltrexone, 101 with both medications and 231 with placebo. We now performed sex-stratified GWAS of time until relapse to any drinking and time until relapse to heavy drinking (i.e., more than 4 drinks/day for men and more than 3 drinks/day for women) based on 3-month treatment outcomes. For each outcome, we performed sex-stratified analyses within each study, and then meta-analyzed the results across studies using METAL.

Results: Of the 1016 study participants in the analysis, 639 (61% of men and 68% of women) relapsed to light drinking and 564 (54% of men and 60% of women) relapsed to heavy drinking. In the GWAS of women, two SNPs (rs7801472 in AUTS2 and rs72949008 in RP11-146N18.1) were associated with time until relapse at a genome-wide significant level ($p = 3.2e-8$ and $p = 4.2e-8$, respectively). One SNP (RP11-73O6.3 rs3818792; $p = 4.8e-8$) was associated with time until relapse to heavy drinking in women. No variants were associated with time until relapse or time until heavy relapse in men at a genome-wide significant level.

Conclusions: We observed two significant genetic associations with time until relapse in women undergoing AUD treatment that map to AUTS2 and RP11-146N18.1. While little is known about RP11-146N18.1, AUTS2 (activator of transcription and developmental regulator) is a gene known to be involved in brain development and regulation of neuronal gene expression. Mutations in this gene cause intellectual disability, microcephaly, and other phenotypes, and common variants in AUTS2 have also been found to be associated with alcohol consumption, alcohol dependence, heroin dependence as well as autism spectrum disorder, impulsivity, ADHD, schizophrenia, and bipolar disorder. We also found significant association between heavy relapse in women and rs3818792, which is in a genomic region that includes L3MBTL3 (L3MBTL histone methyl-lysine binding protein 3), associated with transcriptional repression, SAMD3 (sterile alpha motif domain containing 3), associated with age at depressive onset and RP11-73O6.3, which is antisense lncRNA of L3MBTL3 and SAMD3. None of these findings in women had corresponding significant associations with the treatment response in men. While these results are preliminary, if replicated, they may guide investigation of potential sex-specific differences in genetic contribution to known triggers for relapse (e.g., negative emotional states) or relapse prevention measures (e.g., medication types). If successful, this line of research may contribute to development of biomarker-based, sex-specific recommendations for men and women seeking treatment for AUD.

Keywords: Alcohol Use Disorder - Treatment, Genetic Association Study, Sex-Specific Effects

Disclosure: Nothing to disclose.

P679. CHRNA5 Gene Polymorphism and Early Smoking Onset Effect on Adult Smoking and Alcohol Measures

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Background: The onset of smoking at an early age increases the risk for later nicotine dependence and alcohol use disorder (AUD). A non-synonymous polymorphism (rs16969968) in the CHRNA5 gene has shown a significant interaction with smoking onset age in causing heavy smoking and nicotine dependence in adults. Furthermore, a recent pre-clinical study has identified a sex-dependent association between CHRNA5 mutation and adolescent alcohol or nicotine exposure on subsequent intake of the opposite drug in adulthood. The current study aimed to determine the effect of the rs16969968 polymorphism and age at onset of smoking on adult smoking and alcohol traits.

Methods: This study included 29,423 participants from the UK Biobank study sample who were current smokers and alcohol drinkers. We divided the study participants into early and late smoking onset groups depending on their smoking onset age (early onset = ≤16 years, late onset = >16 years). Smoking and alcohol measures, including the number of cigarettes smoked per day (CPD) and Alcohol Use Disorders Identification Test (AUDIT) scores, were compared between onset age groups and by CHRNA5 (rs16969968) genotype, with age and gender included as covariates. Additionally, we conducted a complementary analysis in the NIAAA study sample that included 188 healthy adult non-AUD drinkers who were also smokers. In this sample, smoking and alcohol measures included Fagerström Test for Nicotine Dependence (FTND), pack-years, AUDIT, Timeline Followback (TLFB), and Lifetime Drinking History (LDH). These measures were compared between smoking onset groups and CHRNA5 genotype groups, with age, gender, and Ancestry Informative Markers scores (AIMs) included as covariates. In all analyses, the main effect of the rs16969968 polymorphism was tested using a dominant model (GG vs. AA/AG) and the effect of smoking onset age was analyzed in the full sample as well as in the GG and AA/AG genotype groups separately.

Results: In the UK Biobank sample, we observed a significant main effect of the CHRNA5 genotype on CPD with higher mean scores in the AA/AG genotype group than the GG genotype group ($p < 0.001$). Individuals with early smoking onset showed an increased quantity of nicotine consumption compared to the late smoking onset group which was consistent across both genotype groups. There was a main effect of the CHRNA5 genotype on AUDIT-C (consumption) with higher mean scores in the GG genotype group than the AA/AG genotype group ($p = 0.016$). However, there was no significant interactive effect of the genotype and smoking onset age on smoking or alcohol measures.

In the NIAAA sample, a main effect of the CHRNA5 genotype and the interactive effect of genotype and smoking onset age groups were not observed on smoking or alcohol measures. The early smoking onset group showed an increased number of years of smoking compared to the late smoking onset group in the full sample and in the GG genotype group but not in the AA/AG genotype group. The AA/AG genotype group showed an increased quantity of cigarette smoking per week and TLFB-total drinks in the early smoking onset group than the late smoking

onset group; this effect was not observed in the full sample or in the GG genotype group.

Conclusions: This study provides further evidence for the main effect of the CHRNA5 (AA/AG) genotype and early smoking onset on increased nicotine consumption. Individuals carrying either the GG or AA/AG genotype are at an elevated risk for increased nicotine consumption in their adulthood if they initiate smoking at an early age. Also, there is an increased risk for a greater quantity of alcohol consumption in smokers carrying the CHRNA5 GG genotype compared to individuals carrying the AA/AG genotype. The risk for increased alcohol consumption was also observed in individuals with CHRNA5 AA/AG genotype who initiated smoking at an early age.

Keywords: CHRNA5, Smoking Onset Age, Adult Smoking and Alcohol Traits

Disclosure: Nothing to disclose.

P680. Stress and Alcohol Exposure Accelerate Epigenetic Aging

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Background: Stress contributes to premature aging and susceptibility to alcohol use disorder (AUD) and AUD itself is a factor in premature aging; however, the interrelationships of stress, AUD and premature aging are poorly understood.

Methods: We constructed a composite score of stress (CSS) from thirteen stress-related outcomes in a discovery cohort of 317 individuals with AUD and controls. We then developed a novel methylation score of stress (MS Stress) as a proxy of CSS comprising 211 CpGs selected by a penalized regression model. The effects of MS Stress on health outcomes and epigenetic aging were assessed in a sample of 615 AUD patients and controls using epigenetic clocks and DNAm telomere length (DNAmTL). Statistical analysis with an additive model using MS Stress and a methylation score for alcohol consumption (MS Alcohol) were conducted. Results were replicated in two independent cohorts (Generation Scotland GS $n = 7028$ and the Grady Trauma Project GTP $n = 795$).

Results: CSS and MS Stress were strongly associated with heavy alcohol consumption, trauma experience, epigenetic age acceleration (EAA) and shortened DNAmTL in AUD. Together, MS Stress and MS Alcohol additively showed strong stepwise increases in EAA. Replication analyses showed robust association between MS Stress and EAA in the GS and GTP cohort.

Conclusions: A methylation-derived score tracking stress exposure is associated with various stress-related phenotypes and EAA. Stress and alcohol have additive effects on aging, offering new insights into the pathophysiology of premature aging in AUD, and potentially, other aspects of gene dysregulation in this disorder.

Keywords: Epigenetics, Aging, Alcohol, Stress, PTSD

Disclosure: Nothing to disclose.

P681. Novel Underlying Biology and Therapeutic Targets for Alcohol Use Disorder and Problem Drinking Identified Using Proteomics and Mendelian Randomization

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Background: Alcohol use disorder (AUD) is a common neuropsychiatric disorder that is a leading cause of morbidity and mortality worldwide; however, only a few pharmacological treatment options are currently available, highlighting the need for novel and safe drug development. While protein biomarkers with causal genetic evidence are promising novel drug target candidates for AUD, scans of brain proteins have not been performed.

Methods: We integrated genome-wide association summary statistics (GWAS) for AUD and alcohol consumption behaviors, i.e., problem items from the Alcohol Use Disorders Identification Test (AUDIT-P), binge drinking, and total drinks per week (DPW), and applied cis-instrument Mendelian randomization (MR) to perform a proteome-wide MR with data from >1,700 brain proteins within the dorsolateral prefrontal cortex (dlPFC) to investigate their causal relevance for AUD and alcohol consumption behaviors.

Results: We identified 34 unique brain protein-alcohol associations that emerged as causal mediators of AUD and alcohol consumption behaviors. Novel proteins not previously implicated in alcohol consumption behaviors included CAB39L, TESC (P-value=1.99×10-7 (AUD)), ERLIN1 (P-value=2.31×10-12 (AUDIT-P)), CPS1 (P-value=6.9×10-6 (binge drinking)), HDGF (SLC5A6 (P-value=1.53×10-7 (DPW)). CAB39L was consistently associated with increased drinking across alcohol phenotypes (with P-values ranging from 8.66×10-10 (AUD) to 1.69×10-114 (DPW)). We were able to replicate proteins using independent dlPFC protein and gene expression datasets. 11 of the proteins also showed evidence of a shared causal variant between the brain protein and respective alcohol consumption behavior, including CAB39L, HDGF, and SLC6A5 with DPW and CPS1 with binge drinking. Single-cell analysis showed enrichment predominantly in excitatory neurons within the dlPFC. Exploratory MR identified corresponding associations for 31 of the 34 alcohol-related proteins with 22 neuropsychiatric endpoints, highlighting pleiotropic associations with a range of disorders and endpoints such as cognition and smoking behaviors. TESC, SLC6A5, HDGF, and CPS1 were not associated with other neuropsychiatric endpoints, suggesting potentially specific roles in alcohol consumption. Finally, several of the proteins are considered actionable drug targets suggesting possible drug repurposing opportunities.

Conclusions: Our findings highlight the power of integrating genetics, proteomics, and transcriptomics in elucidating causal biology underlying AUD and alcohol consumption behaviors, linking them with neuropsychiatric disorders, and identifying novel drug targets that may aid the development of new therapeutics aimed at reducing problematic drinking.

Keywords: Mendelian Randomization, Proteomics, Alcohol Consumption, Alcohol and Substance Use Disorders, Single-cell RNA Sequencing

Disclosure: Nothing to disclose.

P682. Unbridled Amygdalae! Opioid Patients Carrying a Genetic Variant Linked to Over-Response of the Stress (Cortisol) System Show Heightened Resting Connectivity Between the Amygdala and the Ventromedial Prefrontal Cortex (vmPFC)

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Background: Honed by eons of survival-driven priorities, our brain leaps into action at the first whiff of danger – or reward. Within the brain, our amygdalae are critical first responders, well-studied for their rapid response to signals, e.g., for threat, and for rewarding drugs of abuse. In the healthy brain, these important functions are 'bridled', tightly regulated by other (inhibitory) brain regions, so that we are not in a chronic state of fear or reward motivation. As stress can undermine the inhibitory 'bridle', we recently tested and confirmed that cocaine and opioid patients carrying a genetic variant linked to over- response of the cortisol stress system (i.e., the minor G allele of rs3800373, for the FKBP5 gene) have heightened ('unbridled') responding to drug reward cues in motivational circuits (CPDD 2019; SfN 2019). This over-response to appetitive cues underscored an intriguing 'cross-talk' between aversive and appetitive motivational systems in the brain – and also begged a further question: Could the impact of the vulnerability allele be detected even when the brain is at rest, representing a brain that is tuned up and 'over-ready' to respond (to signals for threat or for drug reward)? Indeed, we found (SfN 2021) that cocaine patents who carried the vulnerability ('unbridled') allele had heightened functional connectivity between the amygdala and motivational circuitry nodes (VMPFC and insula). For the current presentation, we tested whether the 'signature' of heightened amygdala resting functional connectivity (for G allele carriers) could also be detected within a cohort of individuals with opioid use disorder (OUD).

Replication in an additional cohort is important, given statistical concerns about studies (whether imaging, behavioral or clinical) that focus on a single nucleotide polymorphism (SNP), even if the examination is hypothesis-driven. This is because most psychiatric disorders and medical conditions are emphatically polygenic. Intriguingly, however, specific allelic variants of FKBP5, including the functional SNP studied here, have been shown to account for a significant outcome variability in complex behaviors, e.g., the vulnerability (high cortisol) allele is linked to healing, vs. progression to chronic musculoskeletal pain, in those with similar initial back injuries. This robust finding was replicated across two large pain cohorts ($n = 948$; $n = 1607$), stimulating the current studies in the appetitive (addiction) domain.

Methods: As part of a large study of brain predictors of relapse to drug use, treatment-seeking opioid outpatients received an fMRI session (BOLD, 3 T) prior to their induction onto depot naltrexone. Resting state was collected at the outset of the session, prior to a variety of fMRI tasks. Resting state data (5 minutes) was analyzed within the SPM8 connectivity toolbox, generating voxel-wise functional connectivity maps for (R; L) amygdalae in carriers of the minor G (vulnerability) allele (TG, GG; $n = 18$) for rs3800373 of FKBP5, and in TT homozygotes ($n = 13$). The resulting correlation maps (r to Z transformed) were examined for each group, and then formally compared across the two genetic subgroups (group difference t maps thresholded $2 < t < 5$ for display; FWE- corrected).

Results: For both opioid allelic subgroups, there was robust positive "intra-limbic" connectivity between the amygdala (R; L) and multiple motivational nodes (e.g., the ventral tegmental area, ventral pallidum, ventral striatum/putamen, and insula) – and both allelic groups lacked inverse ('bridling') connectivity with the dorsomedial prefrontal cortex (PFC) found in studies of healthy controls – indeed, the connectivity was in the positive direction. In addition, carriers of the minor (vulnerability) allele for FKBP5 showed heightened resting connectivity between the amygdala (L and R amyg) and the ventromedial PFC, and the ventral striatum (L amyg), as compared to the TT homozygotes.

Conclusions: Encouragingly, these new data in an opioid cohort do replicate the patterns of findings from the earlier cocaine sample: overall, both the opioid and cocaine patients exhibit the 'unbridled' amygdala relationships with the cortical regulatory regions – and both cohorts show that carriers of the vulnerability alle have heightened connectivity of the amygdala with the subcortical VMPFC, as compared to the TT homozygotes. These replicated results give confidence that the brain findings may be a marker of vulnerability across substance use disorders: carriers of the minor G (vulnerability) allele of rs380037 for the FKBP5 gene may be at additional risk for psychopathologies related to amygdala over-activity, whether in the domain of aversive (e.g., pain /anxiety /stress /PTSD) or appetitive (e.g., drug reward/relapse) motivation. Finally, though the current findings are in adult individuals struggling with addiction, accruing neurodevelopmental cohorts (e.g., the ABCD study) offer the unique opportunity to combine resting state functional connectivity with genetics as potential markers of vulnerability to the future development of affective or substance use disorders. If validated prospectively, these combined brain and genetic markers would encourage future (behavioral, pharmacologic, or neuromodulatory) interventions to boost or to restore regulation of the amygdala, especially for carriers of the vulnerability allele.FKBP5

Keywords: FKBP5, Resting-State fMRI, Cocaine and Opioid Use Disorders, Amygdala, Functional Connectivity

Disclosure: Nothing to disclose.

P683. An Increase in Epigenetic Age Acceleration Among People Who Use Substances

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Background: Substance misuse can have a significant impact on overall health. People who use substances present greater risk of morbidity and mortality than non-users. Biological aging has been proposed as an indicator of the adverse effects of substance misuse on health and frailty. Recently developed epigenetic "clocks" employ cellular DNA methylation (DNAm) as a measure of the aging process. DNAm-based epigenetic clocks were shown to be more sensitive and precise measures of cellular age than other genomic measures (e.g., transcriptome, telomere length). In this study, we tested whether substance misuse (i.e., alcohol, cigarette smoking, and cannabis use) altered epigenetic clocks (i.e., HorvathAge, HannumAge, PhenoAge, GrimAge, and MonoAge). We further examined the mediating role of stress and BMI in substance misuse related epigenetic age acceleration

Methods: All analyses were performed in a community-based sample from Greater New Haven, Connecticut ($N = 509$). All participants were determined healthy without major medical or psychiatric disorders. Substance use was based on self-reported data. Alcohol consumption was measured by using the 10-item Alcohol Use Diagnosis Identification Test (AUDIT). Heavy alcohol drinking was defined as an AUDIT score ≥8 for men and an AUDIT score ≥7 for women. The average AUDIT score was 5.69 among all participants in the cohort. Cigarette smoking and cannabis use were categorized as current, past, and never users as well as quantified number of uses in the past 30 days. DNA methylation in the blood methylome was profiled by using Illumina Human-Methylation Beadchip 450 K. Each clock of interest was estimated using the established algorithms for each sample. Pearson's correlation between DNAm age and chronological age was performed in each group. Epigenetic age acceleration (EAA) was defined as the residuals of regressing DNAm age on chronological age. We calculated the mean difference in EAA between heavy users and non-heavy users, smokers and non-smokers, cannabis users and non-cannabis users. Mediation analysis was conducted to test whether stress and BMI mediated the EAA in each substance misuse

Results: We found significant associations of each epigenetic clock with frequency of alcohol use per week (pHorvathAge = 0.03, pHannumAge=0.02, pPhenoAge=0.007, pGrimAge=0.002, pMonoAge=0.01), AUDIT-10 (pPhenoAge = 0.003 and pMonoAge=0.03), cigarette per day (pHorvathAge = 6.49E-05, pHannumAge=7.38E-05, pPhenoAge=2.27E-06 pGrimAge=2.18E-08, pMonoAge=0.0001 and cannabis use in the past 30 days (pHorvathAge = 0.0013, pHannumAge=0.02, pPhenoAge=0.002, and pMonoAge=0.012). For epigenetic age acceleration, AUDIT score was associated with HorvathAge (p = 0.02), HannumAge (p = 0.001), and GrimAge acceleration (p = 0.0006). Cigarette per day was associated with PhenoAge (p = 0.001) and GrimAge only (p = 5.73E-10). Cannabis use in the past 30 days was significantly associated with GrimAge acceleration (p = 0.002). These results suggest that each epigenetic clock employs slightly different functionalities to capture epigenetic age in each substance misuse type.

Conclusions: Our results demonstrate that substance misuse increases epigenetic age acceleration in a healthy young population. We also show that stress partially mediates the substance misuse-related age acceleration. These findings indicate the importance of early prevention to potentially reserve adverse effects of substance misuse.

Keywords: Epigenetic Age Acceleration, Substance Abuse, Lifetime Stress

Disclosure: Nothing to disclose.

P684. In Vivo Imaging of Brain Cortisol Regulation in Alcohol Use Disorder

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Background: Stress dysregulation is associated with the maintenance of and relapse to alcohol use. Stress is also a potent activator of the hypothalamic-pituitary-adrenal (HPA) axis, initiating the release of glucocorticoid hormones. Levels of glucocorticoids (e.g., cortisol, cortisone) present in the brain are dependent on the enzyme 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), which catalyzes the conversion of cortisone to cortisol and amplifies the action of glucocorticoids in the brain. 11β-HSD1 is located in brain regions critical in the negative feedback of glucocorticoids and in addiction, including the prefrontal-limbic circuit. Thus, high brain glucocorticoid levels, driven by 11β-HSD1 and induced by stress, may contribute to risky drinking. We used positron emission tomography (PET) imaging with the novel 11β-HSD1 specific radioligand [^{18}F]AS2471907 to assess 11β-HSD1 expression in participants with alcohol use disorder (AUD) vs. healthy controls.

Methods: We imaged ten individuals with moderate to severe AUD ($n = 6$ men, $n = 4$ women; mean age=38 years) and 12 healthy controls ($n = 7$ men, $n = 5$ women; mean age=29 years). Participants received 93.5 ± 15.6 MBq [^{18}F]AS2471907as a bolus injection at high specific activity and were imaged for 150-180 minutes on the High-Resolution Research Tomograph (HRRT; 2-3 mm resolution). 11β-HSD1 availability was quantified by [^{18}F]AS2471907 volume of distribution (VT; mL/cm3), the ratio at equilibrium of [^{18}F]AS2471907in tissue to un-metabolized [^{18}F]AS2471907in arterial plasma. A priori regions of interest included amygdala, anterior cingulate cortex (ACC), hippocampus, ventromedial PFC (vmPFC) and caudate, as these corticolimbic regions are involved in HPA axis regulation, stress pathophysiology, and addiction. Individuals were required to be overnight abstinent from drinking. Levels of 11β-HSD1 were correlated with past 30-day alcohol use on the Timeline Followback and self-reported childhood trauma (Childhood Trauma Questionnaire [CTQ]).

Results: Individuals with AUD consumed 53.72 drinks/week and had 5.81 drinking days/week. Healthy controls consumed 2.75 drinks/week and had 1.30 drinking days/week. Preliminary data suggest that 11β-HSD1 levels are higher in amygdala, ACC, hippocampus, vmPFC, and caudate in individuals with moderate to severe AUD compared to healthy controls ($p < 0.03$). The AUD group demonstrated positive correlations between drinks per week and drinks per drinking episode with 11β-HSD1 levels in vmPFC (p = <0.01; r = 0.85 and r = 0.78, respectively) and caudate (p = 0.05; r = 0.67 [drinks per week only]). Childhood trauma was associated with higher 11β-HSD1 levels in ACC in individuals with AUD (p = 0.05; r = 0.66).

Conclusions: This is the first in vivo examination of 11β-HSD1 levels in individuals with AUD. These preliminary findings suggest higher brain cortisol-producing 11β-HSD1 in AUD compared to healthy individuals, and a possible relationship between [^{18}F]AS2471907 VT and self-reported alcohol use and childhood trauma. These results are consistent with work suggesting that childhood trauma may be related to increased stress and alcohol use in adulthood. Future studies will further investigate [^{18}F]AS2471907as a marker of brain cortisol regulation in relation to stress-related drinking.

Keywords: PET Imaging, Cortisol, Alcohol Use Disorder, Stress, Trauma

Disclosure: Nothing to disclose.

P685. Identification of Δ9-Tetrahydrocannabinol (THC) Impairment Using Resting-State Neuroimaging

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Background: Intoxication from cannabis impairs cognitive performance due to the effects of Δ9-tetrahydrocannabinol (THC, the primary psychoactive compound in cannabis) on prefrontal cortex (PFC) function. There are currently no evidence-based methods to detect cannabis-impaired driving, and current field sobriety tests with gold-standard, drug recognition evaluations are resource-intensive and may be prone to bias. We tested whether a portable and inexpensive imaging method, functional near infrared spectroscopy (fNIRS), could be used to objectively detect impairment from cannabis at resting state.

Methods: 169 participants with regular cannabis use were given a single dose of up to 80 mg of dronabinol, an FDA-approved synthetic THC ingredient in MARINOL® Capsules or identical placebo. Participants underwent two fNIRS sessions; one before dronabinol administration ("pre-THC"), and the other at approximately two hours after dronabinol administration ("post-THC"), which is the median peak of pharmacokinetic effects of dronabinol. During each session, 6 minutes of resting-state functional data were collected using a continuous-wave NIRS device, in which 8 Sources and 7 detectors were placed on the forehead, resulting in 20 channels covering PFC regions. fNIRS analysis was conducted using the CONN toolbox (https://web.conn-toolbox.org).

Results: 76 participants had concordant clinical and algorithm ratings indicating that they were impaired/intoxicated following THC at (mean dose of 35.6 mg ± 11.5) and 57 participants had concordant ratings of not impaired (mean dose of 34.8 ± 16.1). The 80 impaired participants had greater subjective (DEQ) and physiologic (heart rate) compared with those who were not impaired post-THC. We found a significant change in resting-state connectivity from pre-THC to post-THC only among those

participants who were impaired (group A, $p < 0.05$; FDR-corrected); those who were not impaired, but received THC, did not show a change in resting-state connectivity (group B). When comparing post-THC to post-placebo, only those who were impaired showed a significant decrease in resting state connectivity (group A, $p < 0.05$; FDR-corrected). Those who were not impaired (group B) did not show any significant differences between post-THC and post-placebo scans, even after receiving equivalent doses of THC.

Conclusions: There is a growing public health need for an objective, reliable, unbiased method to detect impairment due to THC. This is not achievable with per se blood or body fluid THC or metabolite concentration cutoffs. Findings suggest PFC response can objectively determine who is impaired from THC intoxication, with potential applications in roadside settings. Future work is warranted to determine specificity to acute THC impairment.

Keywords: Cannabis, Functional Impairment, Resting State, Functional Near-Infrared Spectroscopy

Disclosure: Inventor: Patent (Self).

P686. Cigarette Smoking Reduces a Marker for Neuroinflammation in People Living With HIV: A [18 F]FEPPA Positron Emission Tomography Study

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Background: Microglia become activated as part of neuroinflammation, which leads to increased expression of the translocator protein 18 kDa (TSPO). Positron emission tomography (PET) studies, using radiotracers that label TSPO, have recently been used to examine TSPO availability as a marker of neuroinflammation in a variety of conditions. In such PET studies, people living with HIV (PWH; HIV+) have been found to have higher levels of radiotracer binding than seronegative individuals (HIV-). In PET studies of cigarette smokers, people who smoke (Smok+) exhibit lower radiotracer binding than non-smokers (Smok-). Based on this prior research, we hypothesized that HIV + individuals (and HIV- controls) who are either cigarette smokers or nonsmokers would have the following order of the marker for neuroinflammation on PET scanning: HIV + /Smok- > HIV + /Smok +≈ HIV-/Smok- > HIV-/Smok + .

Methods: 53 otherwise healthy participants who were in one of four categories completed the study: HIV + /Smok- ($n = 16$), HIV + /Smok + ($n = 11$), HIV-/Smok- ($n = 17$), and HIV-/Smok + ($n = 9$). Participants underwent baseline assessments to confirm eligibility, genetic testing for a genotype known to affect TSPO affinity, PET/computed tomography (CT) scanning for 90-min following bolus injection of the radiotracer [18 F]FEPPA, and magnetic resonance imaging to assist in localization of regions on the PET/CT images. Smokers were scanned in the satiated state. The primary outcome measure was whole brain standardized uptake value (SUV).

Results: An overall analysis of variance with whole brain SUV as the dependent variable, participant group as a factor of interest, and genotype as a nuisance covariate revealed a significant effect of participant group ($F = 3.0$;df=3,48;$p = 0.04$). In clarifying this overall result, smoking status had a significant main effect on whole brain SUV ($F = 5.6$;df=1,48;$p = 0.02$), while main effect of HIV status ($F = 1.5$,n.s.) and the interaction of HIV and smoking statuses ($F = 1.2$,n.s.) did not reach significance. Within the HIV + group, a main effect of smoking status was also found ($F = 7.9$;df=1,24;$p = 0.01$). Group whole brain SUV values (mean + /-SD) were in an order very similar to the hypothesized order cited above: HIV + /Smok- (1.27 + /-0.26), HIV-/Smok- (1.17 + /-0.21), HIV + /Smok + (1.11 + /-0.11), and HIV-/Smok + (1.10 + /-0.24).

Conclusions: PET/CT findings using a marker for neuroinflammation in HIV + smokers were highly consistent with prior research examining the effects of HIV and cigarette smoking statuses separately. HIV + nonsmokers had the highest levels of the marker for neuroinflammation, while cigarette smokers (both HIV + and HIV-) had the lowest levels of the marker for neuroinflammation. Thus, cigarette smoking appears to have an anti-inflammatory effect on the elevated neuroinflammation levels found in PWH.

Keywords: Human Brain Imaging, Tobacco Dependence, Human Immunodeficiency Virus, Positron Emission Tomography

Disclosure: Nothing to disclose.

P687. Acute White Matter Integrity Increases After the Moderate Dose of Alcohol Administration

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Background: Chronic heavy alcohol drinking adversely affects physical and mental health. On the other hand, a protective effect of light to moderate drinking has some biological plausibility. However, it is not well known the effects of modest alcohol intake on brain structure.

In the present study, we aimed to examine the changes in white matter integrity after a modest dose of acute alcohol administration using diffusion tensor imaging (DTI) in healthy subjects. We hypothesized that the acute intake of modest alcohol would increase the white matter integrity.

Methods: We recruited healthy volunteers aged 20-29 who carried ALDH2*1/*2 genotype, understood the study design, and gave written informed consent. The participant received an intravenous alcohol infusion using the alcohol clamp method to keep a target blood alcohol concentration of 0.50 mg/mL. We conducted MRI scans and assessed self-reported clinical responses to alcohol administration (i.e., stimulus, physical, or sedative) using the visual analogue scale (VAS) at baseline (T0), 90 minutes after alcohol administration started (T1), and 90 minutes after alcohol administration ended (i.e., 180 minutes from baseline) (T2).

MRI data were acquired on a 3 T MRI scanner (MR750; GE Discovery) equipped with a 12-channel head coil at Kurihama Medical and Addiction Center. DTI data were obtained via the use of a single-shot echo-planar sequence with diffusion gradients (b = 1000 s/mm2) applied in 30 noncollinear diffusion directions along with five diffusion-unweighted images with b = 0 s/mm2 (TE = 74.5 ms, TR = 16,000 ms, flip angle = 90, 128 × 128 matrix, 1.0×1.0×2.5 mm). After preprocessing using the FMRIB Software Library, we compared whole-brain fractional anisotropy (FA)/mean diffusivity (MD) values and FA/MD values for 48 regions of interest (ROI) extracted from the JHU-ICBM-DTI-81 white matter atlas across time using repeated-measure analysis of variance (ANOVA). Multiple comparisons for significant regions were performed using Bonferroni correction. Additional analyses were conducted to examine the correlation between baseline FA/MD value or FA/MD value changes and changes in behavioral VAS scores. Furthermore, we performed tract-based spatial statistics (TBSS) to compare white matter integrity between conditions (i.e., T0 vs T1, T0 vs T2, T1 vs T2).

Results: Eighteen subjects participated in this study (mean age=25.1 ± 3.0 years, male=7 (38.9%)).

Repeated measures ANOVA showed no significant differences in the whole FA ($F(2,51)=0.26$, $p = 0.77$) or FA values in any DTI ROIs among the three time points. TBSS analysis revealed no significant differences in FA between T1 and T0 or T2 and T1. However, there were significant increases in FA in the cerebral white matter voxel cluster (>1000 voxels) at T2 compared to T0 ($p = 0.001$, FWE-corrected), whereas no significant decreases were found.

For MD values, repeated measures ANOVA indicated no significant differences in the whole MD ($F(2,51)=0.03$, $p = 0.97$) or MD values in any DTI ROIs among the three time points. In addition, TBSS analysis found no significant differences in MD between T1 and T0 or between T2 and T1. However, there were significant decreases in MD in the voxel clusters (>1000 voxels), including right anterior corona radiata, right inferior fronto-occipital fasciculus, corticospinal tract, left uncinate fasciculus, anterior thalamic radiation at T2 compared to T0 ($p < 0.05$, FWE-corrected) whereas no significant increases were found.

The correlation analysis found that the whole FA change from T0 to T1 was negatively correlated with the change in the sedative score from T0 to T1 ($r = -0.51$, $p = 0.03$). This finding did not survive after the multiple comparison correction. The whole FA change between T0 and T2 was not associated with the sedative score change between T0 and T2. No significant correlation was found between the change in the whole FA values and the stimulus or physical score changes ($p's > 0.05$). No significant association was found between whole MD changes and changes in behavioral score changes from T0 to T1 or from T0 to T2. No correlation was shown between baseline FA/MD value and the FA/MD value changes or behavioral score changes from T0 to T1 or T0 to T2.

Conclusions: We found an acute increase in white matter integrity after the modest dose of single alcohol intake. Further research is needed to identify the effect of repeated administration of a modest amount of alcohol on brain structure in the long run.

Keywords: Alcohol, Diffusion Tensor Imaging, Modest Dose, Acute Effect

Disclosure: Nothing to disclose.

P688. Glucocorticoid Receptors in the Orbitofrontal Cortex Mediate Cocaine-Induced Response Biases

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Background: Many addictive drugs, including cocaine, increase circulating stress hormones. Additionally, addictive drugs and corticosterone (CORT) weaken the ability of organisms to select actions in a flexible manner (causing a reliance on habitual behavior), and loss of dendritic spine densities on excitatory neurons in the ventrolateral orbitofrontal cortex (VLO) is common. We hypothesize that cocaine causes decision-making biases by increasing circulating stress hormone levels, activating low-affinity glucocorticoid receptors (GR), ultimately leading to dendritic spine loss in the VLO.

Methods: Here we used pharmacological and site-selective gene silencing strategies to reduce Nr3c1, the gene for GR, in male and female mice. To quantify action selection strategies, mice were trained to generate two distinct responses for food, then required to update response strategies when one behavior was no longer reinforced. We used Thy1-driven YFP for the visualization of layer V excitatory neurons in the VLO. We then used Fos2A-iCreER (TRAP2) transgenic mice to express excitatory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in neuronal ensembles in the OFC when mice must update the association between an action and its outcome.

Results: Cocaine increased circulating CORT, and exogenous CORT exposure was sufficient to disrupt flexible behavior. Cocaine had the same effects, while inhibiting CORT synthesis blocked cocaine-induced response biases. Reducing Nr3c1 in the VLO also protects against cocaine-induced response biases, concurrent with dendritic spine modifications on excitatory deep-layer neurons. Activating neuronal ensembles involved during memory updating in cocaine-exposed mice rescued flexible behavior.

Conclusions: Our findings indicate that cocaine-induced decision-making biases are driven by repeated activation of GRs in the VLO. Additionally, cocaine-induced spine loss may be rescued by GR reduction. Finally, we found that cocaine impedes the ability of OFC neurons to stably store action-outcome memories, thus destabilizing contingency learning.

Keywords: Stress Hormones, Orbitofrontal Cortex, Cocaine, Rodents, Dendritic Spines

Disclosure: Nothing to disclose.

P689. Scarcity Early in Life Alters Both 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. In our laboratory, we use the limited bedding and nesting (LBN) to model mild adversity. Our prior research discovered LBN reduces morphine self-administration in adult males and decreases impulsive choice and risk-taking behavior in the probability discounting (PD) task. 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 beginning to explore 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 (9AM) and ended 6 hours late (3PM) for 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 sequences 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 on day 1 ($F(1,18)=25.94$, $p < 0.0001$). However, there was no difference in lever pressing between LBN and control rats in males ($F(1,18)=.325$, $p = 0.5757$) or females ($F(1,20)=.217$, $p = 0.646$). 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 morphine-taking 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 drug taking in males, but there is no effect on cocaine drug-taking. Furthermore, LBN does not affect cue-driven incubation of morphine craving. LBN also induces sex-specific patterns of gene transcription within the BLA.

Keywords: Basolateral Amygdala, Cocaine, Morphine, Transcriptome

Disclosure: Nothing to disclose.

P690. The Endogenous Ghrelin Antagonist LEAP-2 is Modulated by Alcohol Exposure and Pharmacological Manipulations of the Ghrelin System: Findings From Human Laboratory Studies

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Background: Investigating the neurobiological and pathophysiological mechanisms of excessive alcohol consumption is critical for developing new medications for AUD. One promising area of research is the gut-brain axis and its related endocrine pathways, which may play important roles not only in control of homeostatic and hedonic eating but also in reward processing and addiction. The stomach-derived 'hunger hormone' ghrelin is a key regulator of metabolism, food intake and reward. Consistent with preclinical research, human studies show that ghrelin levels decrease after acute alcohol administration and are positively associated with alcohol craving. Ghrelin administration increases cue-induced alcohol craving and alcohol self-administration, while preliminary human work suggests that the ghrelin receptor (GHS-R1a) inverse agonist PF-5190457 reduces alcohol cue-elicited craving. The liver expressed antimicrobial peptide-2 (LEAP-2) was recently discovered as an endogenous antagonist of the GHS-R1a. LEAP-2, produced predominantly in the liver, jejunum, and duodenum, is a key member of the ghrelin system and LEAP-2:acyl-ghrelin molar ratio is a marker of GHS-R1a function. In preclinical experiments, LEAP-2 administration inhibits ghrelin-induced food intake and growth hormone (GH) release. In humans, contrary results show LEAP-2 administration, decreases food intake and GH levels. Given the close link between alcohol consumption and the ghrelin system, our aim was to investigate LEAP-2 levels and the LEAP-2:acyl-ghrelin molar ratio, in relation to alcohol consumption, ghrelin administration and inverse agonism of GHS-R1a.

Methods: We measured blood LEAP-2 and acyl-ghrelin concentrations via enzyme-linked immunosorbent assay (ELISA) in AUD patients enrolled in three placebo-controlled, human laboratory studies. Study 1 ($n = 18$; NCT01779024) was randomized, crossover, double-blind, placebo-controlled study, and included concomitant intravenous (IV) ghrelin administration and IV alcohol self-administration. Study 2 ($n = 11$; NCT02039349) was a single-blind, within-subject, placebo-controlled, Phase 1b human laboratory study, with oral PF-5190457 (50 mg BID and 100 mg BID) treatment and an oral alcohol challenge. We analyzed LEAP-2 levels with 100 mg BID treatment only in order to make the analyses consistent across Study 2 and 3. Study 3 ($n = 32$; NCT02707055) was a randomized, crossover, double-blind, placebo-controlled Phase 2a study, and included dosing with PF-5190457 (100 mg BID) without alcohol co-administration for up to 14 days. For statistical analysis, we used linear mixed modeling with covariates including baseline concentration, age, sex, body mass index, breath alcohol concentration (BrAC) and acyl-ghrelin level. Bonferroni correction was used to control for multiple comparisons during post hoc analysis.

Results: In Study 1, we established that IV ghrelin administration increases LEAP-2 levels ($p < 0.001$). Under placebo conditions, IV alcohol self-administration does not change LEAP-2 levels significantly, but reduces acyl-ghrelin, resulting in a higher LEAP-2:acyl-ghrelin ratio ($p < 0.05$). Furthermore, acyl-ghrelin levels correlated positively ($r = 0.228$, $p = 0.035$) with LEAP-2, and sex was found to be a significant covariate ($p = 0.016$), with females having higher LEAP-2 levels. In Study 2, we found that LEAP-2 decreases 90 minutes after oral alcohol intake ($p < 0.05$), but ghrelin receptor inverse agonist, PF-5190457 diminishes the effect of alcohol. Consistent with Study 1 results, LEAP-2:acyl-ghrelin ratio increased after oral alcohol administration ($p < 0.001$), because acyl-ghrelin showed a more expressed decrease than LEAP-2. In Study 3, PF-5190457 decreases LEAP-2 levels ($p < 0.001$) and LEAP-2:acyl-ghrelin ratio ($p < 0.05$), when administered for a minimum of 6 days. Interestingly, PF-5190457 administration led to a pronounced decrease of LEAP-2 levels after the first day of dosing, however LEAP-2 showed a compensation via a gradual, modest increase from the minimal levels on the second day of treatment.

Conclusions: In summary, we provide the first description that IV and acute oral alcohol administration results in a higher LEAP-

2:acyl-ghrelin ratio, suggesting an inhibition of GHS-R1a. This is congruent with earlier results showing lower acyl-ghrelin levels after acute alcohol administration. IV ghrelin, co-administered with IV alcohol, increased LEAP-2 levels, while GHS-R1a inverse agonism reduced LEAP-2 and LEAP-2:acyl-ghrelin ratio, and inhibited alcohol's effect on LEAP-2 levels. These results suggest a compensatory interplay between the endogenous ligand (agonist) and antagonist of the GHS-R1a, namely acyl-ghrelin and LEAP-2. Our findings are highlight the complex intersect between the ghrelin system and AUD toward the development of new potential therapeutical targets.

Keywords: Ghrelin, Alcohol and Substance Use Disorders, Alcohol Consumption, IV Alcohol

Disclosure: Nothing to disclose.

P691. Depletion of the Gut Microbiome Influences Morphine Reward and Medial Prefrontal Cortex Gene Expression in an Age-Dependent Manner in Males and Females

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Background: Adolescence is a period of significant development and corresponds to a time of high rates of onset of mental health conditions. In addition to maturation of several crucial brain areas such as the medial prefrontal cortex (mPFC), there are major changes occurring in organ systems throughout the body – including major shifts in the composition of the gut microbiome. Data from our lab has demonstrated that knockdown of the microbiome influences cocaine and opioid reward as well as gene expression in the nucleus accumbens. Yet, it is unknown if perturbation of the microbiome might influence opioid reward and gene expression in an age-dependent manner, since both the microbiome and the brain are undergoing marked changes during this time. The current set of studies investigated the role of the microbiome on morphine reward and gene expression in the mPFC in adolescent and adult mice.

Methods: To test whether microbiome depletion would influence morphine reward in an age-dependent manner, adolescent and adult male and female C57BL/6 J mice ($n = 5$-6 males, $n = 7$-8 females) were given a cocktail of broad-spectrum antibiotics (Abx) in their drinking water for 5 days before the start of behavior. This Abx treatment regimen is shorter than what has been utilized by the lab in the past and was chosen due to the short time that mice remain in adolescence. This was followed by a five-day morphine conditioned place preference (CPP) paradigm at 5.6 and 10 mg/kg morphine. Data was analyzed separately by age using two-way ANOVAs with morphine dose and Abx treatment as factors. Additional planned pairwise comparisons were conducted to assess the effects of Abx within sex and dose and were corrected for multiple comparisons.

Experiment 2 used a separate group of mice to determine the effects of microbiome knockdown on gene expression in the mPFC. For this study, adolescent and adult male and female C57BL/6 J mice ($n = 4$-5) were placed on Abx for five days before receiving daily injections of 20 mg/kg morphine or saline (s.c.) for five days. This generated four treatment groups within each age and sex: H2O Sal (control), Abx Sal, H2O Mor, and Abx Mor. One hour after their last injection, mice were euthanized and their mPFC and cecal contents were removed and flash frozen. DNA was extracted from cecal contents using Qiagen DNA PowerSoil kit before bacterial 16 s sequencing on an Illumina MiSeq. The number of observed taxonomic units was used to assess microbiome diversity; this was analyzed using separate two-way ANOVAs with treatment group and age as factors. Principle component analysis using Unifrac dissimilarity was used to determine microbiome similarity across groups. RNA was isolated from whole mPFC tissue using Qiagen RNeasy kit and was sequenced using an Illumina HiSeq2500. Differentially expressed genes were identified using t-tests with an FDR-corrected $p < 0.05$ against each groups' age- and sex-matched control (H2O Sal). Functional pathways were identified using G:profiler and Ingenuity Pathway Analysis.

Results: Microbiome knockdown reduced morphine place preference in adolescents (Abx effect: $F(1, 47) = 4.356$, $p = 0.0423$, no other significant effects), but not adults (Abx effect: $F(1, 51) = 0.3204$, $p = 0.5739$, significant effect of dose $F(1, 51) = 7.218$, $p = 0.0097$). Planned comparisons indicated that adolescent females with a reduced microbiome demonstrated a place aversion at 10 mg/kg morphine ($p = 0.034$) but adult females were not affected by Abx ($p = 0.2584$). Adolescent males demonstrated reduced place preference at both doses ($p = 0.0232$), but adult males did not ($p = 0.5626$). While there were some interesting sex-specific effects, taken together adolescents showed a significant reduction in morphine place preference that was not demonstrated by adults, suggesting that adolescents are more sensitive to microbiome disruption than adults.

For experiment 2, 16 s analysis confirmed that adolescent and adult male mice, but not female mice had unique microbiomes at baseline. However, males and females of both ages demonstrated shifts in the microbiome compositions after Abx, this was confirmed by reductions in microbiome diversity in both males and females (males: main effect of group: $F(3, 31) = 45.39$, $p < 0.0001$, interaction: $F(3, 31) = 6.604$, $p = 0.0014$, no age effect; females: main effect of group: $F(3, 30) = 99.7$, $p < 0.001$, interaction: $F(3, 30) = 19.94$, $p < 0.0001$, age: $F(1, 30) = 83.40$, $p < 0.0001$).

Finally, in these same groups of mice, the combination of microbiome reduction and morphine treatment had the greatest effect on gene expression in the mPFC in both males and females at both ages. Abx Mor adolescents had a larger number of differentially regulated genes than their sex- and treatment-matched adults. Thus, like that observed in CPP, adolescents were more sensitive to the effects of microbiome depletion on gene expression in the mPFC. Pathway analysis identified more terms related to histone modification in male adolescents than adults, suggesting that alterations to chromatin structure could contribute to the age-specific effects on behavior and gene expression observed here.

Conclusions: This series of studies demonstrated that adolescents are more sensitive to brief microbiome disruption than adults- observed effects were seen in both behavior and gene expression in the mPFC. Further work will demonstrate whether microbiome manipulations during the adolescent period can have long lasting effects on brain and behavior into adulthood.

Keywords: Adolescence, Opioid, Microbiome

Disclosure: Nothing to disclose.

P692. Abstinence From Cocaine Self-Administration Drives Microglial Phagocytosis of Astrocytes

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Background: Prolonged abstinence from drugs of abuse, such as cocaine, produces lasting physiological alterations in the brain, many of which prime the system for susceptibility to relapse. These alterations are not exclusive to neurons; two critical non-

neuronal cell types- astrocytes and microglia- are also affected both by exposure to cocaine and duration of abstinence.

We have reported that prolonged abstinence following long-access (6 h/day) cocaine self-administration leads to a pronounced reduction (~40%) in nucleus accumbens (NAc) astrocyte volume and synaptic colocalization, suggesting significant impairment in the ability of these pathological astrocytes to appropriately regulate synaptic function (Kim et al., 2022.04.06.487393). Separately, reports in the literature indicate that cocaine can directly trigger TLR4-dependent microglial activation. As professional phagocytes, microglia are appreciated for their ability to phagocytose dead or stressed cells and prune synapses in both homeostasis and disease. Thus, we hypothesized that cocaine induces aberrant microglial phagocytosis of astrocyte processes resulting in astrocyte dysfunction during prolonged abstinence.

Methods: To test this hypothesis, we implanted indwelling jugular catheters and microinjected AAV-GfaABC1D-Lck-GFP virus (1 µL/hemisphere) into the NAc of adult male Sprague-Dawley rats for later quantification of astrocyte morphology. After 5 days of recovery, animals underwent 10 days of long-access (6 h/day) cocaine or saline self-administration, followed by either 1 or 45 days of home cage abstinence. We used confocal microscopy and three-dimensional modeling to determine the extent to which astrocyte membranes were internalized to microglia across abstinence (day 1 vs day 45).

In a separate experiment, adult male Sprague-Dawley rats underwent 10 days of long-access (6 h/day) cocaine self-administration, followed by 30 days of home cage abstinence. Prior to self-administration, all rats received intra-NAc microinjection of AAV5-GfaABC1D-Lck-GFP virus (1 µL/hemisphere). Beginning on abstinence day 1, rats received intra-NAc infusion of neutrophil inhibitory factor (NIF) peptide (0.5 µL/hemisphere) or vehicle every 7 days to block microglial phagocytosis throughout abstinence (4 microinjections total). On abstinence day 30, all rats were tested for cue-primed drug seeking behavior, to determine whether blocking microglia phagocytosis would reduce drug seeking.

Results: Microglia from cocaine self-administering rats contained significantly more GFP + inclusions compared to saline controls on abstinence day 45 ($p < 0.001$). This corresponded with an increased colocalization of both the lysosomal marker CD68 ($p < 0.01$) and separately the "eat-me" signal C3 ($p < 0.01$) in microglia from cocaine self-administering rats at this time point. Moreover, we found that NIF peptide-treatment reduced lever-pressing ($p < 0.05$) compared to vehicle-treated controls. Studies are currently underway to determine whether these measures of astrocyte engulfment are present during early abstinence (day 1) and the extent to which NIF peptide-treatment normalizes astrocyte volume and synaptic localization.

Conclusions: These findings indicate that NAc microglia become highly phagocytic and engulf astrocyte processes following cocaine self-administration. Astrocytic pruning occurs throughout prolonged abstinence, which we predict is related to the previously observed astrocyte dysfunction. Drug seeking behavior is reduced by inhibiting phagocytosis during abstinence, which highlights the utility of targeting the neuroimmune system for potential therapeutic interventions.

Keywords: Microglia, Astrocytes, Cocaine Self-Administration, Abstinence, Cocaine-Seeking Behavior

Disclosure: Nothing to disclose.

P693. Chronic Progesterone Treatment Reduces Nicotine Consumption and Accumbens Microglial Activation in a Sex-Specific Fashion

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Background: Nicotine use is a substantial burden to public health. Further, craving and relapse to smoking appear to vary as a function of sex. In women, increases in 17β-estradiol (E2) and progesterone (P4) are associated with addiction vulnerability and resilience, respectively. P4 has been examined clinically as a smoking cessation agent and shows promise for women. However, studies incorporating men have shown unclear or limited to no efficacy. To date, there are no studies evaluating the mechanisms by which P4 may yield sex-specific efficacy, and no studies have been conducted to model the effects of P4 in a nicotine rodent model. Further, steroid hormones including P4 have anti-inflammatory properties. We found that neuroimmune signaling within the nucleus accumbens core (NAcore) is driven by nicotine seeking and consumption in a sex-specific fashion, whereby female rats are more susceptible to nicotine-induced neuroimmune consequences as compared to males. However, it is not yet known if P4 can reduce nicotine-induced neuroinflammatory signaling mechanisms.

Methods: Gonad-intact male and female Long Evans rats ($N = 6$-8/group) underwent nicotine self-administration (0.06 mg/kg/infusion) for 10 sessions, followed by 15 sessions of nicotine self-administration in which rats received either daily systemic P4 (1.75 mg/kg, SC in 0.1 mL sesame oil) or vehicle (0.1 mL sesame oil) 2 h prior to sessions. Rats were then perfused, and NAcore microglia were quantified via 3DMorph. ANOVA or t-tests were used for statistical analyses. Uterine horns from females were also dissected to confirm hormonal treatments.

Results: Here, we show that daily P4 treatments decreased nicotine consumption in female (but not male) rats (ANOVA, $p < 0.05$). We further show that in females, P4 reduced nicotine-induced NAcore microglial activation as compared to vehicle, measured by increased territorial volume ($p < 0.05$) and % occupied volume ($p < 0.05$), indicating that P4 reduces nicotine-induced activation of microglia. Male NAcore microglial morphology is currently being evaluated. Finally, uterine horns were significantly lighter in weight (g) in P4 as compared to vehicle-treated females, demonstrating that P4 treatment impacted the female reproductive system.

Conclusions: Together, inhibiting nicotine-induced chronic activation of NAcore microglia may underlie the sex-specific therapeutic efficacy of P4 seen clinically. However, given that P4 does not reduce smoking in men, our results may justify examination of other possible therapeutics that have anti-inflammatory properties for smoking cessation, which may yield higher efficacy across both biological sexes. Next, we will test necessity of P4 to inhibit NAcore neuroimmune activation to exert therapeutic effects via our newly validated chemogenetic approach.

Keywords: Microglial Activation, Nicotine Addiction, Nucleus Accumbens Core, Progesterone, Sex Differences

Disclosure: Nothing to disclose.

P694. Microglial Acid-Sensing Regulates Ethanol Consumption and Respiratory Depression in Mice

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Background: Alcohol use disorders (AUDs) are prevalent and debilitating. Alcohol has a wide range of effects on the body, engaging systems important for regulation of behaviors, emotions and physiology, all of which all contribute to the cycle of alcohol

use and abuse. Alcohol use represents a potent threat to physiological homeostasis (e.g. neutral pH) through its ability to induce acidosis and neuroinflammation. Maintaining physiological homeostasis is critical for an organism's survival, and threats to homeostasis elicit behavioral, emotional and physiological responses directed toward this goal. Acidosis appears shortly after alcohol use, can last up to 24 hours in plasma and brain, and is positively correlated with withdrawal severity. Most alcohol research has focused on mechanisms underlying the rewarding and aversive aspects of alcohol use as a motivated behavior. However, alcohol's effects on behavior and physiology could also result from a drive to restore pH homeostasis. Therefore, the role of acid-sensing in regulating behavioral and physiological responses to alcohol, and the specific acid-sensing receptors mediating these effects, need to be investigated.

A prominent acid sensor is the T-cell death associated gene 8 (TDAG8) receptor, located on microglia. Our lab recently identified a role for TDAG8 in panic-relevant behavior and physiology. TDAG8 mediates these effects through neuroinflammatory signaling within the subfornical organ (SFO). The SFO is a sensory circumventricular organ that has a leaky blood brain barrier and helps maintain physiological homeostasis by regulating behaviors, respiration and cardiovascular function. Lesion studies show SFO also regulates ethanol consumption, likely though its projections to other regions regulating ethanol consumption like the bed nucleus of the stria terminalis and central amygdala. Given the high comorbidity between panic disorder and AUDs, and the growing evidence for neuroinflammatory regulation of alcohol use/abuse, is it likely that TDAG8 activation in SFO may be a novel neuroimmune mediator of behavioral and physiological responses to alcohol use and regulator of alcohol consumption.

To determine whether TDAG8 is a shared mechanism across panic and AUD, we tested the hypothesis that TDAG8 knockout would reduce voluntary ethanol consumption and microglial activation in the SFO. As acidosis is a respiratory stimulant, we also tested the hypothesis that TDAG8 knockout would exacerbate respiratory responses to ethanol.

Methods: Male and female TDAG8 knockout (TDAG8-/-) or wild-type (TDAG8+/+) littermates were used (8-16 weeks old; $n = 5$-12). To determine if ethanol increases TDAG8 expression, TDAG8-promoter driven GFP expression was quantified by immunohistochemistry (IHC; anti-GFP) in transgenic mice 24 h after injection with 2 g/kg ethanol. To examine the effect of TDAG8 on voluntary ethanol consumption, we used the 4-day drinking in the dark (DID) paradigm. We used the same DID procedure to measure sucrose consumption, water consumption and ethanol consumption by separating the tests by one week. Mice were sacrificed 90 m after the last DID procedure (ethanol exposure). To determine effects of TDAG8 on ethanol-evoked neuroinflammation, fixed brain tissue was stained for microglial marker IBA-1 and microglia soma perimeter was then quantified. In separate cohorts of mice, ethanol's effects on locomotion and respiration were examined using the open field test (locomotor stimulation, 1.25 g/kg ethanol), balance beam test (ataxia, 2 g/kg ethanol) and plethysmography (respiratory depression; 4 g/kg ethanol), respectively. Statistical analysis was preformed using Students t test, ANOVA, 2-way ANOVA or 3-Way repeated ANOVA as needed.

Results: Ethanol increased TDAG8-promoter driven GFP expression within the SFO ($p < 0.05$), but not another circumventricular organ expressing TDAG8, the OVLT. During DID, both male and female TDAG8 deficient mice drank less ethanol than their WT littermates (genotype $p < 0.05$). There was no effect on sucrose or water consumption. TDAG8-/- mice also had reduced microglial soma perimeters compared to TDAG8 + / + mice ($p < 0.05$), which was specific to the SFO. Genotype had no effect on ethanol-evoked locomotor stimulation, but knockout animals demonstrated increased footslips on the balance beam test ($p < 0.05$). TDAG8-/- mice also had increased respiratory depression in response to a high dose of ethanol ($p < 0.05$).

Conclusions: Together, these data point to acid-sensing receptor TDAG8 as a novel regulator of ethanol consumption and aversive outcomes (ataxia, respiratory depression) associated with alcohol use. Further, they suggest that these effects could be mediated through neuroimmune signaling within the SFO. Given the association of TDAG8 with both AUD- and panic-associated outcomes, this suggests SFO-TDAG8 and associated neuroimmune effectors may provide a unique and novel shared mechanism for AUD and panic disorder. Overall, these data support the idea that some behavioral and physiological responses to alcohol use may result from a drive to restore physiological homeostasis after a pH challenge. They also suggest acid-sensing receptors may represent a novel treatment target for alcohol use disorders.

Keywords: Alcohol Use Disorder, Mouse Models, Neuroinflammation, Acid-Sensing

Disclosure: PsyBio Therapeutics, Inc: Grant (Spouse).

P695. Distinct Endothelial and Neuroimmune Responses in the Prefrontal Cortex is Associated With Relapse to Drinking in Alcohol Dependent Female and Male Rats

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Background: The prefrontal cortex (PFC) is important for the development of alcohol addiction. Neuronal activity in the PFC is regulated by immune responses, and neuroimmune 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 neuroimmune response and BBB integrity in the PFC have been minimally explored. Moreover, the sex 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 and male rats were made ethanol dependent by chronic intermittent ethanol vapor (CIE) and ethanol drinking (ED) procedure. Rats were euthanized after relapse session and plasma isolated from trunk blood and brain tissue homogenate of the PFC were analyzed for 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 VE-cadherin via Western blotting and VEGF by ELISA. This allowed us to compare sex differences in the levels of individual cytokines as well as associations among cytokines and expression of tight junction proteins between the plasma and PFC.

Results: CIE increased ED in both female and male rats, with females having higher ethanol consumption during CIE and relapse to ethanol drinking sessions compared with males. In parallel, CIE females had higher levels of interferon-γ (IFN-γ), interleukin-6 (IL-6), IL-10 and VEGF in the plasma compared to CIE males. CIE females had higher levels of TNF-α and VEGF in the PFC compared with control females. However, CIE males had lower levels of IFN-γ, IL-1β and IL-4 compared with control males. Furthermore, in the PFC, abstinence reduced the expression of VE-cadherin in both sexes. However, relapse to drinking increased levels of VE-cadherin in CIE females beyond the control condition and this effect was not observed in CIE males. Claudin-5 expression was not altered in both sexes.

Conclusions: These results reveal significant sex differences in pro- and anti-inflammatory cytokine levels in plasma and PFC that

were associated with BBB disruption after relapse to ethanol drinking, and emphasize their possible role in alcohol addiction.

Keywords: Alcohol Dependence, Neuroimmune Activation, Blood-Brain Barrier

Disclosure: Nothing to disclose.

P696. Preliminary Evidence for an Elevated Neuroinflammatory Signal in Opioid Use Disorder: Findings from a PET TSPO Imaging Study

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Background: Preclinical studies show that repeated opioid administration is associated with elevated levels of glial markers, e.g., Iba1 and GFAP, in the rodent brain. Postmortem analyses of deceased opioid users show elevated levels of glial markers and alterations in neuroinflammatory signaling pathways. Yet, direct evidence that chronic opioid use may be neuroinflammatory in living individuals with opioid use disorder (OUD) has not been published to date. In our previous work, we showed that a single morphine dose acutely increased 18 kDa translocator protein (TSPO) availability, a neuroimmune marker, by 25-32% across brain regions in healthy adult volunteers via a [11 C]PBR28 Positron Emission Tomography (PET) imaging study. In the current study, we build on this work to investigate neuroimmune status of OUD patients and well-matched control subjects using [11 C] PBR28 PET imaging.

Methods: Individuals currently using opioids and who met DSM5 criteria for OUD ($n = 6$) were recruited locally, admitted to a locked inpatient unit, and inducted on buprenorphine. Individuals with OUD were titrated to an individualized dose and stabilized for 5+ days to achieve steady-state plasma buprenorphine levels prior to a 120-minute [11 C]PBR28 PET scan. During the inpatient stay, non-medical opioid abstinence was verified with daily urine screens. Concurrently, healthy control subjects ($n = 18$) were recruited, screened, and underwent identical [11 C]PBR28 PET scanning procedures. On scan day, subjects also completed a computerized cognitive battery, self-report measures, and provided a plasma sample (assayed for biomarkers). During each PET scan, arterial blood was acquired to measure the metabolite-corrected arterial input function. Total volume of distribution (VT), i.e., TSPO availability, was estimated in 10 brain regions of interest (ROIs) using multilinear analysis-1 (MA-1; t*=30). Group differences in regional [11 C]PBR28 VT were evaluated using linear mixed effects models with rs6971 genotype ('high' vs. 'moderate' affinity binders) and diagnostic group (OUD vs. controls) entered as fixed factors, and regional VT as the within-subject repeated factor. Partial correlations, controlling for rs6971 genotype, investigated relationships between regional [11 C]PBR28 VT and subjective and behavioral data among OUD patients.

Results: Groups were well-matched for age, gender, ethnicity, body weight, body mass index, rs6971 genotype, and cigarette smoking status (ps > 0.15). Individuals with OUD were 32.0 ± 6.2 years old, 50% female, mostly white (83.3%), and reported using 6.3 ± 5.2 bags of heroin per day for 6.7 ± 2.1 years on average. Four individuals with OUD reported intranasal heroin use, whereas two reported injection use. Mean length of inpatient stay was 12.3 days (range: 10-15 days) and mean daily buprenorphine dose during stabilization was 7.7 mg (range: 4-16 mg).

Linear mixed effect models showed that individuals with OUD exhibited significantly higher TSPO availability by 20% on average (13-24% across ROIs), relative to controls, $F(1,236)=5.06$, $p = 0.036$. Partial correlations, controlling for rs6971 genotype, indicated that longer inpatient stay prior to scanning, i.e., longer opioid abstinence and time in buprenorphine treatment, was correlated with lower TSPO availability in the prefrontal cortex (PFC; $r = -0.99$, $p = 0.001$). Also, partial correlations, controlling for rs6971 genotype, indicated that lower TSPO availability in the PFC was 'trend-level' correlated with less opioid craving on scan day ($r = 0.80$, $p = 0.059$). Consistent with the literature, individuals with OUD generally exhibited lower levels of peripheral cytokines/chemokines than controls with GM-CSF and IL-8 reaching statistical significance (ps < 0.05; TNF-α and IL-10 were 'trend-level': ps<0.10). Finally, there were no group differences in cognitive task performance (ps > 0.05).

Conclusions: Our preliminary findings show the first in vivo evidence that individuals with OUD, in the first 1-2 weeks of treatment, exhibit elevated levels of neuroimmune signaling as evidenced by PET TSPO imaging. Longer inpatient stay prior to scanning was correlated with lower, i.e., more 'normal', TSPO levels in the PFC, suggestive of neuroimmune recovery with treatment. Further, lower TSPO levels in the PFC were 'trend-level' correlated with less subjective opioid craving. Taken together, our findings suggest that individuals with OUD may exhibit a neuroinflammatory phenotype upon entry to treatment, and that neuroimmune recovery may be possible with prolonged opioid abstinence which may translate to less opioid craving. Our findings highlight the neuroimmune system as a potential therapeutic target in OUD.

Keywords: Opioid Use Disorder, Neuroinflammation, PET Imaging, Neuroimmune Mechanisms, Buprenorphine Maintenance

Disclosure: Nothing to disclose.

P697. Influence of Sleep Quality on Alcohol Use During a Quit Attempt Among Treatment-Seeking Individuals With Alcohol Use Disorder

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Background: Sleep problems are highly prevalent among individuals with alcohol use disorder (AUD). The relationship between alcohol use and sleep quality is conceptualized as bidirectional, in that sleep problems can be induced by chronic alcohol consumption and alcohol may be used to self-medicate sleep problems. Sleep difficulties may begin during early stages of abstinence from alcohol and commonly persist into long-term abstinence. The occurrence of sleep difficulties is clinically important given that sleep difficulties commonly precede alcohol relapse. Although there is established research on sleep difficulties as a risk factor for relapse, research focused on early stages of abstinence are lacking. Thus, the present study tests the relationship between sleep quality and alcohol use during a quit attempt in treatment-seeking individuals with an AUD.

Methods: The present study utilized data from a two-week early efficacy paradigm trial to screen medications for AUD using naltrexone and varenicline. Fifty-three eligible participants were randomized to either naltrexone ($n = 15$), varenicline ($n = 19$), or matched placebo ($n = 19$). Randomized participants reported on their alcohol use during the 30-days prior to randomization using the Timeline Followback and their sleep quality using the Pittsburgh Sleep Quality Index (PSQI). Following a weeklong medication titration period, participants were asked to attend an in-person visit on Day 8 to begin a 7-day practice quit attempt and complete electronic daily dairy assessments (DDA) to report on previous day alcohol consumption, sleep quality, mood, and

craving. On Day 14, participants reported on their alcohol consumption and sleep quality. A logistic regression analysis was conducted to test the relationship between baseline sleep quality (continuous variable) and abstinence (binary variable: abstinent versus not abstinent) during the practice quit attempt. A simple linear regression analysis was conducted to investigate the relationship between baseline sleep quality and total drinks during the 7-day practice quit.

Results: An unadjusted logistic regression analysis revealed no relationship between baseline sleep quality and abstinence during the practice quit attempt, B = (-.009), SE = 0.012, Wald = 0.541, $p < .462$. An unadjusted linear regression analysis showed that baseline sleep quality was not associated with total drinks during the practice quit attempt, B = −.025, SE = 0.044, p = 0.575.

Conclusions: While the bidirectional relationship between sleep quality and alcohol drinking is well documented, these preliminary results suggest that sleep quality is not associated with drinking behavior during the practice quit attempt among treatment-seeking individuals with AUD. Future statistical analyses are underway testing alcohol craving and negative mood as moderators of the relationship between sleep quality and drinking behaviors during the practice quit attempt.

Keywords: Alcohol Dependence, Sleep Disturbances, Alcohol Abstinence, Drinking

Disclosure: Nothing to disclose.

P698. Transcranial Magnetic Stimulation of Dorsolateral Prefrontal Cortex Reduces Craving and Increases Salience Network Connectivity in People Who Smoke Cigarettes

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Background: Repetitive Transcranial Magnetic Stimulation (rTMS), a noninvasive brain stimulation technique, was recently approved by the FDA as a treatment for Tobacco Use Disorder. Although the efficacy of rTMS as a smoking cessation treatment has been demonstrated, the mechanism by which this occurs is not known, and such mechanistic information could improve and optimize treatment outcomes. This data reported here are part of a registered clinical trial, NCT03827265.

Methods: Thirty-two people who smoke cigarettes daily were included. Before data collection, each was asked to remain abstinent from smoking cigarettes overnight (or at least 12 hours prior to stimulation) to heighten cigarette craving, and abstinence was verified by a carbon monoxide level of <10 ppm in expired air. Participants received 3,000 pulses of 10-Hz rTMS (5-second trains with 10-second inter-train intervals over 15 minutes) delivered to the left dorsolateral prefrontal cortex. They were not presented with any smoking-related or craving-inducing cues, and instead were instructed to relax and remain still. Spontaneous craving symptoms were measured by self-report using the Urge to Smoke scale and Shiffman-Jarvik Withdrawal Scale (craving subscale) before and after rTMS, and resting-state functional connectivity was measured by BOLD fMRI. Independent component analysis was used to identify the default mode and salience networks, and connectivity maps were compared before and after rTMS.

Results: Single-session rTMS significantly reduced self-reported craving measurements on both the Urge to Smoke and Shiffman-Jarvik Withdrawal scales (Urge to Smoke: p = 0.01, d = 0.3; Shiffman-Jarvik: p = 0.02, d = 0.39). Effects of rTMS on default mode network connectivity did not survive familywise error correction, but robust and widespread effects on salience network connectivity were found. rTMS significantly increased connectivity between the salience network and clusters with maximum intensity in primary visual cortex (k = 8,551), right putamen (k = 7,020), posterior cingulate cortex (k = 556), supplementary motor area (k = 117), left putamen (k = 52), sensorimotor cortex (k = 47), thalamus (k = 45), anterior cingulate cortex (k = 35), and hippocampus (k = 21).

Conclusions: Brain stimulation targeting the dorsolateral prefrontal cortex alters connectivity of the salience network. This finding suggests that the salience network is a justifiable target for future investigations of its usefulness as a biomarker of rTMS efficacy. The salience network also may itself provide clinically useful targets for stimulation to reduce cigarette craving, as its network hubs are accessible with rTMS.

Keywords: Neuromodulation, Non-Invasive Neuromodulation, Resting State Networks

Disclosure: Nothing to disclose.

P699. Impact of a Substance Use Treatment Program for Women on Negative and Positive Urgency and Relationships to Striatal Responsivity to Reward

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Background: Methamphetamines and opioids are among the most commonly used illegal substances, accounting for approximately 10% disability adjusted life years worldwide. The biological processes that contribute to the initiation and maintenance of substance use disorders are still poorly understood. Identifying such processes could help to better develop targets for interventions to improve outcomes of these disorders. Negative and positive urgency, i.e., responding impulsively to experiences of negative and positive affect respectively, have been proposed as behavioral processes contributing to the maintenance of substance use disorders. Altered ventral striatal dopamine release and activity during reward anticipation has been associated with substance use disorders and individual differences in impulsivity. This investigation examined impact of a comprehensive substance use intervention on positive and negative urgency and striatal anticipation to reward to determine whether these processes could be considered targetable disease modifying processes for addiction.

Methods: Participants ($N = 168$, Mean age = 32.71) were women recruited from the Women in Recovery (WiR) program, a court-ordered mental health diversion program for drug-related offenses. WiR includes comprehensive treatment services, including empirically supported treatment for SUD and mental health and access to pharmacological therapy, parenting classes, GED classes, and occupational assistance. WiR participants remain in the treatment program for an average of 17 months. A total of 74% ($N = 125$) of participants successfully graduated from the WiR program; current analysis focused on $N = 75$ women who completed the UPPS-P and functional magnetic resonance imaging (fMRI) at baseline and one-year after enrolling in the WiR program. Participants mean age = 32.73 (SD = 7.23); 69% reported less than or equal to a high school education; 53% and 24% reported methamphetamine and opiates as their drug of choice respectively. Linear mixed models were used to assess changes in (a) the negative and positive urgency sub-facets of the UPPS-P and (b) left and right nucleus accumbens (NAcc) activity during anticipation of reward (+5 vs +0) and loss (-5 vs -0) on the monetary incentive delay task during fMRI. For NAcc activity, average percent signal change was extracted from Brainnetome Atlas NAcc regions of interest. Regression analyses were used to examine relationships between negative/positive urgency and

striatal reactivity at baseline, as well whether the extent of change in striatal reactivity predicted the one-year outcomes in positive and negative urgency (covarying for baseline urgency). Average motion during fMRI, age, and education were also utilized as covariates across all analyses. Study was registered at clinical trials.gov (NCT02601495).

Results: From baseline to one-year outcome, women reported significant decreases in both positive ($F(1, 79) = 19.38$, $p < 0.001$, $d = -0.99$) and negative urgency ($F(1, 80) = 52.89$, $p < 0.001$, $d = -1.63$) but no significant changes were observed for striatal reactivity to reward or loss anticipation (ps > 0.10). While striatal reactivity did not relate to urgency measures at baseline (ps > 0.10), greater increases from baseline to one-year outcome in right and left striatal reactivity during reward anticipation related to greater decreases in positive (right: t(70)= -2.59, $p = 0.012$, $\beta = -5.69$; left: t(70)= -3.03, $p = 0.003$, $\beta = -6.78$) and negative urgency (right: t (70) $= -2.50$, $p = 0.015$, $\beta = -3.86$; left: $t(70) = -2.62$, $p = 0.011$, $\beta = -4.13$).

Conclusions: Results indicate that comprehensive and extended substance use treatment is effective at reducing negative and positive urgency. Although there were no overall changes in NAcc activation to reward or loss anticipation with treatment, results indicate that the extent to which treatment increases engagement of NAcc when anticipating non-drug rewards be an important neural process contributing to changes in positive and negative urgency. Taken together, this longitudinal study of a comprehensive intervention for individuals with substance use disorder supports the idea that positive and negative urgency may be disease modifying processes on a behavioral level that is linked to changes in neural reactivity to reward anticipation. Future longitudinal and experimental research is needed to identify specific treatment strategies that directly target these processes.

Keywords: Striatum, Reward Anticipation, Substance Use Disorder, Functional Magnetic Resonance Imaging, Impulsivity

Disclosure: Nothing to disclose.

P701. Dynamics of Dopamine Increases During Methylphenidate Challenges in Humans Measured With PET

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Background: The dynamics of dopamine (DA) are relevant to reward signaling including drug rewards, but access to methods that quantify DA dynamics in the human brain have been limited. Here, we show that a simple approach using positron emission tomography (PET) can be used to non-invasively assess the dynamics of dopamine increases induced by methylphenidate (MP) in the human striatum. In simulations, we tested the hypothesis that the time-varying differences in standardized uptake value ratio (SUVr) between [11 C]raclopride scans collected with and without a MP-challenge reflect the dynamics of dopamine increases induced by MP in the striatum. To test the hypothesis that the intensity of the 'high' reflects the rate of dopamine increases in the striatum, we carried out a within-subject [11 C]raclopride PET study with a double-blind placebo-controlled design in healthy adults. We studied dynamic dopamine increases using oral-MP (results in slow brain delivery) and intravenous (IV)-MP (results in fast brain delivery) challenges, in association with measured subjective responses to MP using self-reports of 'high' throughout the scan.

Methods: To simulate effects of time-varying DA concentrations on [11 C]raclopride binding of D2/3 receptors we used the linear extension of the simplified reference region model (LSSRM). We tested twenty healthy adults (36.1 ± 9.6 years old; 9 females) who underwent 90-min long PET scans collected in 3 randomly ordered sessions (placebo, oral-MP, and IV-MP) while simultaneously recording their self-reported 'high' ratings (0-10) under resting conditions, using oral-MP and IV-MP. In each session, each participant received an oral pill (60mg-MP or placebo) 30 min before injection of the PET tracer ([11 C]raclopride), followed 30 min after the tracer by an IV administration (0.25 mg/kg-MP or placebo, manually injected as a ~30-second bolus). List mode PET emission data were acquired continuously for 90 min and initiated immediately after manual injection of [11 C]raclopride (dose = 15.7 ± 1.9 mCi; duration 5-10 seconds). A 3-dimensional ordered-subset expectation-maximization (OSEM) algorithm was used to create PET time series consisting of 48 time windows (30 frames of 1 min, followed by 12 frames of 2.5 min, and 6 frames of 5 min). Standardized uptake values (SUVs) for [11 C]raclopride were calculated after normalization for body weight and injected dose and spatially normalized to MNI space. Relative SUV time series, SUVr(t), were computed in MNI space by normalizing each SUV volume by its mean SUV in cerebellum.

Results: In simulated data we found that the dynamics of endogenous DA increases shape the time-varying SUVr changes (delta SUVr) elicited by MP. To assess the sensitivity of DA's time-to-peak (TTP) increase to MP dose we simulated the normal variability of delta SUVr dynamics within and across individuals (1000 simulations). Specifically, random variations in LSSRM parameters (5%) also caused minimal variability in fitted TTP (SD = 0.55 min). Differently, random variations in MP dose (10%), input function, and LSSRM parameters (5%) showed that fitted TTP was significantly associated with variations of the MP dose ($R2 = 0.88$), but not with variations of the other parameters. Similarly, we assessed the association between fitted TTP and the 'true' DA rate TTP using 4% random variability in all parameters (1000 simulations) and found that fitted and 'true' DA rate TTP had excellent correlation ($R2 = 0.94$). These simulations suggest that fitted TTP can accurately predict DA rate TTP [coefficient of variation ~ 0.1]. The feelings of reward elicited by MP in the participants were stronger for IV- than for oral-MP ($P < 0.0002$), and the gamma cumulative distribution function accurately fitted the dynamics of delta SUVr as predicted by our model. Fitted DA release TTP in putamen was significantly correlated with the difference in peak 'high' ratings between MP and placebo, independently for oral-MP and IV-MP, such that shorter TTP was associated with higher self-reports of "high" from MP (oral: R(13)= 0.76, IV: R(18)= 0.69; $P < 0.003$, two-sided).

Conclusions: Dynamic PET is a unique tool to assess the rate of DA increases induced by potentially addictive drugs (or other rewarding stimuli) in the human brain. Drug-related DA dynamic measures can also be used to advance our understanding of their associations with brain function and behavior simultaneously collected with PET-MRI scanners in humans (or laboratory animals). In simulations, we show that time-varying ΔSUVr parallels the dynamics of DA increases induced by MP in the striatum. In a double-blind placebo-controlled PET study with a within-subjects design we show that the intensity of the 'high' reflects the dynamics of DA increases in the striatum. These findings provide strong evidence that the speed of DA increases in the striatum, which is influenced by the rate of drug uptake in the brain and is modulated by the route of drug administration, accounts for why a drug like MP can be used safely for oral ADHD treatment, whereas it can result in addiction when injected. Our findings that the faster the rate of DA increases, the more intense the "high", would also explain why very large oral doses of MP can also be rewarding.

Keywords: Dopamine Release, Methylphenidate, Reward
Disclosure: Nothing to disclose.

P702. Text Mining of Substance Cessation Support Groups on Reddit Reveals Robust Subjective Experience Phenotype Uniquely Enriched for Anxiety, Disgust, and Gratitude

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Background: Online communities and the posts they generate represent an unprecedented resource for studying subjective emotional experiences, capturing population types and sizes not typically available in the laboratory. Here we mined such a platform to explore a putative specificity of the emotional experience of substance cessation and its cross-substance overlap. Revealing transdiagnostic clues that could ultimately be used for mental health outreach was an important motivation for this exploration. Specifically, we aimed to characterize the emotions associated with cessation of three major substances and compare them to emotional experiences reported in non-substance cessation posts.

Methods: Two million pseudonymous posts made respectively in the Fall of 2020 (discovery dataset) and Fall of 2019 (replication) were obtained from 394 forums at Reddit.com. We tracked emotion word frequencies in posts from three substance cessation forums (for alcohol, nicotine, and cannabis topic categories), contrasting them to general forums. Emotion word frequencies were further tracked on multiple distinct categories of emotions and represented as a multidimensional emotion vector for each forum. We quantified the degree of emotional resemblance between different forums by computing cosine similarity on these vectorized representations. For substance cessation posts with self-reported time since last use, we explored changes in the use of emotion words as a function of abstinence duration.

Results: Compared to posts from general forums, substance cessation posts showed more expression of anxiety, disgust, pride, and gratitude words. 'Anxious' emotion words were attenuated for abstinence durations > 100 days compared to shorter durations ($t12 = 3.08$, two-tailed, $P = 0.00930$). The cosine similarity analysis identified an emotion profile preferentially expressed in the cessation posts across substances, with lesser but still prominent similarities to posts about social anxiety and ADHD. These results were replicated in the 2019 (pre-COVID-19) data and were distinct from control analyses using non-emotion words.

Conclusions: We identified a unique subjective experience phenotype of emotions associated with the cessation of three major substance types, replicable across two time periods. We noted changes to this experience as a function of duration of abstinence. Although to a lesser extent, this phenotype quantifiably resembled the emotion phenomenology of other relevant subjective experiences (social anxiety, ADHD). Taken together, these transdiagnostic results suggest a novel approach for future identification of at-risk populations, allowing for the development and deployment of specific and timely interventions.

Keywords: Natural Language Processing (NLP), Addiction Phenotypes, Alcohol, Nicotine Addiction, Cannabis Use
Disclosure: Nothing to disclose.

P703. Effects of Intermittent- vs Long-Access Cocaine Self-Administration on Addiction-Like Behaviors and NAc Glutamatergic Plasticity

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Background: Long Access self-administration procedures (LgA, 6 h + /day) have become the gold-standard in the addiction field because they produce addiction-like behaviors, and changes in brain not seen following shorter drug access that models drug taking (1-2 h/day). However, Zimmer and colleagues recently developed an intermittent access self-administration procedure (IntA) to better model the patterns of cocaine use seen in addicts. IntA produces more robust addiction-like behaviors compared to LgA (cocaine-motivation, reinstatement, and cocaine-seeking) despite much less drug intake. Whether these distinct patterns of cocaine exposure produce similar or different alterations in nucleus accumbens (NAc) function is poorly understood, and there is limited understanding of the behavioral effects of IntA across sex.

Methods: We used whole-cell patch clamping to evaluate how IntA vs LgA cocaine alters NAc excitatory transmission. Given the role of NAc CP-AMPA receptors in cocaine-seeking following LgA, we measured sensitivity to the CP-AMPAR antagonist Naspm, and sEPSCs. We also examined sex differences in IntA effects on psychomotor sensitization, and how increasing the intermittency of self-administration affects motivation for cocaine and psychomotor sensitization using newly established deep-learning based approaches.

Results: Initial results suggest that IntA produces enhancements in NAc glutamate transmission that are distinct from those seen following LgA. IntA produced more robust psychomotor sensitization compared to LgA, and greater sensitization in females than males. Finally, increased intermittency of cocaine use enhanced motivation for the drug.

Conclusions: Overall, the data suggest that IntA may better model addiction and thus provide additional information about translationally relevant targets for the treatment of substance use disorder.

Keywords: Cocaine Addiction, Nucelus Accumbens, Motivation
Disclosure: Nothing to disclose.

P704. Prenatal Circadian Rhythm Disruption Induces Sex-Specific Substance Use and Mood-Related Phenotypes in Mice

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Background: 20% of Americans are at risk for environmental circadian rhythm disruptions (CRD) due to shift work. Shift workers experience substantial negative health outcomes, but females are especially affected with greater vulnerability for substance use (SU) and adverse outcomes associated with pregnancy. These outcomes not only occur during pregnancy, but offspring are affected at birth and later in life. In mice, prenatal CRD (pCRD) recapitulates these risks, increasing adverse pregnancy outcomes and altering behavior in adult offspring. However, it is unknown whether pCRD affects SU in mature offspring.

Methods: C57Bl/6 J dams were sham handled or disrupted, by reversing the light/dark cycle 4 times during gestation. Reward- and mood-related behaviors were measured in adult offspring. Cocaine reward was measured using conditioned place preference (5 mg/kg cocaine). Contingency degradation was used to measure decision making. Mice were trained to respond on two levers for food, then the likelihood that one of those levers will be reinforced was degraded. Another cohort was trained to respond for food before jugular catheterization. After recovery, mice were trained to respond on a different lever for cocaine. Acquisition, the reinforcing and motivational properties of cocaine, extinction and

cue-induced reinstatement were measured. Throughout, 2-way ANOVAs were used for data analysis (disruption group by sex), with additional repeated measures as appropriate (i.e. session, lever, dose). Significant interactions were followed up with Sidak posthoc tests. $p < 0.05$ was considered significant and $p < 0.1$ trending. Samples sizes of 6-14, depending on the experiment.

Results: Interestingly, females exposed to pCRD developed an anhedonic-like phenotype with decreased food self-administration (interaction, $p = 0.007$), cocaine intake (disruption, $p = 0.008$) and reinforcing properties of cocaine (interaction, $p = 0.01$). On the other hand, pCRD males showed a SU-like phenotype with increased cocaine preference (interaction, $p = 0.08$), higher order food self-administration (disruption, $p = 0.099$) and cocaine reinforcement (interaction, $p = 0.08$). Furthermore, while male pCRD mice maintained goal-directed decision making, responding more on a reinforced lever, female pCRD mice did not, indicating habit formation. Together, these results suggest that male and female mice exposed to pCRD respond differently for rewarding outcomes. In order to determine whether these divergent behavioral outcomes are unique to reward I next measured anxiety-like behavior. As expected, preliminary results in the open field test and elevated plus maze (interaction, $p = 0.005$) paralleled reward-related behavior. Anhedonic-like female pCRD mice showed increased anxiety-like behavior and pCRD males showed decreased anxiety/increased risk-taking behavior.

Conclusions: These results suggest that pCRD may predispose individuals to distinct psychiatric disorders based on sex, mood disorders in females and SU disorders in males. By understanding how disrupted rhythms during pregnancy affect behavior in adulthood, we can develop novel therapeutic approaches for SU and mood disorders in adults.

Keywords: Circadian Rhythms, Substance Use, Sex Differences

Disclosure: Nothing to disclose.

P705. Effects of Acute Cannabis Exposure on Simulated Automotive Driving Behavior: A Longitudinal, Double-Blind, Placebo-Controlled Study

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Background: Acute cannabis intoxication may have significant effects on complex day-to-day activities such as automobile driving that require integrating multiple cognitive and psychomotor functions. The recent legalization of both recreational and/or medicinal marijuana in many states has created an urgent need to better understand the dose dependent effects of Tetrahydrocannabinol (THC) on driving behavior. The present study employed a longitudinal, double-blind, placebo with 2 active doses to investigate the differential effects of THC levels in cannabis on a variety of driving-related behaviors in a controlled, naturalistic, simulated environment. The current study attempts to overcome a limitation of prior cannabis driving studies by challenging driving within naturalistic driving scenarios that better approximate real-world risky situations. Based on previous findings from both simulated and real-world driving, we hypothesized subjects to have increased lane deviations. In addition, we postulated that the driving challenges would be relatively more sensitive than prior studies in identifying dose-sensitive drugged driving differences, such as altered risky overtaking decisions or inconsistent speed during safe passing, fewer fine steering and/or gas pedal adjustments when lane keeping is perturbed by environmental factors, and a relative inability to safely follow a lead car under the influence of cannabis, compared to placebo.

Methods: Data are presented on $N = 38$ subjects ($N = 25$ male, frequent cannabis users, mean age $24.25 + 7.01$), each exposed to a placebo, low and high dose of cannabis on three separate days. On each day, following a single acute inhaled 0.5 g dose of either 0%, 5.9% or 13% of THC via a desktop 'Volcano' vaporizer, subjects drove a virtual driving simulator (RTI Sim Vehicle platform) three times inside an MRI scanner and once out of scanner, randomized, and dispersed throughout an eight hour daily period. During each driving session, a variety of real time behavioral risk measures corresponding to lane-keeping (LK) following simulated wind gusts, lead car following (CF) and safe overtaking (OT) were parameterized using custom Matlab scripts to extract event-locked driving performance information, resulting in a total of 29 dependent variables. Data were analyzed using a generalized linear mixed model framework in SPSS v24 which included dose, time (time since dose in mins), instrument (desktop PC versus MRI), dose*time, dose*instrument, age and sex as primary fixed factors. In addition, subject*dose were modelled as random effects to better capture the variation in slopes across individuals and dose. The above model was further refined on a variable by variable basis by removing the fixed interaction terms if they were found to be non-significant.

Results: The original model revealed no significant fixed interaction terms. The lane keeping paradigm showed that under the influence of cannabis, the number of finer steering adjustments was reduced in a dose dependent manner ($F = 7.57; p < 0.001$). In the car following task, there was an increase in correlation lag between the subject's car and a lead car ($F = 2.99; p < 0.05$) and fewer fine gas pedal corrections ($F = 3.59; p < 0.03$). In addition, albeit non-significant, a trend of decreased time to a potential collision was noted for higher doses ($F = 2.50; p < 0.08$). Consistent with the greater overall complexity of the overtaking task, drugged drivers showed more widespread abnormalities on several driving metrics. These included decreased median total time to pass ($F = 3.26$; $p < 0.04$), lower median minimum speed during overtake ($F = 3.66$; $p < 0.02$), lower median speed entering the oncoming lane ($F = 3.64$; $p < 0.03$). In addition, several comparable metrics involving speed and judgement of when it was safe to pass obstacles reached trend levels of statistical significance, i.e., decreased median gap to passed cars ($F = 2.56; p < 0.08$), median maximum overtaking speed ($F = 3.64; p < 0.1$) and median speed leaving oncoming lane ($F = 2.41; p < 0.09$). Most of the above dose-dependent impairments tended to diminish significantly throughout the day as cannabis effects waned, although to a varying degree across metrics.

Conclusions: From the current study, we draw three main conclusions: A) As expected, we observed widespread dose-related behavioral impairments in naturalistic scenarios that challenge automatic driving behavior. There were relatively more and diverse impairments on these tasks compared to prior reports. B) We were able to detect impairments in several driving behaviors related to fine steering/gas pedal adjustments, altered speed during passing and safe car following that have not been previously reported. C) These new effects were seen only during the high dose and not the low dose, offering more clarity on dose dependency. In general, individuals while driving under the influence of cannabis demonstrated rather 'lethargic' or delayed responses to stimulus events, putting them at risk of responding more slowly to sudden driving challenges.

Keywords: Cannabis, Simulated Driving, Behavioral Pharmacology, Substance Abuse Disorders, Alcohol and Substance Use Disorders

Disclosure: Nothing to disclose.

P706. Cannabis Induced Brain-Behavior Correlates of Simulated Automobile Driving: A Double-Blind, Placebo-Controlled fMRI Study

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Background: Driving is a complex everyday activity that requires the use and integration of different cognitive and psychomotor functions, many of which are known to be affected by cannabis. Given the public health and legal implications of drugged-driving and rapidly increasing use of cannabis nationwide, there is an urgent need to better understand the drug's effects on such functions and their underlying brain mechanisms in the context of driving. This longitudinal, double-blind placebo-controlled study investigated the effects of cannabis on driving brain-behavior relationships in a controlled, simulated environment using functional MRI (fMRI). Based on prior findings on cognitive impairments while driving under the influence of cannabis, and our own prior work on alcohol-impaired driving, we hypothesized that brain regions/circuits related to executive functioning (prefrontal/frontal cortex), attentional control (fronto-parietal, anterior cingulate), information processing (parietal, temporal), motor coordination (cerebellum) and visual (primary and extra-striate visual cortex) would be prominently affected in our current study.

Methods: $N = 29$ frequent cannabis users (22 male, mean/SD age $23.90 + 5.26$ and 7 female, mean/SD age $21.85 + 1.77$ were administered 0.5 grams of 13% THC or placebo flower cannabis via a Stortz+Bickel 'Volcano' vaporizer using paced inhalation, on separate days at least 1 week apart. On each study day, participants drove a virtual driving simulator (steering wheel, brake, gas pedal) inside an MRI scanner approximately 30 minutes post-dosing. Each fMRI driving session presented a naturalistic simulated environment that unobtrusively engaged drivers with scenarios that tested specific driving skills and response. There were three, approximately 10 min epochs where drivers engaged in tasks of lane-keeping/weaving (LK), lead car-following (CF), and safe overtaking from a parked starting place (OT).

fMRI data were prepared for analyses using the Human Connectome Project (HCP) pipeline v4.2. Independent Component Analysis (ICA) identified 40 valid neural networks from both drugged and placebo fMRI sessions. Regional connectivity estimates were averaged within 360 discrete cortical parcels identified by the HCP and another 27 sub-cortical regions. For each subject, a 'placebo minus drug' map of spatial connectivity difference was computed to quantify changes in functional connectivity due to THC. A similar difference score was computed for the 29 behavioral datapoints that described complex driving behavior. Principal component analysis (PCA) reduced these scores to 9 latent factors (3 for each task) that preserved between 80-92% of the driving behavior variance. FSL randomize was used to assess non-parametric statistical associations between connectivity maps and behavioral data. Results were thresholded and displayed at an FWE < 0.05 level adjusted for searching all parcels.

Results: For car-following, Factor 1 correlated with connectivity values in anterior BA 9/46, intra parietal, and superior temporal (STS) parcels. Factor 2 correlated with posterior ACC and visual cortex connectivity. Factor 3 correlated with visual cortex and ventral diencephalon. Similarly, for lane-keeping, Factor 1 correlated with STS/inferior parietal, Factor 2 with inferior frontal and nucleus accumbens, and Factor 3 with ventral area 9-46/10 and nucleus accumbens. Lastly, for the overtaking task, factor 2 correlated with connectivity values from inferior frontal, 9-46, and cerebellum parcels, while Factor 3 associated with inferior frontal, BA9, and parahippocampus.

Conclusions: As hypothesized, cannabis-related changes in connectivity of lateral prefrontal, cingulate, parietal cortex, and cerebellum were associated with drug-related driving task differences. Additional, non-hypothesized regions, including nucleus accumbens were also affected by recent cannabis use. These preliminary results depict complex yet informative links between many key brain areas sensitive to acute cannabis exposure and a profile of driving behaviors within a simulated environment that are also affected by the drug. These drug-related brain circuit alterations also differed significantly from those seen in our prior work on alcohol-impaired drivers. The current links underscore the likelihood that cannabis' acute effects on the brain networks engaged for driving are related to real-world risks to public safety by underlying driving related behavior.

Keywords: Cannabis, Simulated Driving, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P707. Association Between Immune Dysregulation and Response to Naltrexone-Bupropion Combination in Methamphetamine Use Disorder: Findings From the ADAPT-2 Study

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Background: There are no approved pharmacological treatments for methamphetamine use disorder (MUD). We recently showed that while response to naltrexone-bupropion combination was 5-fold higher than placebo, only 13.6% of individuals were able to attain response. Here, we evaluated the association of immune dysregulation with response to naltrexone-bupropion treatment.

Methods: Participants of the Accelerated Development of Additive Treatment for Methamphetamine Disorder [ADAPT-2] study with available week-6 post-treatment initiation plasma samples were included. Immune markers were assayed with the Bioplex Pro™ human cytokine standard 27-plex kit (Bio-Rad Laboratories, Hercules, CA, USA). Principal component analysis was used to identify latent factors of immune function. Logistic regression was used for association between immune factors and response (defined as 3 out of 4 negative urine sample over a 2-week evaluation period). Covariates included age, sex, race, and ethnicity. Post hoc analyses evaluated percentage of individuals with negative urine drug sample based on the levels of immune markers (high versus low, median split).

Results: $N = 49$ and $N = 119$ participants were randomized to naltrexone-bupropion and placebo, respectively. The first two principal components were retained and explained over 75% variance in the immune markers. First principal component (PC1) markers included interferon γ, interleukin (IL) 1β, IL-17A, and IL-2. Second principal component (PC2) markers included IL-4, IL-9, macrophage inflammatory protein 1 beta, and tumor necrosis factor alpha. At week-6, higher levels of PC2 were associated with lower likelihood of response at week-6 to naltrexone-bupropion [odds ratio (OR) = 0.28, 95% confidence interval (CI) = 0.09, 0.90; $p = 0.032$). No similar association were noted for placebo group. Notably, the association between higher levels of PC2 at week-6 and lower likelihood of response to naltrexone-bupropion combination at week-12 was also significant (OR = 0.17, 95% CI = 0.04, 0.70; $p = 0.013$). Low vs. high levels of PC2 were associated with higher proportion of methamphetamine-negative

urine samples with naltrexone-bupropion by week-2 and this difference was maintained until week-12.

Conclusions: We found that alterations in immune markers were significantly associated with poor response to naltrexone-bupropion combination. Future studies are needed to understand the cellular and molecular underpinnings of this association and clinical significance.

Keywords: Methamphetamine Use Disorder, Naltrexone, Bupropion, Stimulant Use Disorder

Disclosures: Acadia Pharmaceuticals, Alkermes Inc, Axsome Therapeutics, Biogen MA Inc, Circular Genomics Inc., GH Research Limited, Janssen, Legion Health Inc, Merck Sharp and Dohme Corp., Mind Medicine (MindMed) Inc., Myriad Neuroscience, Neurocrine Biosciences Inc, Noema Pharma AG, Orexo US Inc, Otsuka, SAGE Therapeutics, Titan Pharmaceuticals Inc,: Consultant (Self),, Alto Neuroscience Inc, GreenLight VitalSign6, Legion Health Inc, Heading Health, Signant Health: Advisory Board (Self).

P708. A Single-Dose of Ketone Ester Decreases Brain Glucose Metabolism in Alcohol Use Disorder

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Background: Acute alcohol intake decreases brain glucose metabolism and increases brain uptake of acetate, a metabolite of alcohol. Individuals with alcohol use disorder (AUD) show elevated brain acetate metabolism at the expense of glucose, and the shift in energy utilization persists beyond acute intoxication. We recently reported that nutritional ketosis and administration of ketone bodies as an alternative energy source over glucose reduce alcohol withdrawal and alcohol craving in AUD. However, the regional effects of nutritional ketosis on brain glucose metabolism have not been studied in AUD.

Methods: Four participants ($n = 2$ male individuals with AUD, 2 non-dependent controls [1 male, 1 female]) underwent two separate study visits, once after an overnight fast (baseline visit), and once after an overnight fast and 1.5 hr. following the consumption of 395 mg/kg (R)-3-hydroxybutyl (R)-3-hydroxybutyrate Ketone Ester (KE) solution (TdeltaS Global Inc.), in randomized order. We measured blood glucose and ketone levels. Brain glucose metabolism was assessed using positron emission tomography with [18 F]fluorodeoxyglucose ([18 F]FDG). Voxel-wise maps of the cerebral metabolic rate of glucose (CMRglc; µmol/100 mL/min) were computed in PMOD v3.7 (PMOD Technologies, Zurich, Switzerland), and further analyzed with SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK).

Results: A single dose of KE elevated blood ketone (beta-hydroxybutyrate) levels compared to the baseline visit ($F3,9 = 24.4$, $p < 0.001$). Although KE administration did not change blood glucose levels ($F3,9 = 1.6$, $p = 0.27$), it decreased whole-brain CMRglc by 18.5% (mean baseline=15.1 + - 1.8 SD, mean KE = 12.3 + - 1.8 SD, $F1,3 = 32.1$, $p = 0.01$). In SPM analyses the largest KE-induced CMRglc reductions were in the frontal cortex (peak MNI = [-22, -2, 64], $k = 5892$, $t = 16.2$, pFWE<0.001), including the bilateral inferior frontal gyrus (peak left [-52, 4, 24], peak right = [58, 14, 24], $k = 132$, $t = 11.3$, pFWE =0.015); occipital cortex (peak = [-14, -86, 0], $k = 206$, $t = 16.0$, pFWE=0.001); temporal gyrus (peak = [-58, 0, -34], $k = 243$, $t = 13.1$ pFWE <0.001); and anterior cingulate cortex (peak = [-8, 16, 26], $k = 410$, $t = 8.4$, pFWE<0.001). KE did not increase CMRglc in any brain region. Sample sizes in this pilot study were too small to compare the AUD and control participants on CMRglc.

Conclusions: These findings provide preliminary evidence of an effect of KE administration in reducing brain glucose metabolism in humans, consistent with a shift from glucose to ketones as a brain energy source. Average reductions in CMRglc of 18.5% are similar to global average reductions documented with 0.5 g/kg alcohol administration. Data collection using this paradigm is ongoing, as are studies of the effects of KE on alcohol consumption. Documenting the clinical and neurometabolic effects of nutritional ketosis will yield fundamental knowledge of its potentially beneficial effects as a novel treatment for AUD and aid in identifying its underlying neural mechanisms.

Keywords: Alcohol and Substance Use Disorders, Positron Emission Tomography, Ketones

Disclosure: Nothing to disclose.

P709. Social Determinants of Health Associated With How Cannabis is Obtained and Used in Patients With Cancer

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Background: Despite increased rates of cannabis use among patients with cancer, there are gaps in our understanding of barriers to accessing cannabis. Social determinants of health (SDoH) are associated with access to healthcare, but few studies have evaluated how SDoH relate to cannabis access and use among cancer patients.

Methods: We examined whether access to and modes of cannabis use associated with lower risk differed across SDoH among patients receiving treatment from a large National Cancer Institute (NCI) designated cancer center. This anonymous cross-sectional survey was developed in collaboration with the NCI Cannabis Supplement consortium. We evaluated the association of race (Black/African-American vs White), gender, income, and age with mode of cannabis use, source of obtaining cannabis (dispensary, friend/family, unlicensed dealer/seller, etc.), what influences their purchase (price, safety, etc.), and medical cannabis certification status.

Results: Overall, 1,053 patients completed the survey and 352 (33.4%) reported using cannabis since their cancer diagnosis. Patients who identified as Black/African-American were less likely to be medically certified ($p = 0.04$). Males and Black/African-Americans were more likely to report smoking cannabis (vs other forms, ps<0.01) and to purchase cannabis from an unlicensed dealer/seller ($p < 0.01$). Lower-income patients were more likely to be influenced by price and ease of access (ps < 0.05). Although cannabis users were younger than non-users, age was not associated with any outcomes.

Conclusions: The current data shed light on how critical drivers of health disparities (such as race, gender, and income) are associated with where patients with cancer obtain cannabis, what forms they use, and what influences their purchase decisions. More research is needed to understand the health outcomes associated with these differences in cannabis use and access-related factors such as the role of clinician-patient communication on certification, education around safe cannabis use, and the impact of statewide policies intended to increase access to cannabis.

Keywords: Cannabis, Cancer, Social Determinants of Health Inequity

Disclosure: Investigator-initiated grant from Novo Nordisk, Inc: Grant (Self).

P710. Applying Behavioral Economics to Screen Medications for Alcohol Use Disorder

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Background: Maladaptive decision-making processes are a hallmark of alcohol use disorder (AUD). A behavioral economic approach to AUD synthesizes concepts and methods from psychology and microeconomics to understand cognitive processes that contribute to overconsumption of alcohol. The excessive delay discounting of temporally distant rewards and increased alcohol demand is characteristic of individuals with AUD and provide support for dysfunctional decision-making processes. The multiple-choice procedure (MCP) was developed to efficiently investigate the relationship between drug preferences and alternative reinforcers. Although behavioral economic paradigms are relevant to alcohol-related pathology, they have not been leveraged to assess medication signals in the human laboratory.

Methods: A total of 40 men and women with current AUD and reporting intrinsic motivation to change their drinking, were randomly assigned to receive naltrexone (50 mg QD), varenicline (1 mg BID), or matched placebo for two-weeks. After a week-long medication titration period, participants began a 7-day practice quit attempt, during which they had daily virtual (online and phone) visits where they reported on their alcohol use. Participants completed an alcohol cue-reactivity paradigm and a behavioral economic battery following study drug administration on the final day of the practice quit attempt (Day 14). The battery included the MCP, wherein participants chose between a standard alcohol drink immediately or a monetary reinforcer in one week, followed by a state-based hypothetical purchase task to assess alcohol demand, and then a delay discounting task. A chi-square test was conducted to examine the relationship between medication group (placebo, varenicline, and naltrexone) and alcohol choice (alcohol now versus money later) on the MCP. One-way analysis of variance (ANOVA) tests were used to examine medication effects on alcohol demand and delay discounting rates.

Results: A chi-square test revealed no relationship between medication group and alcohol choice via the MCP ($\chi 2 = 0.13$, $p = 0.94$). Unadjusted one-way ANOVAs showed that varenicline and naltrexone, relative to placebo, did not influence alcohol demand indices (i.e., intensity [$F = 0.10$, $p = 0.91$], Omax [$F = 1.02$, $p = 0.37$], and breakpoint [$F = 1.15$, $p = 0.33$]), or change delay discounting rates (overall ln[k]; $F = 1.13$, $p = 0.32$).

Conclusions: Previous work demonstrates that varenicline and naltrexone have modest efficacy in reducing alcohol consumption. In this 2-week human laboratory trial, these medications did not influence behavioral economic indices suggesting that longer treatment periods may be necessary to influence decision-making processes that subserve AUD. Future statistical analyses will examine medication effects by considering the interplay between behavioral economics and alcohol cue-reactivity.

Keywords: Behavioral Economics, Alcohol Use Disorder - Treatment, Medication Development

Disclosure: Nothing to disclose.

P711. Attention-Deficit/Hyperactivity Disorder and Self-Control Moderate Effects of the COMT Inhibitor Tolcapone on Alcohol Consumption and Control-Related Brain Activation

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Background: Individuals with Alcohol Use Disorder (AUD) have higher levels of trait impulsivity than those without AUD (Sanchez-Roige et al., 2014), and are disproportionately likely to also have Attention-Deficit/Hyperactivity Disorder (ADHD) (van Emmerik-van Oortmerssen, 2012). A shared deficit in AUD, ADHD, and impulsivity is cognitive control. A key neurobiological influence on cognitive control is dopamine signaling in the prefrontal cortex (PFC), where catechol-O-methyltransferase (COMT) is the primary regulator of dopamine tone. The COMT inhibitor tolcapone, which potentiates elicited cortical dopamine release and improves performance on a variety of cognitive control tasks, could particularly benefit individuals with AUD and either ADHD or low self-control.

Methods: This study was a secondary analysis of a recently published randomized controlled trial of tolcapone (Schacht et al., 2022) in which 90 non-treatment-seeking individuals with AUD (59% male, 88% of European-American descent, mean age = 26.5 [SD = 5.1], mean drinks per day = 7.0 [SD = 2.3]) were prospectively genotyped for the COMT rs4680 (val158met) single nucleotide polymorphism and randomized, based on their rs4680 genotype, to tolcapone (titrated to 200 mg t.i.d.) or placebo for 8 days. To assess cortically dependent cognitive control, participants completed a visual spatial working memory (SWM) task at baseline and after 7 days of study medication; data from this task have not previously been reported. ADHD diagnosis, as assessed by the World Health Organization Adult ADHD Self-Report Scale (WHO-ASRS), and Barratt Impulsiveness Scale (BIS-11) self-control subscale score were analyzed as moderators of the effect of tolcapone, relative to placebo, on the number of drinks consumed during the medication period. ADHD diagnosis was also analyzed as a moderator of tolcapone effects on cortical activation during the SWM task.

Results: ADHD diagnosis significantly moderated the effect of medication group on drinks per day during the medication period ($F(1, 80) = 6.42$, $p = 0.013$), such that, among individuals with ADHD ($n = 15$), tolcapone, relative to placebo, reduced the number of drinks consumed per day ($F(1, 80) = 5.09$, $p = 0.027$; mean difference = 3.2 drinks per day, 95% CI = 0.4-6.0), while among those without ADHD, there was no significant difference between tolcapone and placebo. BIS-11 self-control subscale score also moderated the effect of medication group on drinks per day during the medication period at a trend level, such that tolcapone, relative to placebo, reduced drinking more among individuals with lower self-control ($F(1, 80) = 3.77$, $p = 0.056$). ADHD diagnosis also significantly moderated the effect of medication on cortical activation in the SWM task; between baseline and Day 7, task-related activation decreased, likely due to task habituation, but among individuals with ADHD, activation of the left dorsolateral PFC (DLPFC) was less reduced in the tolcapone group than the placebo group ($z > 2.3$, cluster-corrected $p < 0.05$). Greater DLPFC activation on Day 7 was associated with less drinking during the medication period at a trend level ($r(77) = -0.21$, $p = 0.069$), suggesting that sustained activation of this area during a task requiring cognitive control was associated with greater ability to resist drinking.

Conclusions: These data suggest that tolcapone may be particularly effective in reducing drinking and increasing cortical function among individuals with AUD who also have ADHD and/or low levels of self-control. Among these individuals, tolcapone may "rescue" cognitive control by increasing cortical dopamine release when individuals make efforts to control their behavior. We are currently evaluating this hypothesis further in a sample of individuals with comorbid AUD and ADHD (R01AA026859).

Keywords: ADHD, Impulsivity, Alcohol Use Disorder - Treatment, COMT Inhibitor, Cognitive Control

Disclosure: Nothing to disclose.

P712. Cannabis Challenge Effects on fMRI-Measured Brain Activity During Time Estimation

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Background: Cannabis is widely popular recreational drug in the US. The drug is known to alter the subjective experience of time. However, its effects on time estimation at a brain level are still largely unexplored. Our goal was to investigate acute effects of cannabis on an fMRI time estimation task by evaluating brain activation differences between cannabis and placebo conditions. We hypothesized that participants' time estimation accuracy and corresponding BOLD response would be altered during the active cannabis condition, compared to placebo.

Methods: In this placebo-controlled, double-blind, randomized trial, a total of $N = 44$ participants had 3 dose visits, at each of which they received either high-dose cannabis (0.5 gm of ~12.5% THC flower), low dose cannabis (0.5 gm of ~5.7% flower) or 0.5 gm placebo, using paced inhalation from a Volcano vaporizer via vaporizer. Drug material was supplied by NIDA/RTI. For the current study we analyzed fMRI data from the first placebo and high dose fMRI sessions throughout each dosing day, in which participants performed a time estimation task. Participants were asked to respond with a mouse click as to which box of two boxes displayed for different intervals was displayed on the screen longer. Both sub-second and supra-second temporal intervals were tested, with a range of easy to hard discriminations. Prior studies have indicated that these different intervals often differentially recruit cerebellar or basal ganglia regions into interval timing networks, respectively. Both were of interest in this study given the abundance of cannabinoid receptors in these regions. We used the Human Connectome Project processing pipeline to prepare fMRI data for GLM modeling of activation using the FSL FEAT toolbox. This model estimated the unique effect sub-second (short) and supra-second (long) interval discrimination, their average effect, and their difference. From these contrasts, the mean activation amplitudes within 387 brain parcels from the Human Connectome cortical atlas were extracted. Robust statistics in R software estimated a paired t test equivalent using the bootdpci function to assess the difference between placebo and the high dose drug conditions for each contrast.

Results: Results for the short sub-second and long supra-second intervals were highly similar, so these were collapsed into a single condition for reporting purposes. Brain regions whose activity significantly differed between $p < 0.05$ and $p < 0.001$ for the average of short and long duration stimuli included premotor cortex, somatosensory and motor cortex, posterior cingulate cortex, visual area, medial temporal cortex, paracentral and midcingulate cortex, anterior cingulate and medial prefrontal cortex, inferior frontal cortex, tempo-parieto-occipital junction, premotor cortex, somatosensory motor cortex, posterior cingulate cortex, medial temporal cortex, orbital and polar frontal cortex, hippocampus. Only premotor cortex survived False Discovery Rate corrections for searching all 387 parcels across the entire brain for the average of short and long temporal estimation conditions. Next, we were interested whether cannabis might have a greater or lesser effect with different length intervals. In contrast to the main effect of cannabis on task activity, differences $p < 0.05$ were observed mostly in visual cortex, including parcels in the ventral stream visual cortex, dorsal stream visual cortex, and early visual cortex. Long versus short-interval brain activity also differed to cannabis in anterior cingulate and medial prefrontal cortex parcels ($p < 0.05$).

Conclusions: The current study observed multiple brain activation differences for the initial post-dose, acute high-dose cannabis vs. placebo conditions. The strongest effect was seen for a premotor cortical region, which was the only parcel to survive multiple comparison correction for searching the whole brain. However, the other effects were noteworthy. A post hoc power analysis showed that adding 10 additional subjects to this sample would achieve significance with multiple comparison correction for medium effect sizes at alpha=0.05. This study identifies for the first time which brain regions engaged for mental time estimation are altered by recent cannabis use. Future studies that examine all doses and tasks would elucidate how the effects unfold longitudinally post-dose and determine if any effects are dose-dependent.

Keywords: Cannabis, fMRI, Time Estimation

Disclosure: Nothing to disclose.

P713. COVID-19 Pandemic Impact on Alcohol Consumption and Pharmacodynamic Responses: Insights From Neurofunctional Domain Analysis

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Background: The COVID-19 pandemic continues to impact individuals, communities, and the global economy. Social isolation, health anxiety, and financial stress can all impact alcohol-related outcomes among individuals across the spectrum of alcohol use disorder. Changes in alcohol use and related problems have been reported; however, there has been substantial heterogeneity in magnitude and directionality of these effects. A major determinant of changes in alcohol use is in the pharmacodynamic response to alcohol, which can be considered in the context of the neurofunctional domains of incentive salience or reward, negative emotionality or stress-reactivity, and executive function or loss of control over drinking. These domains are characterized in the Addictions Neurochemical Assessment (ANA) framework that is aligned with the 3-domain cycle of addiction. The objective of this study was to examine changes in alcohol use and consequences related to the COVID-19 pandemic in a sample of individuals with and without alcohol use disorder (AUD), and to examine these changes in relationship to alcohol response measures aligned with the ANA framework.

Methods: 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. Participants completed subsequent surveys ranging from weekly to every six months for two years beginning June 2020. Assessments included demographics, the Alcohol Use Disorder Identification Test (AUDIT) to measure alcohol consumption and problems, and a COVID-19 stress scale. Incentive salience measures included the Self-Report of the Effects of Alcohol Scale (SRE), Anticipated-Biphasic Alcohol Effects Scale (A-BAES), and

Penn Alcohol Craving Scale (PACS); negative emotionality measures included the Perceived Stress Scale (PSS) and the Positive Affect Negative Affect Scale (PANAS); and executive function measures included the Impaired Control Scale (ICS). Data were analyzed using general linear models covarying for history of AUD, sex, age, household income, enrollment phase, and time since participation in the natural history study.

Results: The study sample included 391 participants (mean age = 44.8 years; 48.0% female). Data from the AUDIT scale indicated a wide variation in pandemic-related changes in alcohol use, with 29% of participants reporting increases, 33% reporting decreases, and 38% reporting no change in AUDIT-Consumption scores. Participants were categorized into three groups based on their AUDIT score change from pre-pandemic to during the pandemic: no change, decrease, or increase. Participants who had an increase in their AUDIT scores from pre-pandemic reported significantly higher scores on incentive salience (PACS, $p < 0.001$; A-BAES sedation, $p < 0.001$; and trending for SRE, $p = 0.06$), negative emotionality (PSS, $p < 0.001$, Negative Affect Scale, $p < 0.001$), and executive function (ICS failed attempts to control drinking, $p < 0.005$) than those that had a decrease or no change in AUDIT scores. Individuals with a History of AUD was significant in all models (all p's<0.01). Household income was significant in negative emotionality and executive function models (all p's <0.01). Age was negatively associated with negative emotionality, while males showed significantly higher scores on incentive salience measures. Finally, those enrolled in the early pandemic phase scored higher on negative emotionality.

Conclusions: This study indicated significant shifts in alcohol consumption, both increases and decreases, associated with the pandemic, although a substantial proportion of individuals showed no change in alcohol consumption as well. An increase in AUDIT scores during the pandemic was associated with higher scores in measures across all three neurofunctional domains, compared to groups that showed a decrease or no change in their AUDIT scores. Individuals with a history of AUD showed significant main effects, highlighting potential biobehavioral correlates of vulnerability to problem drinking and addiction vulnerability. The neurofunctional domain lens may provide additional insights into the underlying pharmacodynamic mechanisms that impact alcohol responses and risk for AUD.

Keywords: Pharmacodynamics, the COVID-19 Pandemic, Incentive Salience, Negative Emotionality, Executive Function

Disclosure: Nothing to disclose.

P714. Sex Dependent Effects of Cannabis: Comparison of Self-Administration and Positive Subjective Drug Effects Between Female and Male Volunteers

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Background: In recent years, rates of cannabis use and cannabis use disorder (CUD) have increased significantly among females; epidemiological studies point to an accelerated progression from first cannabis use to development of CUD in females compared to males. Preclinical studies suggest possible biological sex-based differences in the reinforcing effects of cannabinoid receptor 1 (CB1) agonists such as delta-9-tetrahydrocannabinol (THC), the primary psychoactive component of the cannabis plant, which may provide insight into why females may be more vulnerable to developing CUD. However, these preclinical findings have yet to be translated to humans. This study sought to explore sex differences in cannabis self-administration and self-reported positive subjective drug ratings in healthy male and female volunteers.

Methods: Data from two within-subject randomized controlled studies comparing the subjective and reinforcing effects of smoked active cannabis (~25 mg THC) versus placebo cannabis (0 mg THC) were pooled for this analysis. Healthy non-treatment seeking males ($n = 55$) and females ($n = 13$) were matched for current cannabis use and included in this analysis. During two outpatient laboratory sessions, participants smoked experimenter administered placebo (0% THC) or active (~5.5% THC) cannabis (one drug condition per session). Subjective mood and drug effects were measured via the Subjective Effect-Visual Analog Scale (SE-VAS) and Cannabis Rating Form (CRF) at several timepoints post-cannabis administration. In the afternoon, participants had an opportunity to self-administer up to 3 puffs (cost: $1/puff) of the cannabis type that was smoked earlier in the session. Using generalized linear mixed models, subjective mood and drug effects were examined as a function of cannabis strength (active versus placebo), sex (female versus male), and time, and self-administration was analyzed as a function of cannabis strength (active versus placebo) and sex (female versus male).

Results: Active cannabis increased positive subjective ratings including 'Liking', 'Take again', and 'Good Effect' relative to placebo cannabis ($p < 0.01$), and females reported significantly higher ratings relative to males on these measures after active cannabis administration ($p < 0.05$). Placebo cannabis was self-administered by 12 (19.0%) males and 2 (15.4%) females ($p = 0.89$). Active cannabis was self-administered by proportionally more females than males (53.8% versus 36.3%), but a significant sex difference was not detected (interaction $p = 0.18$).

Conclusions: Under active cannabis conditions, females reported higher ratings of positive subjective drug effects relative to males. Despite these differences, females were not significantly more likely to self-administer active cannabis. These findings suggest that there may be other sex- or gender-based factors that modify the relationship between heightened sensitivity to certain positive subjective drug ratings and reinforcing effects of cannabis. Given the small number of females in this secondary exploratory analysis, future research with a higher number of females is needed to test sex-based differences in cannabis' acute subjective drug, mood, and reinforcing effects.

Keywords: Cannabis, Cannabis Use Disorder, THC, Sex Differences

Disclosure: Canopy Growth Corporation: Consultant (Self)

P715. Baseline Affective Symptomatology Moderates Acute Subjective Effects of High Potency THC and CBD Cannabis Concentrates

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Background: As cannabis legalization spreads throughout the US, products with high concentrations of Δ9-tetrahydrocannabinol (THC) become more commonly consumed. However, relatively little is known about the effects of these highly potent products and their relationship to baseline levels of affect, and the impacts of Cannabidiol (CBD) dominant concentrates on affect and intoxication are also not well understood. Thus, the current study took an observational approach to assess the relationship between baseline affective symptoms and use of high THC or high CBD cannabis concentrates as they relate to acute mood and intoxication responses to cannabis.

Methods: Using a naturalistic at-home administration study design, participants were randomly assigned to ad libitum administration of either a THC-dominant (84.99% THC and THCa, <1% CBD) or CBD-dominant (74.7% CBD, 4.1% CBDa, 4.5% THC and THCa) concentrate product. 54 adults (48% female; Mean age 29.87 (9.52 SD)) were assessed at a baseline appointment and then five days later in a mobile pharmacology lab before, immediately, and one hour after ad libitum administration of their assigned concentrate. Models were run regressing each outcome on time, condition, baseline affective symptom measures, and their two and three-way interactions. Tests of simple effects/slopes were conducted for models with significant interactions. In the case of simple effects, levels of baseline affect were held at one standard deviation (SD) above ('high'), below ('low'), or at the mean ('average') of scores.

Results: Participants at the follow-up appointment demonstrated cannabinoid levels in blood that were commensurate with their assigned concentrate group (THC dominant or CBD dominant). There was a significant two-way interaction between condition and baseline depression levels on acute changes in positive mood ($F = 9.47$, $p < 0.005$) such that higher levels of depression were significantly associated with higher levels of elation for the THC-dominant group but not for the CBD-dominant group. There was also a three-way interaction between condition, baseline anxiety, and time on acute changes in tension ($F = 5.55$, $p < 0.01$) such that tension decreased in the CBD-dominant group at average and high anxiety levels, but not low anxiety levels or among the THC-dominant group. Finally, there was a significant three-way interaction between condition, baseline anxiety levels, and time on acute intoxication ($F = 3.72$, $p = 0.03$). Specifically, THC users at all anxiety levels were more intoxicated than CBD users immediately post-use, but at one hour post-use, THC users were comparably intoxicated to CBD users at high anxiety levels but more intoxicated than CBD users with average and low anxiety. These data were drawn from a fully powered study of the effects of concentrate use on psychological, functional, and motor outcomes, but the current study was an exploratory moderation analysis based on post-hoc observations.

Conclusions: This is one of the first studies to examine whether baseline affective symptoms moderate the acute effects of ad libitum use of high potency cannabis concentrates, including both THC- and CBD-dominant products. These exploratory analyses suggest an important relationship between a user's baseline levels of anxiety or depression as they pertain to one's subjective drug experience. As concentrate products continue to gain popularity on the legal market, these preliminary findings may be especially relevant towards informing future research on cannabis concentrate misuse or withdrawal.

Keywords: Cannabis Use, Cannabis Concentrates, Affective Psychopathology

Disclosure: Nothing to disclose.

P716. Consuming Oral Cannabidiol (CBD) Prior to a Standard Alcohol Dose Prolongs Physiological and Subjective Effects of Alcohol Without Impacting Impairment, Affect or Craving: A Pilot Human Laboratory Study

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Background: Cannabidiol (CBD) is a cannabinoid commonly found in the cannabis plant. CBD thought to be safe, non-intoxicating and well-tolerated in humans. In recent years, CBD has garnered considerable attention as a potential treatment for numerous medical and psychiatric conditions, including substance use disorders. In particular, rodent studies have demonstrated that CBD appears to reduce alcohol consumption and decrease other markers of alcohol dependence in murine models of alcohol use disorder (AUD). The mechanism(s) through which CBD may serve to reduce alcohol intake is not well understood, and it is also presently unknown whether CBD decreases alcohol consumption and influences other AUD phenotypes in humans. Further, the existing human laboratory work exploring the acute effects of CBD and alcohol is limited and conflicting. The present placebo-controlled, crossover study aims to assess the effects of oral CBD on alcohol-induced psychomotor performance, self-reported affect, alcohol craving and subjective responses to alcohol in a sample of $N = 36$ heavy-drinking human participants.

Methods: Heavy drinkers (males and females) who do not regularly use cannabis and were not CBD-naïve were recruited to participate in this 3-session pilot study. At each session, eligible individuals consumed either 30 mg CBD, 200 mg CBD, or placebo prior to drinking a standardized dose of alcohol designed to bring their blood alcohol content (BAC) to .06 g/dL. Participants were blind to which dose they received at each session. All three sessions were identical except for the CBD dose administered. Participants completed sessions in randomized order. At each session, following the CBD (or placebo) administration and alcohol consumption period, participants completed measures of psychomotor impairment, affect, alcohol craving, subjective responses to alcohol and had their breath alcohol content (BrAC) measured. Blood was drawn at the start of the experiment, at 25 minutes after CBD administration and once more 60 minutes after the alcohol administration period. Differences in these outcomes across the three CBD conditions and by sex were explored using multilevel structural equation models. Separate models were run for each of the following dependent variables of interest: BrAC, alcohol craving, low arousal positive affect, high arousal positive affect, alcohol-induced sedation, alcohol-induced stimulation and psychomotor impairment. Due to the research design, where each participant completed each condition (placebo, 30 mg CBD and 200 mg CBD) and within each condition there were 7 repeated measures of interest, MSEMs were run with a latent growth curve on the within-level to capture change over time. Condition was treated as a within-person variable and sex was treated as a between-person variable. To examine whether condition (30 mg CBD, 200 mg CBD, placebo) influenced changes over time, we regressed the latent slope parameter defined by the latent growth curve model on a within-person condition variable. To determine if sex moderated the condition on change relationship we created a random slope of condition predicting the latent slope variable and regressed that random slope on the between-person sex variable. This is referred to as the random coefficient prediction method. We also used this method to determine if the overall change over time was moderated by sex by regressing the random latent slope parameter on the between-person sex variable. We also examined CBD blood levels during each session before CBD ingestion, 25 minutes post-ingestion and 1-hour post-alcohol consumption to ensure that participants showed expected increases in blood-CBD following ingestion of each CBD dose.

Results: Plots of CBD-blood during the experiment were consistent with the CBD doses administered, such that individuals showed increased blood-CBD over the course of the experiment, and CBD blood-levels were higher for the 200 mg CBD condition (average peak CBD = 96.39 ng/ml) than the 30 mg CBD condition (average peak CBD = 8.19 ng/ml). BrAC and high arousal positive affect were significantly reduced over the course of the descending limb of the BAC curve. All conditions showed significant differences in change in in BrAC over the descending limb of the BAC curve. Specifically, the placebo condition had the steepest negative BrAC slope, followed by 30 mg CBD, and 200 mg

CBD had the flattest BrAC slope. A significant difference emerged between 200 mg CBD and placebo on changes in stimulation, with placebo having a steeper negative slope. There was a significant difference between 30 mg and placebo for sedation, with placebo having a steeper negative slope. No significant sex differences or moderating effects of sex emerged for any outcomes

Conclusions: Results from this pilot study indicate that pre-treatment with oral CBD prior to consuming a dose of alcohol appears to extend the physiological effects of alcohol (i.e., BrAC) and prolong the subjective effects of alcohol (specifically, stimulation and sedation). Notably, in this study, CBD did not impact alcohol positive affect or craving, suggesting that craving-reduction is likely not a mechanism through which CBD serves to reduce alcohol intake. Although preliminary, these findings suggest that CBD should be further explored for its potential to aid individuals who wish to reduce their drinking, and that extending the physiological and subjective effects of alcohol may be one mechanism through which CBD could help to reduce alcohol consumption.

Keywords: Cannabidiol, Alcohol, Subjective Effects

Disclosure: Nothing to disclose.

P717. Short-Term Anxiolytic and Harm-Reducing Effects of Cannabidiol in Cannabis Flower

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Background: Cannabis is increasingly used to self-treat anxiety; however, data are mixed on anxiolytic effects. Additionally, the primary cannabinoids Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have varying pharmacological actions that may render different short-term effects on anxiety.

Methods: These are the first data on the primary hypotheses from a fully powered NIDA-funded R01(DA044131; PI Bidwell; ClinicalTrials.gov Identifier: NCT03491384) that uses experimental methodologies to examine the impact of different cannabinoids in individuals with anxiety. Using an at-home administration procedure compliant with federal law, the present study examined the acute effects of three cannabis flower chemovars with different THC to CBD ratios to test whether chemovars with a higher CBD content produce different effects in anxious cannabis users. Participants who reported at least mild to moderate anxiety (Score of ≥ 5 on the Generalized Anxiety Disorder-7 (GAD-7) scale) and current use of (or intent to use) cannabis to cope with anxiety were recruited for the study [N = 201 adults (Male = 91, Female = 110); Mean cannabis use days past 14 days = 6.36 (SD = 5.24)]. Participants were randomly assigned to ad libitum administration of one of three chemovars (THC dominant: 24% THC, 1% CBD; THC + CBD: 9% THC, 10% CBD; CBD dominant: 1% THC, 23% CBD) and assessed in a mobile pharmacology lab before (pre-use) and immediately after (post-use) ad libitum administration of their assigned chemovar. A sex-matched group of non-users with mild to moderate anxiety (GAD-7 ≥ 5; $n = 41$) were recruited and evaluated over these same time points. Using a mixed model ANOVA design, with four groups, two assessment points (pre- and post-use, and an average ICC = 0.5 between assessment points, a total $n = 240$ (60 per group) allowed us to detect a groupXtime interaction effect as small as f = 0.12.

Results: Plasma cannabinoids as well as subjective mood and intoxication effects were assessed at each time point. Participants who used the CBD-dominant and THC + CBD chemovars had less THC and more CBD in plasma after cannabis use compared to participants who used the THC-dominant chemovar. The CBD dominant chemovar was associated with acute reductions in anxiety and tension as compared to the THC dominant and the THC + CBD chemovars. In addition, the use of all three strains was associated with intoxication and positive subjective mood effects. Results pointed to graduated drug reward effects across strains, with the highest levels of intoxication and positive mood being present in the THC dominant chemovar, the next highest in the THC + CBD chemovar, and the lowest levels present in the CBD dominant chemovar. Similarly, adverse effects, including paranoia, demonstrated a graduated effect across strain groups, with the highest levels in the THC dominant chemovar, the THC + CBD chemovar falling in the middle, and the CBD dominant showing significantly lower levels than the other two strain groups. Non-users did not show significant change in either objective or subjective mood and intoxication effects between the study time points.

Conclusions: In one of the first studies to examine the differential effects of ad libitum use of cannabinoids on measures relevant to individuals with anxiety who use cannabis, the CBD dominant chemovar was associated with short-term anxiolytic effects as compared to a THC + CBD and a THC dominant chemovar. Participants using the CBD dominant and THC + CBD chemovars also reported lower THC plasma levels and yet still some level of intoxication and positive drug effects, which is intriguing from a harm reduction perspective. Our study contributes novel data on the effects of various THC to CBD ratios using chemovars that are widely available in state-regulated markets, with an emphasis on the effects of CBD. Further research is needed to clarify the anxiolytic and harm reduction potential of CBD in cannabis products.

Keywords: Cannabis, Delta9-Tetrahydrocannabinol, CBD, Anxiety, Generalized Anxiety Disorder

Disclosure: Nothing to disclose.

P718. THC Modulates Pain Sensitivity Among Persons Receiving Opioid Agonist Therapy for Opioid Use Disorder: A Within-Subject, Randomized, Placebo-Controlled Human Laboratory Study

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Background: The opioid and cannabinoid receptor systems are inextricably linked, overlapping at the anatomical, functional, and behavioral levels. Seminal preclinical studies have reported that cannabinoid agonists can induce opioid-sparing effects (i.e., a reduction in the effective dose of an opioid agonist). In the grip of the opioid crisis, a growing number of states has authorized the medicinal use of cannabinoids — in some cases, even replacing opioids — to treat two often co-occurring conditions: Pain and opioid use disorder (OUD). These rapid changes in public policy are in stark contrast with the lack of experimental data on the effects of cannabinoid agonists among persons with OUD — leaving clinicians, patients, and stakeholders without high-quality evidence to guide their decisions. To address this consequential knowledge gap, we have designed the first experimental human study to investigate the acute effects of the main constituent of cannabis, delta-9-tetrahydrocannabinol (THC), among persons with OUD receiving methadone — the most common opioid agonist therapy for OUD.

Methods: We conducted a Phase I within-subject, crossover, human laboratory study including 27 persons with OUD who were receiving methadone therapy. Across three test sessions,

each lasting for 5 hours, participants were randomly assigned to receive single doses of oral THC (10 mg or 20 mg), administered as dronabinol; or placebo. Before each test session, abstinence from non-medical substance use was confirmed by a urine laboratory immunoassay. 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 liability 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 THC dose, and interactions between THC (10 mg, 20 mg) and methadone doses (low-dose methadone defined as < 90 mg/day; and high-dose methadone defined as ≥ 90 mg/day). At a significance level of 0.05, this exploratory study had 80% power to detect medium effect sizes (d ≥ 0.56).

Results: Participants were aged 47.3 ± 12.2 years old. Approximately 76% of participants self-identified as male, and 24% self-identified as female. The average methadone dose was 96.6 ± 35.1 mg/day. Approximately 48% of participants were receiving low-dose methadone (62 ± 22.7 mg/day), and 52% were receiving high-dose methadone (118 ± 19.7 mg/day). Preliminary results show a main effect of THC dose on pain sensitivity, indexed by the MPQ total pain score ($F(2,104)=5.33$, $p = 0.006$): Pain sensitivity was lower under 10 mg THC ($d = 0.65$) and 20 mg THC ($d = 0.39$) than under placebo. Post-hoc analyses indicated that the lower MPQ total pain scores under THC were due to a reduction of sensory pain ($F(2,104)=5.93$, $p = 0.003$), rather than of affective pain. Although there were no main effects of THC dose, nor THC by methadone dose interactions for CPT outcomes, 20 mg THC was associated with higher CPT pain tolerance among persons receiving low-dose methadone ($d = 1.01$) than among persons receiving high-dose methadone. Further, we observed a main effect of THC dose for abuse liability ($F(2,463)=4.05$, $p = 0.02$), as 20 mg THC produced greater DEQ stimulatory effects ($d = 0.57$) than placebo. Lastly, we found a main effect of THC dose for cognitive performance ($F(2,39)=3.72$, $p = 0.03$): Compared to placebo, 20 mg THC reduced HVLT delayed recall ($d = 0.47$). No interactions between THC and methadone doses were observed for abuse liability and cognitive outcomes.

Conclusions: The study had several notable findings. First, likely due to cross-tolerance between opioid and cannabinoid agonists for analgesia, relatively high doses of THC may be required to produce acute analgesic effects among persons receiving therapeutic doses of methadone. Yet, persons receiving lower doses of methadone may be more likely to derive analgesic effects from THC. Second, our findings support the notion that, in this population, THC modulation of acute pain is mediated primarily by sensory nociception, rather by affective pain processes. Third, the analgesic effects of THC among persons with OUD receiving methadone therapy may occur at the expense of abuse liability and cognitive deficits — similar to analgesia, the cross-tolerance between opioid and cannabinoid agonists for reinforcing and cognitive effects is incomplete. Collectively, these data have key implications for future studies investigating opioid-sparing effects of cannabinoids among persons receiving opioid agonist therapy for OUD. It is critical to examine the risk/benefit ratio of sparing opioids by administering cannabinoids when opioid agonist doses are lower in OUD treatment — during initiation and taper. Future studies are warranted to ascertain whether, during these vulnerable periods in OUD pharmacotherapy, the trade-off between therapeutic and adverse effects of cannabinoids can be optimized to reduce the harm from opioids, including overdose deaths.

Keywords: Cannabis, Opioid Agonist Treatment, Analgesia, Mu-Opioid Receptor Agonist, Cannabinoid Receptor

Disclosure: Nothing to disclose.

P719. The Trajectory of Cannabis Withdrawal in Cannabis and Tobacco Co-Users: A Preliminary Investigation

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Background: The cannabis withdrawal syndrome, consisting primarily of mood and behavioral symptoms, is well-established in people with frequent cannabis use (CU) and follows a distinct trajectory. Withdrawal symptoms begin 24 hours after cessation, peak within 7 days, and dissipate after 28 days of abstinence. In animal models, reduced endocannabinoid activity, indexed by low levels of endocannabinoids (anandamide and 2-arachidonoylglycerol, 2-AG), has been linked to the presence and severity of cannabis withdrawal-like symptoms. However, the neurobiological substrates underlying cannabis withdrawal in humans have not yet been identified.

Tobacco co-use is highly prevalent among people with CU. Notably, relative to cannabis-only users, co-users have increased difficulty quitting cannabis demonstrated by their higher and accelerated relapse rates. This may reflect that greater proportions of co-users, compared to cannabis-only users, experience cannabis withdrawal and experience it at greater severity levels. Our previous work has shown that when individuals with CU were parsed according to tobacco dependence severity (rather than the presence/absence of tobacco use), associations with cannabis withdrawal severity were even more robust.

Notably, the few studies assessing the relationship between tobacco co-use and cannabis withdrawal have been cross-sectional. Thus, it is unknown whether among co-users cannabis withdrawal severity is transiently elevated or remains persistently elevated for the duration of the withdrawal syndrome (i.e., 28 days).

Aim 1: To examine if cannabis withdrawal is elevated and remains persistently elevated in severity in people with CU and high tobacco dependence following 28 days of cannabis abstinence relative to individuals with CU and low tobacco dependence. Aim 2: To test associations between endocannabinoid levels and cannabis withdrawal severity and tobacco use following overnight abstinence.

Methods: Men with CU ($N = 20$) were parsed according to their tobacco dependence severity using the Fagerstrom Test for Nicotine Dependence (FTND), which is optimal for classifying low (FTND ≤ 4) and high tobacco dependence (FTND ≥ 5). Participants underwent 28 days of cannabis abstinence facilitated by contingency management and weekly supportive therapy. Abstinence was biochemically-verified with twice weekly urine analysis and self-reported cannabis use. Cannabis withdrawal severity was assessed weekly using the Marijuana Withdrawal Checklist (MWC). Further, in an independent sample of men with CU ($N = 8$), following overnight cannabis abstinence, we evaluated relationships between serum-derived anandamide and 2-AG levels and cannabis withdrawal severity and tobacco consumption.

Results: The majority of participants ($n = 14$) achieved biochemically-verified cannabis abstinence, five participants significantly reduced their cannabis use by >70%. Among these 19 individuals, $n = 12$ were classified with low tobacco dependence and $n = 7$ were classified with high tobacco dependence. Controlling for age, between-group differences revealed a significant difference in baseline withdrawal severity ($p < 0.01$) that persisted across all 28 days of abstinence (Group x Time interaction effect, $F(4,64) = 4.40$, $p < 0.01$, $\eta 2 = 0.22$). Correlations between cannabis withdrawal severity and endocannabinoid levels revealed negative associations between cannabis craving and anandamide ($r = -0.78$; $p = 0.02$) and 2-AG ($r = -0.84$;

$p = 0.01$). Moreover, we found that tobacco consumption (i.e., cigarettes per day) negatively correlated with anandamide levels ($r = -0.71$; $p < 0.05$).

Conclusions: Our preliminary data showed that following 28 days of cannabis abstinence, people with CU with high tobacco dependence exhibited significantly elevated cannabis withdrawal symptoms which remained persistently elevated compared to people with CU and low tobacco dependence. The unique withdrawal trajectory observed in individuals with CU and high tobacco dependence is of great clinical importance given that longer episodes of elevated withdrawal severity would prolong the time that these individuals remain at high risk for cannabis relapse, hindering long-term recovery. Reduced endocannabinoid levels may underlie increased cannabis withdrawal severity in people with CU, and tobacco co-use may further decrease endocannabinoid levels in a dose-dependent manner. Thus, greater tobacco consumption in individuals with CU may lead to more severe withdrawal symptoms. Our findings have important treatment implications for developing interventions for cannabis-tobacco co-users.

Keywords: Cannabis Use Disorder, Tobacco, Withdrawal, Abstinence, Endocannabinoids

Disclosure: Nothing to disclose.

P720. Influence of Suvorexant Maintenance on Commodity Demand Following Cocaine Challenge: A Cross-Over, Double-Blind Comparison

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Background: A promising target for the regulation of drug use motivation is the orexin system. Preclinical evidence indicates a connection between drug-regulated behaviors and orexin producing cells in the lateral hypothalamus and brain limbic areas (e.g., the nucleus accumbens and ventral tegmental area). Existing in vivo evidence from preclinical rodent models shows that antagonism of the orexin system attenuates the behavioral effects of, and motivation for, varied drug classes. A broadly consistent preclinical finding is this orexin antagonism or knockdown, especially of the orexin1 receptor, increases demand elasticity (i.e., indicating lower drug use motivation) while leaving consumption at unconstrained price (i.e., demand intensity) unchanged. Suvorexant is a dual orexin1 and orexin2 receptor antagonist that is clinically approved and of interest for clinical translation. This analysis focused on drug demand data from a within-subject human laboratory study that determined the influence of suvorexant maintenance on cocaine pharmacodynamics.

Methods: Eight (4 women; 2 White, 5 Black, 1 Multiethnic) non-treatment seeking participants with cocaine use disorder were enrolled in a cross-over, double-blind, randomized residential study (one participant with partial data due to the COVID-19 pandemic). Participants were maintained on 0, 5, 10 and 20 mg oral suvorexant/day in randomized order with experimental sessions completed after at least three days of maintenance on each target suvorexant dose. Experimental sessions included administration of a sample dose of 0, 10, and 30 mg/70 kg of intravenous cocaine. Analyses focused on standardized behavioral economic demand tasks completed 15 minutes after the cocaine sampling dose. Commodities in these tasks included the sampled cocaine dose as well as chocolate, cigarettes, and alcohol. The dependent measures were observed demand values (i.e., intensity, Pmax, Omax, and breakpoint). Mixed-effect models evaluated demand outcomes with Suvorexant Dose (0, 5, 10 and 20 mg/day) and Cocaine Dose (0, 10 and 30 mg/70 kg) as within-subject factors.

Results: A main effect of Cocaine Dose was observed for all cocaine demand measures (p values < 0.001) reflecting a dose-related increase in demand for the blinded cocaine dose. A main effect of Suvorexant Dose was observed for cocaine Pmax ($p = 0.035$) reflecting an increase in cocaine demand (higher Pmax) for the 10 mg cocaine dose when participants were maintained on 10 mg suvorexant ($p = 0.009$) and 20 mg suvorexant ($p = 0.039$) relative to placebo. Cocaine challenge did not alter demand for alcohol, but a main effect of Suvorexant Dose was observed for alcohol Pmax ($p = 0.021$) and Omax ($p = 0.022$) reflecting increases in alcohol demand when participants were maintained on the 10 mg suvorexant dose regardless of sampled cocaine dose that session. Cocaine challenge did not alter demand for cigarettes, or chocolate (p values > 0.107) nor were effects of suvorexant maintenance observed for cigarette or chocolate demand. Exploratory analyses indicated no differential effects based on participant gender.

Conclusions: Suvorexant maintenance increased demand for a low dose of cocaine, as well as for alcohol, in a dose-related manner. These findings contrast with preclinical findings, which may be explained by differences in acute versus subchronic dosing or the lack of orexin receptor subtype selectivity of suvorexant. These data also support the validity of demand procedures for measured blinded drug demand by showing an expected dose-related increase in demand for blinded doses of cocaine and low responding for placebo. Future research should test more selective orexin antagonists in combination with cocaine and other substances to determine dual versus single orexin receptor targeting effects in clinical populations.

Keywords: Dual Orexin Receptor Antagonist, Behavioral Economics, Cocaine, Addiction, Alcohol

Disclosure: Nothing to disclose.

P721. Voluntary Adolescent Binge Alcohol Exposure Enhances the Ability of Cocaine Microinjected Into the Poster VTA to Stimulate Dopamine Release in the Nucleus Accumbens Shell

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Background: Most adolescents will consume alcohol. The biological consequences of the normal experimentation with alcohol and drugs of abuse during adolescence is enhanced by binge alcohol consumption. Adolescent Binge Alcohol Exposure (ABAE; alcohol BEC levels > 80 mg%) produces persistent alterations in the adult brain that increases the likelihood of adult alcohol consumption and self-administration of other drugs of abuse. Previous research has indicated that when cocaine is microinjected into the posterior VTA, there is an increase in extracellular levels of dopamine (DA) within the Nucleus Accumbens Shell (AcbSh). The current experiment examined the effects of ABAE on the ability of cocaine microinjected into the posterior VTA to stimulate DA release in the AcbSh.

Methods: Alcohol preferring (P) rats were given 24-hr free-choice access to water, 15%, and 30% EtOH from post-natal day (PND) 28-48. The control group (CON) was only given access to water during this time. From PND 48-89, rats were paired housed in standard shoebox cages. On PND 90, rats were surgically implanted with a guide cannula aimed at the posterior VTA and AcbSh (ipsilateral). All animals were handled and monitored for health. On PND 98, all rats had a microdialysis probed inserted

into the AcbSh the night before the microinjection-microdialysis experiment. ABAE and CON were microinjected with 0 (aCSF), 25, 100, or 250 pmol cocaine HCL into the posterior VTA (30 microinjections over a 5 min period; 5 sec pulse microinjection – 15 sec timeout). Dialysis samples from the AcbSh were collected every 20 minutes. Sampling occurred 2 hours before cocaine microinjections and 4 hours after.

Results: Adolescent P rats consumed more than 7 g/kg/day EtOH, which has been shown to produce BECs > 80 mg%. In CON rats, 100 and 250 pmol cocaine microinjected into the posterior VTA increased DA in the AcbSh (50 and 82%) for 40 or 60 minutes. ABAE P rats microinjected with 25, 100 or 250 pmol cocaine into the posterior VTA increased DA in the AcbSh (126%, 192%, and 164%, respectively) in the AcbSh for 80 or 100 minutes.

Conclusions: The data demonstrate that ABAE produced neuroadaptations within the posterior VTA that increased the sensitivity to, and responsiveness to, cocaine. The cross-sensitization to other drugs of abuse produced by ABAE indicates that ABAE produces neuroadaptations that mediate a common system that is activated by drugs of abuse. Future research must determine the biological neuroadaptations produced by ABAE that mediate these effects.

Keywords: Adolescence, Adolescent Alcohol, Cocaine Sensitivity, Dopamine, Microdialysis

Disclosure: Nothing to disclose.

P722. Novel Positive Allosteric Modulators Augment 5-HT2A Receptor Functionality

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Background: The serotonin 5-HT2A receptor (5-HT2AR) is a modulator of cortical circuitry and serotonergic psychedelics (e.g., psilocybin) are inviting attention based upon the postulation that 5-HT2AR agonists may "liberate" cortical dysfunction in disease states. The selective chemotype targeting of the 5-HT2AR is challenging given the similarity of the orthosteric sites across the 5-HT2R family. Targeting 5-HT2AR allosteric site(s), which differ from the orthosteric site for endogenous 5-HT, creates new opportunities to optimize 5-HT2AR signaling in disorders marked by cortical dysfunction. We have crystalized a chemical biology strategy to potentiate 5-HT2AR functional responses with positive allosteric modulators (PAMs). Herein, we report the first-generation series of 5-HT2AR PAMs, unlocking an entirely new and innovative line of investigation.

Methods: Newly designed and synthesized molecules were screened for efficacy to enhance 5-HT-induced intracellular calcium (Cai2+) efflux in stable Chinese hamster ovary (CHO) cell lines expressing the human (h) 5-HT2AR relative to h5-HT2BR or h5-HT2CR cell lines. Selected 5-HT2AR PAMs (CTW0404, CTW0419) were evaluated in a series of in vitro adsorption, distribution, metabolism and excretion (ADME) and secondary competition binding. Drug metabolism and pharmacokinetics (DMPK), and blood-brain barrier penetration analyses were conducted in male Sprague-Dawley rats. In rats trained to discriminate the 5-HT2AR agonist 2,5-dimethoxy-4-iodoamphetamine [(-)-DOI] from saline in a two-lever, water-reinforced task, we assessed the PAMs in substitution and potentiation tests. Rats were also monitored for the head twitch response following treatment with 5-HT2AR PAMs as a proxy for hallucinogenic potential. In silico molecular docking with receptor X-ray crystal structures was used to identify potential PAM binding sites.

Results: CTW0404 and CTW0419 failed to exhibit intrinsic activity in the three cell lines, but did potentiate 5-HT-evoked Cai2+ in the 5-HT2AR-, but not the 5-HT2BR or 5-HT2CR, cells. In vitro ADME analyses demonstrated that CTW0404 and CTW0419 possess drug-like qualities, including long half-lifes (T1/2) and limited cytochrome P450 (CYP450) enzyme inhibition. CTW0419, but not CTW0404, is predicted to be a p-glycoprotein substrate. In vivo DMPK studies detected both compounds in rat brain with a greater brain to plasma ratio for CTW0404, relative to CTW0419. Neither CTW0404 nor CTW0419 substituted for the discriminative stimulus of (-)-DOI nor did these compounds evoke head twitches. In silico molecular docking has identified potential binding site to less conserved sites, rather than the orthosteric binding pocket.

Conclusions: We have designed, synthesized, and evaluated a novel series of 5-HT2AR PAMs with promising profiles in vitro and in vivo. Our ongoing studies are focused on providing the basis for choosing new molecular entities with optimized profiles, necessary for medication candidate selection and preclinical development.

Keywords: 5-HT2A Receptor, Positive Allosteric Modulators, Drug Discovery

Disclosure: Nothing to disclose.

P723. Region-Specific Effects of Psilocin on Neuronal Activity in Male and Female Rodents

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Background: The psychedelic compound psilocybin is currently characterized as a drug of abuse and is listed as a Schedule I controlled substance by the United States Drug Enforcement Agency. Recent work suggests that psilocybin, or it's active metabolite psilocin, may have therapeutic potential in the treatment of psychiatric conditions like anxiety, depression, and substance use disorders. Despite the clinical potential of drugs like psilocybin as novel therapeutics, the underlying cellular actions and brain region-specific effects of these drugs remain poorly understood.

Methods: Electrophysiological recording in prefrontal cortex (PFC) and central amygdala (CeA) neurons were performed in male and female C57BL6/J mice (Jackson) and or 5-HT2AR-eGFP-CreERT2 mice (provided by Bryan Roth). Fiber photometry recordings using genetically-encoded calcium sensors (jGCaMP7f) injected into the CeA were performed in male and female Sprague Dawley rats. Paired or unpaired t-tests will be used to compare means for single-factor analyses. Two-way ANOVAs (with sex and drug exposure as between-subjects factors) were used to compare differences among conditions and groups. Repeated measures ANOVAs were used where appropriate.

Results: Focal application of psilocin (10 uM) produced variable changes in firing in PFC neurons from C57Bl6/J mice but consistent increases in firing were observed in 5-HT2A PFC neurons from 5-HT2AR-eGFP-CreERT2 mice. No sex differences in the effects of psilocin on PFC firing were observed. In contrast, focal psilocin application produced consistent decreases in firing in non-specified and 5HT2A CeA neurons from C57Bl6/J and 5-HT2AR-eGFP-CreERT2 mice, respectively. Psilocin effects on firing in both the PFC and CeA were mediated by the 5HT2A receptor. Calcium dynamics in the CeA of male and female rats produced consistent increases in time-locked CeA reactivity to an aversive air puff stimulus. Administration of psilocin (2 mg/kg, sc) increased stimulus-locked CeA reactivity in female but not male rats as compared to vehicle controls. Male rats displayed consistent reductions in stimulus-locked CeA reactivity that persisted for

28 days. Sex differences in stimulus-locked behavioral responses (active and passive) and effects of psilocin on stimulus-locked behavioral response were also observed.

Conclusions: Collectively, these findings provide important brain region- and context-specific effects of the psychedelic compound psilocin, providing an improved mechanistic understanding of the neurobiology of these compounds which is essential for therapeutic consideration.

Keywords: Amygdala, Medial Prefrontal Cortex, Psychedelics, Psilocybin, Psilocin

Disclosure: Nothing to disclose.

P724. (S)-Ketamine Reinforcement and Abuse Liability in Rats is Mediated by Mu Opioid Receptor Activation

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Background: (S)-ketamine is an enantiomer of (R,S)-ketamine and an FDA-approved medication for treatment-resistant depression and major depressive disorder with acute suicidal ideation or behavior. (S)-ketamine is regarded as a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, but it also binds to and activates the mu opioid receptor (MOR) in vitro. However, the contribution of MORs to (S)-ketamine's in vivo pharmacological actions and intravenous self-administration (IVSA), a method used to screen drugs for potential abuse liability, is not well understood.

Methods: We implanted adult rats ($n = 8$) with jugular vein catheters. Rats underwent (S)-ketamine IVSA (0.5 mg/kg/infusion) and their IVSA dose-response curve was determined. Rats were then exposed to additional IVSA sessions at the unit dose (0.25 mg/kg/infusion, corresponding to the peak of the ascending limb of the dose-response curve) that were preceded by either saline (vehicle), naltrexone (3 mg/kg, SC), or the NMDA antagonist MK-801 (0.1 mg/kg, SC) pretreatment 15 min prior to IVSA.

Functional ultrasound imaging (fUSI), a neuroimaging modality used to measure brain-wide changes in cerebral blood volume (CBV) was then performed. Rats ($n = 10$) were pretreated with saline or naltrexone (3 mg/kg, SC) followed by a single IV injection of saline or 0.5 mg/kg (S)-ketamine 15 min later while imaging the mPFC and NAc.

We used positron emission tomography (PET) and [18 F] fluoroethyl-diprenorphine (FE-DPN) to examine in vivo MOR occupancy by a reinforcing dose of IV (S)-ketamine. Rats ($n = 12$) were anesthetized and then injected IV with a bolus of [18 F]FE-DPN (~650 μCi) followed immediately by PET imaging acquisition while receiving an IV infusion of saline ($n = 6$) or (S)-ketamine ($n = 6$, 20 mg/kg/h). Next, we exposed these rats to 8 daily sessions of IV saline or (S)-ketamine infusions (20 mg/kg/h, 60 min) and scanned them on day 9 using PET and [18 F]FE-DPN. To confirm that the BPND changes were due to lower MOR density, the rats were euthanized 24 h after PET and MOR density was assessed using [3H]DAMGO. Adjacent brain sections were exposed to DAMGO- (10 μM) or (S)-ketamine-stimulated (10 μM) [35 S] GTPγS autoradiography. Kappa opioid receptor (KOR) and NMDA receptor densities were assessed using [3H]U69,593 and [3H] MK801.

Rats were then exposed to 8 daily IV saline ($n = 9$) or (S)-ketamine ($n = 10$) (20 mg/kg/h, 60 min) infusions. On day 9, rats started daily 3-h training sessions for heroin IVSA (5 days at 100 μg/kg/infusion, 5 days at 50 μg/kg/infusion). After training, we performed a dose-response assessment of heroin IVSA.

Results: Rats acquired (S)-ketamine IVSA and showed an inverted U-shaped dose-response function with highest responding to a 0.25 mg/kg/infusion unit dose. Naltrexone ($t(7)=6.47$; $P = 0.03$) and MK-801 ($t(7)=6.65$; $P = 0.03$) significantly decreased (S)-ketamine infusions at this dose.

Naltrexone pretreatment significantly decreased (S)-ketamine-induced CBV increases in the nucleus accumbens (NAc) core (corrected $P = 0.03$) and shell (corrected $P = 0.036$).

Compared to saline, acute (S)-ketamine significantly decreased [18 F]FE-DPN BPND across several brain regions, indicating that reinforcing doses of (S)-ketamine occupy MOR in these regions in vivo. Repeated IV (S)-ketamine led to significant decreases in [18 F]FE-DPN BPND in NAc ($P = 0.018$), thalamus ($P < 0.0001$), superior colliculus ($P = 0.001$), ventral midbrain ($P = 0.0042$), medulla ($P = 0.0032$) and periaqueductal gray ($P < 0.0001$). Consistent with PET, (S)-ketamine exposure significantly decreased [3H]DAMGO binding in mPFC ($t(8) = 2.681$, $P = 0.028$), NAc ($t(8) = 2.45$, $P = 0.04$), and thalamus ($t(8) = 2.4$, $P = 0.043$) compared to control. DAMGO increased [35 S]GTPγS binding in both saline and (S)-ketamine-infused rats but its effect on [35 S]GTPγS binding in (S)-ketamine-infused rats was significantly lower compared to saline-infused rats in NAc ($t(8) = 4.066$, $P = 0.0036$). 10 μM (S)-ketamine increased [35 S]GTPγS binding in saline-treated rats in mPFC ($t(3) = 6.05$, $P = 0.009$), CPu ($t(3) = 4.971$, $P = 0.016$) and NAc ($t(3) = 3.4$, $P = 0.042$). (S)-ketamine did not significantly increase [35 S]GTPγS binding in rats exposed to repeated (S)-ketamine infusions. (S)-ketamine did not affect KOR or NMDA receptor density.

Repeated IV (S)-ketamine significantly increased subsequent heroin IVSA and intake. Both saline and (S)-ketamine-infused rats acquired heroin IVSA, but rats infused with repeated (S)-ketamine had significantly more heroin infusions compared to saline-infused rats (2-way RM ANOVA: session x group interaction: $F(9, 153) = 2.96$; $P = 0.002$). Heroin infusions for saline- and (S)-ketamine-infused rats followed the typical inverted U-shaped function, but (S)-ketamine-infused rats had significantly greater infusions than saline-infused rats, specifically at the peak (6.25 and 12.5 μg/kg) unit doses (Mixed-effects ANOVA: dose x group interaction: $F(6,97) = 2.98$; $P = 0.01$) indicating that repeated exposure to reinforcing doses of (S)-ketamine increased subsequent heroin IVSA.

Conclusions: Our findings indicate that (S)-ketamine, at reinforcing, self-administered doses, rapidly binds to and activates MORs in the mPFC, NAc and other brain regions in vivo. Upon repeated exposure, (S)-ketamine induced MOR desensitization and increased heroin intake, likely due to tolerance. Our results suggest that MOR activation contributes to (S)-ketamine self-administration, and possibly its abuse liability.

Keywords: Esketamine, Mu-Opioid Receptors, Heroin Self-Administration

Disclosure: Nothing to disclose.

P725. Fos-Expressing Neuronal Ensembles in Rat Nucleus Accumbens Encode Initial Cocaine Seeking in Rats

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Background: Synchronized activity of neuronal ensembles within the (NAc) has been shown to drive cocaine seeking in well-trained rats. However, it is unclear how rapidly this circuitry is recruited to control cocaine-seeking behavior. Here, we tested the necessity of NAc neuronal ensembles in initial cocaine seeking behavior using the Daun02 inactivation procedure.

Methods: We trained male and female transgenic Fos-LacZ rats to self-administer cocaine for 3 hr daily sessions until rats met acquisition criteria (>30 active lever presses, >75% responding on active lever). Upon meeting acquisition criteria, we subjected rats to an additional 30 min cocaine self-administration session to reactivate neuronal ensembles associated with cocaine-seeking behavior. Ninety min later, we infused Daun02 to selectively inactivate NAc Fos expressing ensembles activated by cocaine self-administration. We tested the rats' cocaine seeking behavior 2 days later in a 30 min non-reinforced recall test.

Results: Rats readily learned to self-administer cocaine and all rats reached acquisition criteria within 9 days. The average number of sessions to reach acquisition criteria was 4.1 sessions. There were no differences between groups that would go on to receive vehicle or Daun02 on induction day. We found that inactivation of NAc neuronal ensembles with Daun02 reduced cocaine-seeking after initial cocaine self-administration on test day ($t32 = 2.2$, $p = 0.03$). Daun02 infusions decreased Fos-expression after the test, indicating ablation of Fos-expressing neuronal ensembles by Daun02.

Conclusions: These results suggest that NAc neuronal ensembles are formed during initial learning of cocaine self-administration and required for initial cocaine-seeking behavior.

Keywords: Stimulants, Cocaine Self-Administration, Memory and Learning

Disclosure: Nothing to disclose.

P726. Differential Sensitivity to Punishment by Signaled Versus Unsignaled Footshock in Male and Female Rats Self-Administering MDPV

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Background: When allowed to self-administer 3,4-methylenedioxypyrovalerone (MDPV), or structurally related synthetic cathinones, approximately 30% of rats ("high-responders") develop unusually high levels of dysregulated drug-taking similar to the binge-like patterns of cathinone (e.g., "bath salts") use that has been reported in humans. The goals of the current studies were threefold: 1) to assess whether high-responder rats were less sensitive to punishment of MDPV-maintained responding than low-responder rats; 2) to evaluate if the punishing effects of footshock were altered when a stimulus signaling an impending footshock was introduced; and 3) to determine if these effects varied as a function of sex or the unit-dose of MDPV available for self-administration.

Methods: Adult male ($n = 20$) and female ($n = 20$) Sprague-Dawley rats were surgically prepared with an indwelling venous catheter and trained to self-administer MDPV (0.032 mg/kg/inf) under an FR1:TO5-sec schedule of reinforcement during daily 90-min sessions. Once responding stabilized under an FR5:TO5-sec schedule of reinforcement, rats were categorized as high- or low-responders based on the proportion of active lever responses that occurred during the post-infusion timeouts (≥20% = high responder, <20% = low responder). Inhibition functions were first generated for unsignaled footshock (0.05-0.9 mA; paired with ~50% of infusions, and increasing intensity across sessions) in rats self-administering 0.01, 0.032, and 0.1 mg/kg/infusion MDPV. Subsequently, rats were then trained on a signaled footshock procedure, in which a stimulus (flashing light) was introduced after two responses to signal to the rat that completion of the FR5 would result in an infusion paired with a footshock (intensities individualized to each rat's IC50); footshocks could be avoided by withholding responding for 30 sec. After rats acquired the signaled shock procedure, footshock intensities were systematically varied (0.05-0.9 mA; increasing across sessions) to generate inhibition functions for signaled footshock in rats self-administering 0.01, 0.032, and 0.1 mg/kg/infusion MDPV.

Results: When footshocks were unsignaled, sensitivities to the punishing effects of footshock were not different between high- and low-responder rats (mean IC50s- high-responders: 0.30 mA; low-responders: 0.36 mA); however, high-responder rats received significantly more total current than low-responder rats (mean: 45 mA vs 18 mA, respectively). When footshocks were unsignaled, high-responder rats were less good at withholding responding during signaled trials and avoided fewer footshocks than low-responder rats. Compared to low-responder rats, high-responder rats responded more in the presence of the signal indicating impending shock and avoided a smaller proportion of shocks. Under both signaled and unsignaled conditions, the effectiveness of footshocks to punish responding was inversely correlated with the unit-dose of MDPV available for infusion. Though the pattern of effects did not differ, females were more sensitive to punishment by footshock than males.

Conclusions: Even though the punishing effects of footshock did not differ between high- and low-responder rats when they were unsignaled, providing information (a signal) that continuing to respond would result in punishment resulted in clear differences between the phenotypes with high-responders avoiding punishment at a significantly lower frequency than low-responder rats. Together, these studies suggest that signaled procedures might provide a more sensitive measure of punishment, and that the high levels of drug-taking observed in the high-responder rats represent a compulsive-like pattern of responding, which may be related to the DSM criteria for substance use disorder, continued use despite adverse consequence.

Keywords: MDPV Self-Administration, Punishment, Sex Differences

Disclosure: Nothing to disclose.

P727. Metformin Effects on Cocaine Conditioned Reward in Male and Female Rats

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Background: Cocaine use disorder (CUD) remains a significant problem in the United States as cocaine-associated overdoses rates have risen to dramatic levels in recent years. There is no current FDA-approved treatment for CUD leading to high attrition rates for those in recovery, and therefore high rates of relapse and overdose. We aim to investigate the therapeutic potential of metformin, a Type II Diabetes drug, to influence the rewarding effects of cocaine in a conditioned place preference (CPP) model. Metformin acts as an indirect activator of adenosine monophosphate activated protein kinase (AMPK), a metabolic energy sensor responsible for maintaining intracellular homeostasis after a stressor depletes ATP levels. When activated by phosphorylation, pAMPK rebalances the ratio of AMP to ATP throughout the body as well as in the brain. Recent studies have shown a decrease in pAMPK within the nucleus accumbens core (NAc) after repeated cocaine use. Our lab recently demonstrated that metformin microinjection into the NAc can reduce cue-induced reinstatement of cocaine seeking. The current experiments extend this study to the systemic administration of metformin as a more translational approach to drug delivery.

Methods: Cocaine conditioned place preference (CPP) was performed in adult male and female Sprague Dawley rats in a 2-chamber box (MedAssociates). Rats were conditioned for 5 days in twice daily 30-minute sessions, control and treatment, 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.

Results: Sex differences were observed in the conditioned rewarding effects of cocaine with female rats ($n = 11$; $p = 0.0002$ for cocaine vs saline paired chamber during post-test) but not male rats ($n = 9$; $p = 0.19$ for cocaine vs saline paired chamber during post-test) acquiring CPP for a 20 mg/kg dose of cocaine. Data were analyzed by two-way repeated measures analysis of variance (ANOVA) followed by Sidak's multiple comparisons. Preliminary data show a lack of CPP in males ($n = 4$; $p = 0.83$) and females ($n = 8$; $p = 0.86$) for a 10 mg/kg conditioning dose of cocaine. A pretreatment of 175 mg/kg metformin had no effect on CPP for 20 mg/kg cocaine in female rats. In ongoing studies, we will add animals to increase power and complete dose response analysis for cocaine and metformin.

Conclusions: Dose-dependent effects of cocaine conditioning have been observed in female and male rats. We are interested in determining if a similar dose-dependent effect for metformin pretreatment may be observed. Our previous research suggests increased sensitivity to the effects of metformin in female rats. We find no effect of metformin pretreatment on acquisition of cocaine CPP in our preliminary results, but aim to further assess metformin effects on expression or reinstatement of this behavior. The continuation of this study will inform the potential development of metformin as a pharmacotherapy for cocaine use disorder.

Keywords: Cocaine, Adenosine Monophosphate Activated Protein Kinase (AMPK), Conditioned Place Preference, Cocaine Sex Differences, Metformin

Disclosure: Nothing to disclose.

P728. Interactions Between Gabapentinoids and Opioids in Rats

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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, primarily those involving opioids. The number of gabapentinoid prescriptions is also on the rise, with the majority of these prescriptions 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 effects of opioid drugs. This study examined the effects of gabapentinoids on heroin-induced ventilatory depression and its reversal by naloxone, and on self-administration of heroin under a progressive ratio schedule of reinforcement.

Methods: Five male Sprague Dawley rats were pretreated with gabapentin (10-100 mg/kg), pregabalin (1-32 mg/kg), or saline i.v. 30 minutes prior to receiving increasing doses of heroin while ventilation was measured using whole-body plethysmography. Three infusions of heroin were administered at 3-minute intervals resulting in cumulative doses of 0.1, 0.32, and 1.0 mg/kg, respectively. Five minutes following the third heroin infusion animals received saline or naloxone (0.0056-0.01 mg/kg). The primary outcome of this study was minute volume, the total volume of air ventilated per minute. In a second experiment seven male and seven female Sprague Dawley rats were trained to self-administer heroin (0.01 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 heroin self-administration and stable responding for the training dose of heroin under the progressive ratio schedule of reinforcement saline was substituted for heroin until behavior stabilized at fewer than 5 infusions per session. Then, animals were randomized to a dose of heroin (0.0032-0.1 mg/kg/infusion). Animals self-administered each dose of heroin for at least 3 days until consecutive days differed by no more than 1 infusion earned. The following day animals were pretreated with gabapentin (1-10 mg/kg) or pregabalin (0.1-1 mg/kg) i.v. 15 minutes prior to the self-administration session. Heroin unit dose and pretreatment condition were evaluated in a pseudo-random order until heroin 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 was compared across pretreatment conditions and sex¬. All procedures were approved by the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee.

Results: Heroin dose-dependently reduced minute volume with 1 mg/kg suppressing minute volume to <50% of baseline in all animals. Pretreatment with gabapentin or pregabalin did not significantly alter the effects of heroin on minute volume. Naloxone dose-dependently reversed the ventilatory depressive effects of heroin with 0.0056 mg/kg restoring ventilation to baseline levels. Following pretreatment with gabapentin (100 mg/kg) or pregabalin (32 mg/kg) this dose of naloxone was no longer sufficient to restore normal ventilation when administered 5 minutes after the largest dose of heroin. A larger dose of naloxone (0.01 mg/kg) was required to return minute volume to baseline levels. Animals self-administered heroin in a dose-dependent fashion with breakpoints significantly greater than for saline. In most animals 0.032 mg/kg/infusion of heroin maintained the highest breakpoint and number of infusions earned. Gabapentin increased the reinforcing effects of heroin in some, but not all animals.

Conclusions: Pretreatment with gabapentinoids did not alter the ventilatory depressive effects of heroin but reduced the potency of naloxone to reverse heroin-induced ventilatory depression. Gabapentinoids increased the reinforcing effects of heroin in some subjects, consistent with reports in humans that gabapentinoids can enhance the positive/euphoric effects of opioids. Future studies will expand these findings to other opioids such as fentanyl and confirm the selectivity of the enhancement of the reinforcing effects of heroin for opioids by determining the effects of gabapentinoids on self-administration of fentanyl and cocaine. The current study suggests that there might be significant interactions between gabapentinoids and opioids related to both opioid overdose and substance misuse that warrant further investigation.

Keywords: Opioid Abuse, Opioid Overdose, Drug-Drug Interaction, Gabapentin, Pregabalin

Disclosure: Nothing to disclose.

P729. Effects of Methocinnamox, a Long-Acting Mu Opioid Receptor Antagonist, on Fentanyl Choice in Monkeys Responding Under a Food Versus Drug Choice Procedure

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Background: Methocinnamox (MCAM), a long-acting mu opioid receptor antagonist, attenuates the reinforcing and ventilatory-depressant effects of opioids in rats and nonhuman primates, suggesting it could be an effective treatment for opioid use disorder and overdose. Previous studies examined effects of MCAM on heroin and fentanyl self-administration under fixed-ratio schedules of drug reinforcement. The current study extends characterization of the potential therapeutic utility of MCAM to examine effects on responding under a food versus drug choice procedure wherein changes in reinforcing effects are evaluated by changes in behavioral allocation from responding for drug to responding for a non-drug alternative such as food. Most studies to date administered MCAM within 1 hr of the test session. This study also compared pretreatment times to determine whether the duration of action of MCAM depends on temporal proximity of MCAM administration to test sessions.

Methods: Five rhesus monkeys (2 females and 3 males) lever-pressed for food or i.v. infusions of fentanyl under a concurrent fixed-ratio 30 schedule of reinforcement. Daily 4-hr sessions were divided into blocks with each block comprising 2 forced trials followed by up to 6 choice trials. Responding on one lever delivered a 300-mg sucrose pellet, whereas responding on the other lever delivered a unit dose of fentanyl that increased across blocks each session from 0.1 to 3.2 µg/kg/infusion. MCAM (1.0 mg/kg) was administered i.v. either 1 or 20 hr prior to test sessions, and, for comparison, the opioid receptor antagonist naltrexone (0.1 mg/kg) was administered i.v. 15 min prior to a test session.

Results: Choice of fentanyl increased with increasing unit doses of fentanyl from less than 10% infusion choice with unit doses of 0.1 and 0.32 µg/kg/infusion to greater than 90% infusion choice with unit doses of 1.0 and 3.2 µg/kg/infusion. Naltrexone decreased choice of fentanyl on the day of treatment, shifting the fentanyl dose-effect curve for percent infusion choice rightward and downward; naltrexone was no longer effective 24 hours later with choice returning to control. MCAM also decreased choice of fentanyl; however, effects of MCAM lasted for 2 to 3 days following a single injection. The time to recovery of responding following MCAM administration did not differ across pretreatment times.

Conclusions: Fentanyl dose-dependently increased choice of drug and decreased choice of food with increasing unit doses. Naltrexone and MCAM treatment decreased choice of fentanyl and prompted a reallocation of responding toward food. These results confirm that effects of MCAM in decreasing responding for opioids under other conditions is selective insofar as in this study responding for food increased when responding for fentanyl decreased. Effects of naltrexone lasted less than 24 hr, whereas effects of MCAM lasted for several days. The duration of effect of MCAM did not appear to differ between pretreatment times. These data are consistent with previous studies showing long-lasting and selective attenuation of opioid self-administration. Demonstration that MCAM promotes the reallocation of responding from drug to a non-drug reinforcer provides additional support for the notion that MCAM might be a safe and effective treatment for opioid use disorder.

Keywords: Opioid Abuse, Opioid Antagonist Treatment, Nonhuman Primates, Choice Procedure, Fentanyl

Disclosure: Nothing to disclose.

P730. Zinc Modulates Cocaine-Related Behaviors and Dopamine Transmission as a Function of Sex

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Background: Zinc (Zn2+) is an essential life element that is implicated in neurophysiological homeostasis, and its dysregulation is associated with various disorders, such as substance use disorder (SUD). Within a subset of glutamatergic neurons, synaptic Zn2+ is co-released with glutamate. The striatum receives a dense innervation of Zn2 + -containing neurons (zincergic). Previous studies showed that synaptic Zn2+ is an endogenous modulator of striatal dopamine neurotransmission. Moreover, Zn2+ potentiates the effects of cocaine by binding the dopamine transporter (DAT), resulting in the enhancement of cocaine-mediated behaviors. For instance, we reported that the absence of synaptic Zn2+ reduces conditioned place preference, locomotor sensitization and self-administration to cocaine. Interestingly, previous evidence demonstrated that estrogen reduces total and synaptic Zn2+ in the brain. In line with these findings, Zn2+ exerts sexually dimorphic effects on locomotion and at skilled motor learning tasks. However, the interaction between synaptic Zn2+ and sex on dopamine transmission has not been characterized. In addition, we investigated the role of Zn2+ in the dorsal striatum (DS) in the development and expression of behavioral sensitization by cocaine. Finally, we studied the proportion of zincergic neurons that project to the striatum.

Methods: To study if Zn2+ alters dopamine transmission in the striatum in a sex dependent manner, we conducted autoradiography assays in brain tissue from C57BL/6 J female and male mice ($n = 5$/group). We used the cocaine analog [3H]WIN-35,428 and [3H]SCH23390 to quantify binding to the dopamine transporter (DAT) and dopamine D1 receptor (D1R) respectively, in the presence of physiological concentrations of Zn2 + . Then, to study the role of Zn2+ on cocaine-induced locomotor sensitization, we bilaterally microinjected saline or TPEN (Zn2 + chelator) into the striatum of C57BL/6 J female and male mice ($n = 8$/group), followed by cocaine injections (10 mg/kg; i.p.) for 5 days. After 2 weeks of withdrawal, mice were challenged with cocaine (10 mg/kg; i.p.) to study the expression of the locomotor sensitization. Finally, in order to study the proportion of glutamatergic/zincergic neurons that project to the striatum. We combined retrobeads and fluorescent in-situ hybridization (RNAscope), this approach allows to trace the neurons projecting to the striatum, and of those, what proportion are zincergic in the cerebral cortex, amygdala, and paraventricular thalamus of C57BL/6 J mice ($n = 4$).

Results: Our autoradiography analysis revealed that [3H]WIN-35,428 binding to DAT was significantly lower in the DS of male compared to female mice ($t = 3.08$, $p = 0.015$). Interestingly, physiological concentrations of Zn2 + (10 µm) significantly increased [3H]WIN-35,428 binding to DAT in both female ($t = 8.490$, $p = 0.001$) and male mice ($t = 4.98$, $p = 0.001$). Notably, this effect was larger in males than in female mice ($t = 4.06$, $p = 0.003$). We observed no significant differences in [3H]SCH23390 binding to the D1R neither between female and male mice ($t = 0.94$, $p = 0.37$) nor in the presence of Zn2 + ($t = 0.01$, $p = 0.82$). We also found that bilateral Zn2+ chelation in the DS of mice significantly decreased the development and expression of the locomotor sensitization in male ($p = 0.001$ by post hoc Tukey comparison) but not in female mice. Finally, by using RNAscope, our preliminary results show that in the cerebral cortex, zincergic

neurons account for 55% of glutamatergic neurons that project to the striatum.

Conclusions: Our findings suggest that part of the effects of Zn2+ on dopamine transmission is by binding the DAT and not the D1R, and this effect is dependent on sex. In addition, our results suggest that Zn2+ augments the locomotor activity induced by cocaine in male mice. Finally, we show that a population of cortical neurons that project to the striatum bear the machinery to release Zn2+ in the striatum. Further studies will address the zincergic circuits that modulate cocaine-seeking behaviors.

Keywords: Cocaine, Dopamine Transporter, D1 Dopamine Receptors, Zinc

Disclosure: Nothing to disclose.

P731. Prenatal Heroin Exposure Alters Brain Morphology and Connectivity in Adolescent Mice

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Background: The United States is experiencing a dramatic increase in maternal opioid misuse and, consequently, the number of individuals exposed to opioids in utero. Prenatal opioid exposure has both acute and long-lasting effects on health and wellbeing. Effects on the brain, often identified at school-age, manifest as cognitive impairment, attention deficit, and reduced scholastic achievement. Understanding the neurobiological basis for these deficits is critical to identifying affected individuals and developing effective interventions. Here, we examine how in utero exposure to the highly addictive and widely misused opioid heroin affects brain development into early adolescence in a mouse model.

Methods: Pregnant C57BL/6 J mice received escalating (1-10 mg/kg, 10 ml/kg, s.c.) doses of heroin twice daily on gestational days 4-18. Control mice received twice daily vehicle injections (physiological saline, 10 ml/kg, s.c.) or no intervention. Litters remained in their home cages with the dam and were not weaned. The brains of offspring were assessed on postnatal day 28 using 9.4 T diffusion magnetic resonance imaging (MRI) of postmortem specimens at 36 micron resolution. Whole brain metrics and the metrics of 166 bilateral regions (332 ROIs) were compared between randomly selected heroin exposed (NHeroi$n$ = 14 (10 male, 4 female) and control (Ncontrol = 14 (8 male, 6 female)) offspring. Two approaches were employed in the comparison of the brains of heroin exposed and control mice: (1) A comparison of scalar phenotypes was conducted to identify changes in global and regional brain volumes, and (2) Connectome/tractography analysis was performed to identify differences in global and regional connectivity. To compare group-mean volumes for every atlas-defined brain region, we used the Kruskal–Wallis analysis of variance with the Benjamini-Hochberg false discovery rate correction. To compare group-mean global and regional connectomes, we used a dimension reduction strategy and multivariate analysis of variance with the Bonferroni correction.

Results: Offspring from heroin-exposed and control dams did not differ in body weight (heroin-exposed mean ± SD: 13.2 ± 2.75 g; control mean ± SD: 13.8 ± 1.25 g; two-sided Wilcoxon Rank Sum test, p = 0.4079). Whole brain volume, however, was reduced in heroin-exposed mice (mean ± SD: 411 ± 34.6 mm3), as compared to controls (mean ± SD: 447 ± 9.41 mm3; two-sided Wilcoxon Rank Sum test, p = 0.0063). After standardizing for whole brain volume, we identified bilateral heroin-associated volume changes in 29 regions (Benjamini-Hochberg adjusted p-values <0.05). Regions with bilaterally reduced standardized volumes in heroin-exposed offspring relative to controls include the ectorhinal and insular cortices. Regions with bilaterally increased standardized volumes in heroin-exposed offspring relative to controls include the periaqueductal gray, septal region, striatum, and hypothalamus. Leveraging microscopic resolution diffusion tensor imaging and precise regional parcellation, we generated whole brain structural MRI diffusion connectomes. Global brain connectivity was unchanged between the heroin-exposed and control offspring. In individual connectome profiles of all 332 ROIs, a single region met the Bonferroni criteria: the left septal region, a region that acts as a hub for limbic regulatory actions.

Conclusions: Consistent with clinical evidence, our findings suggest that prenatal opioid exposure may have effects on brain morphology, connectivity, and, consequently, function that persists into adolescence. Future studies are needed to dissect how stress, postnatal mother-pup interactions, and offspring sex interact with gestational opioid exposure to bring about changes in brain structure and behavior. This work expands our understanding of the risks associated with opioid misuse during pregnancy and identifies biomarkers that may facilitate diagnosis and treatment.

Keywords: Prenatal Drug Exposure, Opioids, Magnetic Resonance Imaging, Mouse Models, Brain Structural Connectivity

Disclosure: Nothing to disclose.

P732. Activation of 5-HT1B Receptors Attenuates the Acquisition of Nicotine Reward in Adolescent Male Rats

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Background: Nicotine addiction continues to be a significant concern, particularly in adolescent populations. Activation of serotonin (5-HT)1B receptors decreases the rewarding and reinforcing effects of stimulant drugs, such as cocaine and methamphetamine. However, the result of 5-HT1B activation on nicotine reward has not been examined. In the present study, we examined the hypothesis that administering CP 94,253, a 5-HT1B receptor agonist, would reduce nicotine preference in adolescent male rats using a 10-day Conditioned Place Preference (CPP) procedure, a well-established animal model of drug reward.

Methods: On postnatal day (PD) 28, baseline preference for a two-sided apparatus was assessed during a 15 min session. In two-day cycles, rats received an injection of CP 94,253 (0 or 5.6 mg/kg, IP) 15 min before the administration of nicotine (0, 0.2, or 0.6 mg/kg, n = 12-14) on one day and saline (n = 12-14) administration on the other day before being confined to one side of the two-chamber apparatus for 15 min. This two-day cycle was repeated over the next 6 days. On day 10, the preference for the nicotine-paired chamber was assessed for 15 min.

Results: Rats exhibited nicotine-induced CPP when conditioned with either 0.2 or 0.6 mg/kg of nicotine ($p < 0.05$). Administration of CP 94,253 (5.6 mg/kg) before nicotine (0.2 or 0.6 mg/kg) resulted in a decreased preference for the nicotine-paired compartment ($p < 0.05$).

Conclusions: The present findings demonstrate that activation of 5-HT1B receptors with CP 94,253 attenuated the acquisition of nicotine-induced CPP in male adolescent rats. Overall, these findings further add to a growing body of literature that points to the 5-HT1B receptor as a pharmacological target for treating

psychostimulant addiction. Future studies should examine the effects of 5-HT1B receptors on nicotine reward in female rats.

Keywords: Nicotine Addiction, Adolescence, Reward

Disclosure: Nothing to disclose.

P733. The Impact of Eating a High Fat or Ketogenic Diet on Sensitivity of Rats to Dopaminergic Drugs

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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 laboratory chow enhances the sensitivity of rats to the behavioral effects of drugs, including drugs that act on dopamine systems, such as methamphetamine and quinpirole (a dopamine D2/D3 receptor agonist). Previous preclinical research has primarily focused on exploring the effects of traditional high fat diets that are high in fat and carbohydrates on drug sensitivity. In contrast, a ketogenic diet is high in fat, but very low in carbohydrates. While high fat/high carbohydrate diets lead to weight gain, ketogenic diets can promote weight loss. It is not known if eating a ketogenic diet might enhance sensitivity of rats to the behavioral effects of drugs that act on dopamine systems, similarly to what has been shown with traditional high fat/high carbohydrate diets. This project tested the hypothesis that rats eating a high fat/high carbohydrate diet would be more sensitive to the behavioral effects of methamphetamine and quinpirole, but that rats eating a ketogenic chow would not differ from rats eating a standard (low fat) laboratory chow.

Methods: To test this hypothesis, male ($n = 24$) and female ($n = 36$) Sprague-Dawley rats eating high fat chow (60% kcal from fat), ketogenic chow (90.5% kcal from fat) or standard chow (17% kcal from fat) were tested once weekly with cumulative doses of either methamphetamine (0.1-3.2 mg/kg; i.p.) or quinpirole (0.0032-3.2 mg/kg; i.p.). To assess drug sensitivity, methamphetamine-induced locomotion and sensitization were examined, as well as quinpirole-elicited yawning. Average locomotor activity counts and yawning were analyzed using two-way repeated measures ANOVAs with diet and dose as factors, and Tukey or Sidak post hoc comparisons where appropriate.

Results: Rats eating high fat chow were more sensitive to the locomotor-stimulating effects of smaller and intermediate doses of methamphetamine (e.g., 0.32, 1.0 mg/kg) than rats eating standard chow. Rats eating ketogenic chow were also more sensitive than rats eating standard chow to the locomotor-stimulating effects of methamphetamine, but only at the largest dose tested (3.2 mg/kg). Rats eating high fat chow were also more sensitive to quinpirole-induced yawning than rats eating standard chow. In contrast, quinpirole-induced yawning was comparable between rats eating a ketogenic chow and rats eating standard chow.

Conclusions: These results suggest while eating a high fat/high carbohydrate diet can enhance the sensitivity of individuals to dopaminergic drugs, this effect might not translate identically to ketogenic diets. These results add to the growing literature suggesting that ketogenic diets and traditional high fat/high carbohydrate diets differ with regard to their impacts on health.

Keywords: Methamphetamine, Dopamine, High Fat Diet, Ketogenic Diet, Rats

Disclosure: Nothing to disclose.

P734. Sex Differences in GABA Regulation of Dopamine Release in the Nucleus Accumbens and Its Role in Cocaine Use Disorder

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Background: While sex differences in the pervasiveness and prognosis of neuropsychiatric disorders have long been known to exist, there are few instances where approaches to pharmacological treatment of these disorders differ between the sexes, which likely contributes to ineffective treatments in women. In substance use disorder (SUD) women exhibit increased propensity to use drugs, a faster transition to addiction from first use, greater problems maintaining abstinence, and relapse at a higher rate than men. At the center of sex-differences in addiction vulnerability is the mesolimbic dopamine system. While work has focused on sex differences in the anatomy of dopamine neurons and relative dopamine levels, an important characteristic of dopamine release from axon terminals in the nucleus accumbens (NAc) is that it is rapidly modulated by local regulatory mechanisms independent of somatic activity. GABA released from local microcircuitry in the NAc has been shown to play a critical role in regulating dopamine release at the terminals through ionotropic GABA-A and Gi-coupled GABA-B receptors and has also been implicated in cocaine-induced processes. Here we define basal sex differences in dopamine release regulation via GABA in the NAc and show how this is dysregulated by chronic cocaine exposure.

Methods: To dissociate dopamine terminal regulation from somatic regulation we utilize ex vivo fast scan cyclic voltammetry in striatal brain slices. Dopamine release was evoked from terminals, and GABA receptor modulation of this signal was determined via the application of picrotoxin (GABA-A antagonist), muscimol (GABA-A agonist), saclofen (GABA-B antagonist), and baclofen (GABA-B agonist) bath application to slices. This was done at baseline in both males and females as well as after chronic cocaine exposure.

Results: First we found that both GABA-A and GABA-B receptors modulate dopamine release on a rapid time scale directly at the terminals in the NAc. There were sex differences in this regulation, with much greater regulation of dopamine release by GABA receptors in males as compared to females. Importantly, chronic cocaine exposure resulted in receptor-specific plasticity in this microcircuit regulatory mechanism.

Conclusions: The results of these studies will contribute to the understanding of how sex fits into the comprehensive framework for dopamine release regulation and how dysregulation of these processes influences the trajectory of CUD in both males and females.

Keywords: Nucleus Accumbens, Cocaine Use Disorder, GABA-A Receptors, GABA-B Receptors, Fast Scan Cyclic Voltammetry

Disclosure: Nothing to disclose.

P735. Effect of Sex and Age on Nicotine Vapour Pharmacokinetics, Reward, Withdrawal, and Functional Connectivity in Rats

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Background: Nicotine use is constantly evolving, with vaping now making up a significant portion of consumed tobacco products. Though vaping likely represents a safer alternative to smoking, it is not without risks, many of which are not well understood. A particular area that requires attention is the effects vaping has on populations that are especially vulnerable to nicotine, such as women and adolescents. Here we evaluate the sex- and age-dependent vulnerabilities to nicotine vapour in a rat model of nicotine vapour exposure.

Methods: Passive nicotine exposures were conducted via JUUL e-cigarettes via a custom-built OpenVape apparatus. Animals were evaluated for reward-like behaviour in a place conditioning paradigm, locomotion in an open field, precipitated withdrawal following i.p. mecamylamine injection (1.5 mg/kg), nicotine and nicotine metabolite brain and plasma pharmacokinetics, and functional magnetic resonance imaging.

Results: Nicotine and nicotine metabolite plasma and brain levels were similar between adults and adolescents, although females did show higher plasma nicotine and cotinine plasma levels at 10 minutes compared to adolescent females. Adult females had greater nicotine and nicotine metabolite concentrations than adult males in both blood plasma and brain supernatant. This trend was similar in adolescent female brain supernatant but not plasma where results were not significantly different. Female, but not male adults, displayed conditioned place preference (CPP) at a high dose of nicotine vapour. Female adolescents did not acquire CPP at any dose tested. Both adult and adolescent males displayed similar levels of precipitated nicotine vapour-induced withdrawal. Female rats did not display any precipitated nicotine withdrawal. Passive nicotine vapor exposure resulted in hyperlocomotion in both adult and adolescent males, but not females. Functional MRI revealed a single network consisting of 12 edges and 13 nodes that displayed reduced connectivity when controlling for age and sex. An additional significant group by sex interaction effect was found with 5 edges and 6 nodes showing further reduced connectivity in females compared to males.

Conclusions: Our findings suggest that nicotine vapour pharmacokinetics as well as behavioural and neural impacts are affected by both sex and age, with unique reward, withdrawal and pharmacokinetic profiles, as well as functional connectivity changes in regions involved in nicotine cue reactivity, withdrawal, and dependence.

Keywords: Electronic cigarette (e-cigarette), Nicotine Metabolism, Resting State Functional Connectivity, Nicotine Exposure, Nicotine vapor

Disclosure: Nothing to disclose.

P736. Effects of Sex and Estrous Cycle on Intravenous Oxycodone Self-Administration and Stress-Induced or Cue-Induced Reinstatement of Oxycodone Seeking in Rats

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Background: The abuse of prescription and illicit opioids has culminated in a national healthcare crisis in the United States. Oxycodone is among the most widely prescribed and misused opioid pain relievers and has been associated with a high risk for transition to compulsive opioid misuse. While sex differences have been reported for the abuse-related effects of other opioids (e.g., heroin) in both humans and experimental animals, only a small number of studies to date have examined sex differences specifically with respect to oxycodone, with conflicting results reported. Here, we sought to assess potential sex differences in the reinforcing effects of oxycodone, as well as stress-induced or cue-induced oxycodone-seeking behavior, using IV oxycodone self-administration and reinstatement procedures. We also assessed whether these measures varied in females as a function of estrous cycle phase.

Methods: In experiment 1, adult male ($n = 5$) and female ($n = 4$) Long-Evans rats were first trained to self-administer 0.03 mg/kg/infusion oxycodone according to a fixed-ratio 1 schedule of reinforcement in daily 2-hr sessions (5-6 days per week). A dose-response function was then established (0.003 – 0.03 mg/kg/infusion) with doses presented in descending order. In experiment 2, a separate group of adult male ($n = 8$) and female ($n = 17$) Long-Evans rats were trained to self-administer 0.03 mg/kg/infusion oxycodone for 8 days, followed by 0.01 mg/kg/infusion oxycodone for 10 days. Responding was then extinguished in daily 2-hr sessions during which active-lever responses had no scheduled consequences. Animals then underwent sequential reinstatement tests following exposure to either intermittent, unpredictable footshock (test #1) or reintroduction of an oxycodone-paired cue light (test #2), with reinstatement tests separated by additional extinction sessions. Vaginal smears were collected daily from female subjects for histological confirmation of estrous cycle phase (proestrus, estrus, metestrus, diestrus).

Results: In the dose-response experiment, IV oxycodone produced a typical inverted U-shape function when response rate was used as the dependent measure, with 0.01 mg/kg/infusion representing the "peak"-effective dose in both sexes. Oxycodone functioned as a more effective reinforcer in females than males as evidenced by an upward shift of the dose-response curve, particularly at the two lower doses tested (0.003 and 0.01 mg/kg/infusion; main effect of sex, $p < 0.05$). This upward shift in responding was not accompanied by a significantly greater number of oxycodone infusions earned in females. In the second experiment, females again exhibited higher rates of responding than males at both the 0.01 and 0.03 mg/kg/infusion doses of IV oxycodone. In females, within-subjects analysis revealed that rates of responding for IV oxycodone were significantly higher during metestrus/diestrus as compared to proestrus/estrus (main effect of estrous phase, $p < 0.05$). In reinstatement tests, neither males nor females displayed appreciable footshock-induced reinstatement of oxycodone seeking, while females exhibited a robust cue-induced oxycodone-seeking response (main effect extinction vs. reinstatement, $p < 0.05$) that was not observed in males and which was independent of estrous cycle phase.

Conclusions: We report here that IV oxycodone functions as a more effective reinforcer in female rats as compared to male rats. Females also exhibited a more robust drug-seeking response following re-exposure to oxycodone-associated discrete cues than males under the present testing conditions, suggesting that sex differences may not only apply to oxycodone taking, but also to oxycodone seeking. We also reveal for the first time that the reinforcing effects of IV oxycodone in females are modulated by estrous cycle phase, an effect that is likely mediated by fluctuations in ovarian hormones although additional experiments are required to confirm this supposition. Taken together, our results confirm and extend previous work suggesting that females may be more vulnerable than males to the abuse-related effects of oxycodone and may also be more susceptible to certain modalities of relapse, although the magnitude of these risks may vary across the menstrual cycle.

Keywords: Oxycodone, Sex Difference, Estrous Cycle, Reinstatement, Self-Administration

Disclosure: Nothing to disclose.

P737. Adolescent Nicotine Vapor Exposure Increases Nicotine Vapor Seeking Behavior in Adult Male Rats

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Background: In recent years there has been an increase in nicotine vapor consumption via electronic nicotine delivery systems, particularly in adolescents. While the health effects of nicotine vapor continues to be investigated, its distinct effects on the brain and behavior remain unclear. Previous studies have used intravenous self-administration to investigate nicotine seeking and taking behaviors. The primary objective of this study was to use a nicotine vapor self-administration (NVSA) system to assess changes in motivation for nicotine vapor intake during adulthood, in rats with a history of adolescent and/or early adulthood nicotine vapor exposure.

Methods: Male Sprague-Dawley rats ($N = 24$) were passively exposed to 0 mg/mL vehicle control (50/50 propylene glycol/vegetable glycerin, PG/VG) or 24 mg/mL nicotine vapor for 10 daily 90-minute sessions during late adolescence (PND 58-67) and/or early adulthood (PND 159-168), resulting in 4 groups defined by early life passive nicotine vapor exposure (adolescent exposure-early adulthood exposure, 0-0, 24-0, 0-24, 24-24, $n = 6$/group). To assess nicotine vapor's short-term effects on motivation for reward, lever pressing for food pellets was assessed using a progressive ratio schedule of reinforcement, immediately after each adolescent and early adulthood passive vapor exposure session. Approximately 25 weeks after early adulthood exposure, motivation for nicotine reward was assessed by testing the rats for 6 mg/mL NVSA across 4 daily 1-hour sessions. Responding in both the progressive ratio task and NVSA was not assessed until acquisition of the task was identified with a criteria of less than 15% variability across 2 consecutive days. Mixed model ANOVA was used to compare effects of passive nicotine vapor exposure on lever pressing for food in the progressive ratio task, as well as nicotine vapor deliveries and active nosepoke entries during NVSA. One-way ANOVA, Fishers LSD, and independent samples t-test were used for post-hoc analyses.

Results: Analysis of lever presses for food rewards immediately following passive nicotine vapor exposure during adolescence revealed a significant interaction of treatment group and day ($F(9,189)=2.61$, $p < 0.01$, $\eta p2 = 0.11$), with t-tests showing higher responding in the 24 mg/mL nicotine vapor group, relative to 0 mg/mL vehicle control group, on test days 1,5,6, and 7 (ps < 0.05). No statistical differences were seen between treatment groups when responding for food rewards following passive vapor exposure during early adulthood. For NVSA, no effect of treatment groups were observed for total nicotine vapor deliveries. However, analysis of total active nosepoke entries identified a main effect of treatment group ($F(3,19)=3.31$, $p < 0.05$, $\eta p2 = 0.34$), with Fishers LSD showing the 24-0 and 0-24 nicotine vapor exposure groups being significantly different from the 0-0 vehicle control group across test days (ps < 0.05). The 24-0 group was also identified as significantly different from the 24-24 group across test days ($p < 0.05$). One-way ANOVAs comparing treatment groups on each of the 4 NVSA test days revealed significant increases in reward seeking nosepokes for the 24-0 (test days 1 and 2) and 0-24 (test day 1) exposure groups, relative to the 0-0 group ($p < 0.05$). Notably, rats in the 24-0 group also showed more reward seeking nosepokes than rats in the 24-24 group on test day 2 ($p < 0.05$).

Conclusions: Our findings demonstrate that adolescent-only and early adulthood-only exposure to nicotine vapor causes immediate and long-term increases in motivation for rewards and may promote e-cigarette use later in life. Interestingly, when nicotine vapor exposure occurred during both adolescence and early adulthood, no increases in nicotine vapor seeking behavior were observed. Additional studies on the neurobiological and behavioral effects of nicotine vapor will be necessary for the development of effective treatment strategies, educational programs, and public policies aimed at curbing recreational use of e-cigarettes.

Keywords: Nicotine Vapor, Self-Administration, Motivation

Disclosure: Nothing to disclose.

P738. Sex-Specific Cholinergic Regulation of Dopamine Release Mechanisms Through Nicotinic Receptors in the Nucleus Accumbens

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Background: For many psychiatric disorders, such as anxiety, depression, and substance use disorder (SUD), sex is a critical biological variable and women represent a particularly vulnerable population. The mesolimbic dopamine system is involved in the expression of sex-specific behaviors and is a critical mediator of many psychiatric disease states. While work has focused on sex differences in the anatomy of dopamine neurons and relative dopamine levels, an important characteristic of dopamine release from axon terminals in the nucleus accumbens (NAc) is that it is rapidly modulated by local regulatory mechanisms independent of somatic activity. One of the most potent regulators of dopamine terminal function is through α4β2* containing nicotinic acetylcholine receptors (nAChRs). In this series of studies, we define the molecular mechanism underling these unique sex differences in dopamine terminal regulation and show how it relates to motivated behaviors.

Methods: We measured sub second release of dopamine using ex vivo fast-scan cyclic voltammetry in NAc brain slices from males ($n = 7$ – 10 slices), naturally cycling (intact, $n = 7$ - 10) and ovariectomized females ($n = 7$ – 10). We assessed the effects of a range of agonists and antagonists of nAChRs on dopamine release with nicotine, Dhβe, α-conotoxin PIA, and Methyllcaconitine (MLA). Additionally, we measured the potentiating effects of 17β-estradiol (E2) on dopamine release mechanisms with and without application of the estrogen receptor antagonists MPP and PHTPP or the nAChR antagonist Dhβe. Lastly, we defined the sex-specific effects of ChAT interneuron stimulation on a dopamine-dependent reinforcement task using Gq-DREADDs injected into the NAc of male ($N = 13$) and female ($N = 15$) ChAT-cre + /-.

Results: We find that nAChR regulation of dopamine release is not present in intact females; however, ovariectomy rescues this regulation in females – indicating that ovarian hormones play a significant role in this process. Critically, we define the molecular mechanism underling these distinctive sex differences in dopamine regulation. Through a series of experiments, we find that acute E2 actions on dopamine terminals increases dopamine release via actions on α4β2*-nAChRs. Specifically, we find that the acute potentiating effects of E2 on dopamine release are blocked by antagonism of α4β2*-nAChRs, but not α6*- or α7*-nAChRs or estrogen receptors. Finally, using chemogenetic approaches in awake and behaving animals, we link these sex differences to sex-specific motivated behaviors.

Conclusions: Here we show that this textbook mechanism of dopamine terminal regulation is not present in females under most conditions. Second, through these studies, we discovered a novel unstudied mechanism by which nAChRs are a substrate for

estradiol effects on the brain, independent of canonical estrogen receptor signaling mechanisms.

Keywords: Dopamine, Sex Differences, Nicotinic Acetylcholine Receptors, Nicotine/Substance Use Disorder

Disclosure: Nothing to disclose.

P739. Evaluation of Kappa and Mu Opioid Receptor Occupancy by CVL-354 Using PET in Nonhuman Primates

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Background: CVL-354 is a potent kappa opioid receptor (KOR) antagonist with significant in vitro selectivity over the Mu opioid receptors (MOR) as measured in cell lines expressing each subtype. Subsequent in vivo receptor occupancy studies in mice showed that CVL-354 displaced [3H]PF-04767135 a KOR preferring radioligand with an ID50 of 0.1 mg/kg and [3H]carfentanil (here denoted CFN), a MOR specific ligand, with an ID50 of 4 mg/kg, confirming KOR selectivity. The objective of this study was to measure receptor occupancy at both Kappa and Mu receptors with varying doses of CVL-354 using radiotracers [11 C]LY2795050 and [11 C]CFN in non-human primates. The hypothesis was that CVL-354 would block receptors in a dose-dependent manner, with a higher affinity for Kappa than Mu. Data from this study would then facilitate dose selection for a clinical study with same tracers.

Methods: For KOR imaging, two rhesus macaques (1 male and female) were scanned once at baseline and several times following administration of varying doses of CVL-354. Similarly, for MOR imaging, two female rhesus macaques were scanned once at baseline and one- or two-times following administration of varying doses of CVL-354. On all scan days, [11 C] LY2795050

or [11 C]CFN were injected as a 3-minute bolus in <10 mL. On all blocking scans, CVL-354 bolus + constant infusion was initiated ~15 minutes before the start of the [11 C] LY2795050

or [11 C]CFN injection. This delivery consisted of a 2-minute bolus followed by a maintenance infusion through the end of the scan. Dynamic scan data were reconstructed with a filtered back projection algorithm with corrections for attenuation, normalization, scatter and randoms. Binding potential (BPND) estimates of [11 C]LY2795050 and [11 C]CFN were produced using the SRTM method with the cerebellum as a reference region. Receptor occupancy (RO) was calculated using the BPND estimates. PK samples were taken throughout the delivery of CVL-354 and later submitted for analysis. Plasma concentrations were used to calculate IC50 for both KOR and MOR RO using the standard Emax model.

Results: Among evaluated regions of interest (amygdala, brainstem, caudate, cerebellum, cingulate cortex, frontal cortex, globus pallidus, hippocampus, insula, nucleus accumbens, occipital cortex, pons, putamen, centrum semiovale, substantia nigra, temporal cortex and thalamus), CVL-354 produced dose-dependent occupancy at both KOR and MOR as measured by [11 C]LY2795050 and [11 C]CFN, respectively. However, as expected, the molecule was at least 10-fold more potent at the KOR.

Conclusions: These data confirm dose-dependent target binding of CVL-354 to both KOR and MOR receptors in nonhuman primates. The results also confirm greater selectivity for the KOR. CVL-354 consistently demonstrated greater affinity towards KOR than MOR (10-40 fold) across the various preclinical evaluations of receptor affinity/activity

Keywords: PET Imaging, Kappa Opioid Receptor Antagonist, Non Human Primate

Disclosure: Cerevel Therapeutics: Employee (Self).

P740. Preclinical Assessment of the Effects of Cannabidiol on Alcohol Dependence

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Background: Cannabidiol (CBD), a non-psychoactive constituent of the cannabis plant, has received attention for its potential to decrease drug and alcohol use given its anti-inflammatory, antioxidant, and neuroprotective effects.

Methods: Here we used a multidisciplinary approach, combining state of the art behavioral models, immunohistochemistry, and electrophysiology to evaluate the effects of chronic (60 mg/kg/day) CBD treatment in several alcohol-related behaviors and on the alcohol-induced neurodegeneration in alcohol dependent male and female rats. We used two different animal models to induce alcohol dependence in rats: the chronic intermittent ethanol vapor exposure (CIE) model and the recently developed ethanol vapor self-administration model (EVSA). The new EVSA model highlights the volitional aspects of alcohol dependence.

Results: We found that chronic CBD treatment prevents the development of alcohol dependence in the EVSA model, by reducing alcohol-induced neurodegeneration in the nucleus accumbens shell (NAcSh) and the dorsomedial striatum (DMS). In animals treated after the establishment of alcohol dependence (CIE model), CBD reduces alcohol drinking, somatic and emotional signs of withdrawal. Finally, the treatment was also effective in reducing alcohol seeking and stress-induced reinstatement, possibly by reverting the reduction of neuronal excitability induced by alcohol in the basolateral amygdala (BLA).

Conclusions: These results extend to the current literature and indicate a profile of potential benefit of CBD for the treatment of alcohol dependence.

Keywords: Alcohol Dependence, Cannabidiol, Basolateral Amygdala, Neurodegeneration

Disclosure: Nothing to disclose.

P741. A Novel, Short-Acting Kappa Opioid Receptor Antagonist Blocks the Analgesic Effects of U50,488 and Attenuates Symptoms of Spontaneous Oxycodone Withdrawal in Rats

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Background: A large body of preclinical evidence has demonstrated that the neuropeptide dynorphin (DYN), which acts at kappa opioid receptors (KOR), is a key player in opioid withdrawal (Koob, 2009; Bruchas et al., 2010). Chronic opioid exposure increases DYN and KOR activation and is thought to produce negative affective states including anhedonia, anxiety-like, and aversive behaviors. Importantly, KOR antagonism has been shown to reduce opioid withdrawal signs and escalation of opioid self-administration in rodent models. However, KORA compounds used in these preclinical models (e.g., norBNI, JDTic) have extremely long KOR antagonist actions—on the order of weeks. As such, the development of a selective and short-acting KOR antagonist has the therapeutic potential to facilitate

discontinuation of opioid use with a pharmacological profile better suited for clinical development. CVL-354 is a potent, selective and short-acting KOR antagonist that was tested in a rat model of spontaneous oxycodone withdrawal.

Methods: Adult male Sprague Dawley rats were used. To determine dose and time course effects of CVL-354 KOR antagonism, rats were treated with CVL-354 (0.0 – 1.0 mg/kg, SC) followed by the KOR agonist, U50,488 (30 mg/kg, SC) and nociceptive responses were measured in the Tail Flick (TF) and Hot Plate (HP) thermal pain assays.

To determine the effects of CVL-354 on oxycodone somatic withdrawal signs, rats were subcutaneously implanted with iPRECIO minipumps (Alzet, model SMP 200) programmed to deliver an escalating dose regimen of oxycodone or saline 2x/day for 14 days. For oxycodone, the escalating dose regimen was 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg/infusions (2 infusions/day; 7:00-9:00AM/PM), with the 0.5 mg/kg dose administered for 2 days and the remaining doses administered for 3 days each.

After the 14-day escalating dose oxycodone (or saline) regimen, spontaneous opioid withdrawal signs emerged, including diarrhea, ptosis, wet dog shakes, teeth chattering, and body flattening. To determine the effects of CVL-354 on somatic withdrawal, rats were administered CVL-354 (0.0, 0.1, 0.3, 1.0 mg/kg, SC; $n = 7$-19) at 6-h (Wdrl d0) and 24-h (Wdrl d1) after cessation of drug delivery. Opioid withdrawal can also alter locomotor activity, with directionality dependent on factors such as novelty and area of the test arena. As such, we tested the effects of CVL-354 on oxycodone withdrawal-induced alterations in locomotor activity in Open Field chambers (Med Associates) on Wdrl d1, immediately after somatic withdrawal measurements ($n = 7$-11). Activity was digitally recorded and later scored using DeepLabCut (Mathis et al., 2018). As a control for attenuation of spontaneous withdrawal signs, we administered the a2 noradrenergic agonist lofexidine (0.64 mg/kg) to separate rats. At the end of behavioral testing on Wdrl d1, rats were euthanized via rapid decapitation to collect plasma for ELISA-based corticosterone analysis ($n = 6$-13). Statistical analyses used were: One-way ANOVA (somatic withdrawal, corticosterone) and two-way ANOVA with repeated measures on time (open field test, tail flick and hot plate tests). Dunnett's posthoc tests comparing treatment to control groups were done when appropriate.

Results: A dose range of 0.1-1.0 mg/kg CVL-354 blocked U50,488-induced analgesia in the TF and HP tests at 1- and 4-h, but not at 24-h. Within this same dose range, CVL-354 reduced spontaneous oxycodone somatic withdrawal signs ($p < 0.01$). In neither the pain nor the somatic withdrawal assays did CVL-354 have an effect on its own. Lofexidine, the current standard of care for mitigation of acute opioid withdrawal symptoms, also significantly reduced somatic withdrawal signs suggesting predictive validity of this model ($p < 0.01$). Locomotor activity was significantly decreased during spontaneous oxycodone withdrawal compared to activity in control rats ($p < 0.05$). Lofexidine exacerbated withdrawal-induced decreases in locomotor activity ($p < 0.01$), whereas CVL-354 had no effect. Finally, spontaneous oxycodone withdrawal resulted in significantly elevated levels of unbound plasma corticosterone compared to control rats ($p < 0.01$). Interestingly, lofexidine pre-treatment significantly potentiated plasma corticosterone levels compared to oxycodone withdrawn rats pre-treated with vehicle ($p < 0.01$), whereas CVL-354 (0.3 mg/kg) pre-treatment showed a trend to decrease oxycodone withdrawal-induced plasma corticosterone levels.

Conclusions: These data demonstrate that CVL-354 has KOR antagonist actions in thermal pain assays for at least 4, but less than 24, hours, and that it is effective at reducing somatic withdrawal signs in a model of spontaneous oxycodone withdrawal model. Intriguingly, lofexidine treatment suppressed locomotor activity and exacerbated levels of the stress biomarker, corticosterone. CVL-354 did not produce these negative effects, suggesting that KOR antagonism may provide better overall efficacy for mitigation of opioid withdrawal symptoms.

Keywords: Kappa Opioid Receptor, Kappa Opioid Receptor Antagonist, Opioid Dependence, Pharmacotherapy, Animal Model, Withdrawal

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

P742. The Ghrelin System as a Potential Target for Treatment of Alcohol Use Disorder: Pharmacological Evidence

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Background: Alcohol use disorder (AUD) is a chronic and relapsing neuropsychiatric disease that is a highly prevalent public health issue. Binge drinking is very common, harmful, and is an important step in the AUD spectrum. Yet, there are only a few Food and Drug Administration (FDA)-approved medications for the treatment of AUD. It is, therefore, important to develop and identify new medications to treat AUD. Towards that end, we investigated the effects of novel compounds that target the ghrelin system on binge-like drinking in a mouse model of AUD. Ghrelin is a stomach-derived peptide hormone with known roles in regulating appetite and food intake. Ghrelin receptors (GHSR-1a) are expressed both in the brain and the periphery. Recent studies show that the ghrelin system is also implicated in alcohol-related behaviors. For example, ghrelin administration increases alcohol intake, whereas blockade of GHSR-1a with specific antagonists such as JMV 2959 decreases alcohol intake. Most of these studies utilized male subjects only and did not measure binge-like drinking.

Methods: "Drinking in the Dark" (DID) is a model of binge drinking that has been validated in C57Bl6 mice. The DID procedure takes advantage of the most active circadian period in mice, which is 3 hours into the dark cycle. During this time, we replace the animal's water bottle with a sweetened alcohol solution or a non-alcohol containing sweetened solution for 2-4 hours each day, leading to pharmacologically significant blood alcohol levels. In the present study, we manipulated the ghrelin system by 1) blocking the ghrelin receptor and 2) sequestering biologically-active ghrelin. First, we investigated six GHSR-1a blockers for their ability to block binge drinking. The prototype GHSR-1a antagonist JMV 2959 has been shown to decrease drinking in 2-bottle choice and operant models but has not been tested in binge-drinking. Other compounds tested include PF-5190457, PF-6870961, HM-04, YIL-781, which are novel and have not been tested in a model of binge drinking. PF-5190457 is a GHSR-1a inverse agonist and represents the only GHSR-1a blocker that has moved forward to human research in AUD. Of note, in humans, it was recently discovered that PF-5190457 administration leads to a major hydroxy-metabolite, PF-6870961, which has specific activity as a GHSR-1a antagonist. Other GHSR-1a antagonists we tested are HM-04 and YIL-781, and the recently discovered endogenous GHSR-1a antagonist LEAP-2. We hypothesized that pharmacological blockade of GHSR-1a would decrease binge-like alcohol intake in mice of both sexes. Additionally, we performed another experiment that used an anti- ghrelin vaccine to sequester biological activity of the endogenous receptor ligand, acyl-ghrelin. We hypothesized that blocking acyl-ghrelin would decrease binge-like alcohol intake in mice of both sexes. We used 48 male and 48 female C57Bl6 mice of 11 weeks of age, (8-11 male and female mice per compound, per solution). The sample size was chosen based on a power analysis and our previous work. A within-subjects, Latin-square design was employed. Alcohol intake

was measured in g/kg of body weight and results were analyzed by two-way ANOVA.

Results: Our results showed that systemic administration of JMV 2959 ($p < 0.0001$), PF-5190457 ($p = 0.0002$), PF-6870961 ($p = 0.0109$), and HM-04 ($p = 0.0059$) reduced alcohol intake in mice of both sexes. YIL-781 reduced intake in male ($p = 0.0108$) but not female ($p =$ NS) mice. LEAP-2 had no effect on alcohol intake ($p =$ NS). For the cohort of animals who received sweet solution with no alcohol content. PF-5190457, PF-6870961, YIL-781, and LEAP-2 had no effect on intake. JMV 2959 reduced intake of this solution ($p = 0.003$) as did HM-04 for females ($p = 0.0029$). HM-04 had no effect on intake of this solution in males ($p =$ NS). In another experiment, anti-ghrelin vaccine administration had no effect on binge drinking compared to sham vaccine administration ($p =$ NS). Finally, to investigate the sedative and locomotor effects of the drug, we performed 2 validated tests of locomotion. The rotarod and circular corridor tests were used to assess motor coordination/ataxia and spontaneous locomotion, respectively. The majority of the drugs had a significant effect on locomotion ($p < 0.05$).

Conclusions: Ghrelin receptor blockade preferentially reduces alcohol intake, and there is little difference in intake between males and females in response to ghrelin receptor blockade. Additionally, circulating acyl-ghrelin has no effect on alcohol intake. Our findings provide novel information that supports the role of the ghrelin system in binge drinking and identifies that system as a potential target for pharmacological interventions against AUD. It appears that the receptor blockade is a more promising approach than modulating the acyl-ghrelin molecule itself. This could be due to the intrinsic activity of the ghrelin receptor, which can be up to 50% without the acyl-ghrelin molecule bound. We also addressed a gap in the literature regarding the paucity of information on the effects of sex on the ghrelin system. Future studies will need to elucidate the biological mechanisms by which the ghrelin system affects alcohol drinking and their modulation by sex and individual differences. Finally, this work has translational relevance as PF-5190457 is the first GHSR-1a inverse agonist that has moved to the clinic for medication development.

Keywords: Alcohol Use Disorder - Treatment, Binge-Drinking, Ghrelin, Alcohol Consumption, Ghrelin Receptor

Disclosure: Nothing to disclose.

P743. Behavioral and Neurobiological Consequences of Chronic Vaporized Δ9-Tetrahydrocannabinol (THC) Self-Administration in Female Rats

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Background: Vaping of cannabis and cannabis extracts containing Δ-9-tetrahydrocannabinol (THC, the primary psychoactive constituent of cannabis) is on the rise. Developing a model of THC vapor self-administration in rodents is critical for increasing our understanding of the behavioral and biological consequences of chronic vaporized THC use.

Methods: Female Sprague-Dawley rats ($N = 48$; 24 per group) were allowed to self-administer either THC vapor (50 mg/ml) or vehicle vapor (VEH: 100% propylene glycol) on an intermittent (e.g., every other day) basis. Initially, rats self-administered 3 s vapor puffs under a fixed ratio 1 (FR1) schedule for 20 sessions. Subsequently, the FR was gradually increased from 1 to 5 (≥5 sessions at each FR). Next, the concentration of THC in the e-liquid was adjusted from 5-200 mg/ml in randomized order (≥5 sessions at each concentration) to assess whether number of THC vapor puffs obtained were titrated in a concentration dependent manner. To evaluate differences in anxiety-like behavior between THC vapor and VEH vapor self-administering rats ($N = 10$/group), subjects were tested in an open field test 24-hrs following their last vapor self-administration session. At the termination of behavioral studies, rats ($N = 3$-4/group) were euthanized, and Diffusion Tensor Imaging (DTI) was performed ex vivo on fixed brains. Total length of time of intermittent vapor self-administration prior to brain collection was 8 months.

Results: Under an FR1 schedule of reinforcement, VEH animals acquired a greater number of vapor puffs per session compared with THC animals (Mean ± SEM: THC puffs: 8.8 ± 1.1, VEH puffs: 12.2 ± 1.1; $F(1,34)=4.51$, $p < 0.05$). However, when the effort required to obtain vapor puffs increased from FR1 to FR5, THC animals maintained their levels of puffs obtained, while the amount of puffs obtained by VEH animals decreased as the FR requirement increased (Drug x FR interaction; $F(4,136) = 6.39$, $p < 0.01$). As the THC concentration was adjusted from 5-200 mg/ml, THC animals titrated the number of puffs obtained, self-administering a greater number of puffs at lower drug concentrations (Drug x Concentration interaction; $F(1,34) = 22.5$, $p < 0.001$). Rats who self-administered THC vapor displayed increased anxiety-like behavior in the open field test when assessed 24-hrs post-session ($t(18)=2.99$, $p < 0.01$). Analysis of DTI metrics revealed decreased microstructure and impaired diffusivity in major white matter tracts, including the corpus callosum, in rats that self-administered THC vapor compared with VEH vapor (fractional anisotropy, FA; Mean ±SD: Control = 0.63 ± 0.02, THC = 0.57 ± 0.03, $p = 0.015$).

Conclusions: THC vapor maintained self-administration behavior in female rats. Chronic THC vapor self-administration resulted in increased anxiety-like behavior and reduced white matter integrity. Preclinical models of vaporized THC self-administration are important for translational value and informing our understanding of the effects of cannabis constituents and consequences of chronic THC vapor exposure.

Keywords: THC, Vapor, Diffusion Tensor Imaging (DTI), Cannabis, Drug Self-Administration

Disclosure: Nothing to disclose.

P744. Repeated Binge-Like Intake of Methamphetamine and the Cathinone Derivative Methyledioxypyrovalerone (MDPV) Produce Differential Effects on Prefrontal Neuroinflammation and Cognitive Flexibility

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Background: Methamphetamine (METH) abuse is associated with impairments in executive functioning, including working memory, impulse control, and cognitive flexibility, which may result from drug-induced dysfunction of the prefrontal cortex (PFC). Recent evidence suggests that METH produces increases in immune signaling, microglial activation, and neurotoxicity in this region. The current study sought to investigate the ability of repeated binge-like intake of METH to induce neuroinflammation in the PFC, and the potential role of inflammatory processes in METH-induced deficits in cognitive flexibility. For comparison purposes, we also examined the effects of the synthetic cathinone derivative methyledioxypyrovalerone (MDPV), which exerts potent cocaine-like monoamine reuptake blockade and has been reported to induce neuroinflammation and cognitive dysfunction.

Methods: Separate groups of male and female rats were allowed to intravenously self-administer either METH (0.05 mg/kg/

infusion), MDPV (0.05 mg/kg/infusion), or saline in three binge-like access sessions, each 96-hr in length and separated by 72-hr of forced abstinence in the home cage. Three weeks following the end of the third 96-hr session, brain tissue was harvested for immunohistochemical assessment of microglia and astrocyte density and morphology, or multiplex ELISA analysis of cytokine levels. To assess drug-induced changes in cognitive flexibility, both prior to and after drug access, additional groups of male rats underwent assessment of cognitive flexibility in an Attentional Set Shifting Task (ASST). The effects of the non-steroidal anti-inflammatory drug parecoxib on post-drug ASST performance was also examined.

Results: Rats self-administering METH demonstrated increased levels of CXCL1, CXCL2, fractalkine, IFN-gamma, IL-1a, IL-1b, IL-2, IL-6, IL-18, leptin and MCP-1) in the PFC as compared to animals self-administering saline. In rats self-administering MDPV, only increases in levels of IL-6 in the PFC were observed, which were specific to male rats. No differences in PFC astrocyte density were observed, although we found an unexpected decrease in the density of microglia in this region. PFC microglia showed reduced territorial volumes and ramification, suggesting an activated inflammatory state. With regards to cognitive function, animals with a history of METH intake showed deficits in extradimensional shift strategy as compared to prior to METH intake. However, animals treated with parecoxib following METH intake showed no difference in set-shifting ability between pre- and post-METH assessments. Finally, animals with a history of MDPV intake showed no drug-induced deficits in ASST performance, while saline control animals showed a slight improvement in performance during post-intake testing.

Conclusions: These results show that neuroimmune activation in the PFC persists several weeks into abstinence following binge-like METH intake, with parallel deficits in PFC-mediated cognitive flexibility. Effects effects of MDPV on neuroimmune activation appear to be less robust. The anti-inflammatory agents parecoxib appeared to attenuate METH-induced cognitive impairments. These findings suggest that targeting neuroimmune response to METH may prove to be effective in facilitating recovery of cognitive function following chronic METH intake.

Keywords: Methamphetamine Self-Administration, Synthetic Psychoactive Cathinones, Neuroinflamation, Cytokines, Binge

Disclosure: Nothing to disclose.

P745. Ethanol Potentiates Fentanyl-Induced Respiratory Depression

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Background: Drug overdose deaths involving opioids continue to increase in the United States, now topping 100,000 annually (Centers for Disease Control, 2021). Opioid overdose deaths are primarily due to the respiratory depressant effects of opioids, which inhibit both peripheral and central areas responsible for maintaining respiratory rhythm and flow. Alcohol misuse, which at high doses can result in respiratory depression and death, is also frequently reported in deaths involving heroin and fentanyl with estimates of co-involvement of around 30% in 2017. This is clinically relevant because while naloxone may be very effective in reversing opioid-induced overdoses, its efficacy might be lower for the reversal of overdoses from combined alcohol and fentanyl use. Therefore, the investigation of the interactions of fentanyl and alcohol on respiratory depression and lethality is relevant to help develop targeted interventions for co-occurring alcohol/opioid overdoses.

Aim: To characterize the effects of concomitant administration of fentanyl and ethanol on ventilation measures.

Methods: Using whole body plethysmography, we analyzed ventilation parameters on a breath-by-breath basis. We have previously observed that fentanyl (12, 25, and 50 ug/kg) reduces minute ventilation, frequency of breathing, and peak inspiratory flow while increasing the duration of the inspiratory time, end-inspiratory-pause and apneic pauses in male and female rats. Therefore, we chose the intermediate fentanyl dose for the current study. Twelve female and 12 male Long-Evans rats underwent intravenous (i.v.) catheter surgery. After habituation to plethysmography chambers, the rats were tested in a within-subjects, Latin-square design with four tests one week apart. In each session, all rats received 5 mL/kg, i.v. of sterile water, fentanyl (25 ug/kg), ethanol (1.18 g/kg), or a combination of fentanyl and ethanol over 1 min. At the end of the experiment, we collected blood for fentanyl and ethanol measurement.

Results: Only the combination of fentanyl and ethanol resulted in mortality (~42% females, ~33% males) and the administration of naloxone did not rescue them. When compared to water, treatment with fentanyl, ethanol, and the fentanyl and ethanol combination led to a reduction in minute ventilation ($F3,30 = 14.84$, $p < 0.0001$), frequency ($F3,30 = 4.03$, $p = 0.02$), peak inspiratory flow ($F3,30 = 30.66$, $p < 0.0001$), and an increase in inspiratory time ($F3,30 = 17.47$, $p < 0.0001$) and apneic pauses ($F3,30 = 5.63$, $p = 0.003$). The fentanyl and ethanol combination led to a more pronounced reduction in minute ventilation than fentanyl alone ($p = 0.007$) and greater inspiratory time and apneic pauses than fentanyl alone ($p = 0.03$) or ethanol alone ($p = 0.02$). There were no significant sex differences in any of the variables analyzed.

Conclusions: Ethanol alone induces robust respiratory depression, but a combination of fentanyl and ethanol increases the risk of death. Further investigations will explore what drives the increases in apneic pauses caused by the combination of ethanol and fentanyl and other mechanisms of drug interactions that make their concomitant use lethal in animal models. This research contributes to the neurobiological understanding of simultaneous substance misuse, which will be valuable to future investigations of compounds that seek effective alternatives and complementary approaches to reverse respiratory depression and prevent overdose deaths.

Keywords: Opioid Overdose, Ethanol, Whole-Body Plethysmography, Fentanyl, Respiratory Depression

Disclosure: Nothing to disclose.

P746. A Translational Rodent Model of Gestational Opioid Exposure: Effects of Morphine Compared to Buprenorphine on the Maternal Brain, Maternal Behavior, and Offspring Outcome

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Background: Due to the opioid epidemic, the number of pregnant women receiving Medications for Opioid Use Disorder (MOUDs), including buprenorphine (BUP) has increased drastically within the last several years. Clinically, BUP produces preferable outcomes for exposed infants as compared to methadone or opioid misuse. However, there is a dearth of knowledge about BUP's effects on maternal caregiving behaviors and underlying neural networks that are critical during the transition to

motherhood. BUP's mechanism of action (partial mu-agonist/kappa antagonist) varies significantly from morphine's (full mu-agonist), which may result in a different impact on the maternal brain during a critical neuroplasticity period.

Methods: We used a translational rodent model to mimic chronic opioid (mis)use (morphine exposure, 3-6 mg/kg/day, b.i.d.) or opioid maintenance drug (BUP exposure, 1 mg/kg/day) to investigate the behavioral and neurochemical consequences of gestational opioid exposure on dams and their offspring. Opioid or saline administration to female rats ($N = 50$) via subcutaneous injections began 7 days prior to mating and continued daily throughout pregnancy until postpartum day 2 (PD2) or was discontinued on gestational day 19 to allow for drug clearance before parturition. Dams' maternal behaviors were monitored through detailed observations of pup-directed and non-pup directed behaviors and dams and pups underwent a series of behavioral tests, including a pup retrieval test, a hunting task, and a two-chamber pup-odor preference test. Pups (both males and females) and dams were sacrificed on postnatal day 2, and brains and trunk blood were collected for subsequent analysis. Data were analyzed with factorial or repeated measures ANOVAs.

Results: Our findings indicate that BUP exposure, continued and discontinued, resulted in more maternal care deficits (i.e. less time spent on pup-directed behaviors), increased postpartum pup mortality (9% for BUP, 4.8% for morphine, 1.8% for Veh; $p = 0.04$ (BUP vs. Veh) and maternal deficiencies in the pup retrieval and pup-odor preference tests, but not in the hunting task, as compared to our control (and partly morphine) groups. Conversely, care behavior and survival rates of the morphine groups varied little from controls. Preliminary results further suggest that each opioid also resulted in a unique change in the microbiome profile of the maternal gut. Using high performance liquid chromatography, we will further analyze neurotransmitter levels and their metabolites in the maternal brain (collected on PD2) to investigate potential neurological effects of these opioids on the maternal brain network.

Conclusions: Our results suggest that BUP exposure during pregnancy negatively influences pup survival rates, which may be due to BUP's unique pharmacological action on the maternal brain interfering with maternal caregiving behaviors. More research is critical to elucidate how BUP mechanistically interacts with the neural network during the transition to motherhood to help alleviate possible negative consequences for mothers and their offspring.

Keywords: Opioid Dependence, Pharmacotherapy, Animal Model, Withdrawal, Pregnancy, Maternal Behavior

Disclosure: Nothing to disclose.

P747. Corticotrophin-Releasing Hormone in the Prelimbic Medial Prefrontal Cortex is Critical for Driving Stress-Enhanced Alcohol Drinking in a Mouse Model of AUD

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Background: Alcohol use disorder (AUD) is characterized as a chronic and relapsing neuropsychiatric disorder, with stress as one of the main contributing factors that drives relapse. Corticotrophin-releasing hormone (CRH) is a stress neuropeptide that has long been the focus of alcohol research in the context of extended amygdala circuitry. And while alcohol activates the CRH system, these studies often exclude the influence of stress, which might contribute to why recent CRH antagonists in clinical trials have failed to show consistent positive effects. Our understanding of the role of CRH in stress and alcohol is limited to its function in the extended amygdala and the hypothalamic circuitry, however CRH is expressed fairly ubiquitously throughout the cortex and many subcortical regions, including key regions implicated in regulating alcohol consumption like the medial prefrontal cortex (mPFC).

Methods: Here we utilize a number of transgenic mouse lines to interrogate the role of CRH in the prelimbic (PL) region of the mPFC in regulating stress-enhanced alcohol consumption. First, we characterized the expression, distribution and molecular phenotype of CRH + neurons in the PL in order to better understand how changes in CRH neuron activity in response to alcohol and stress my be influencing PL activity. We then characterized the effects of chronic stress (forced swim stress, FSS) and alcohol (chronic intermittent ethanol, CIE) exposure on CRH neuron physiology in the PL using Crh-Cre x Ai14 reporter mice and slice electrophysiology recordings. We next utilized Crh-Flox mice to conditionally knockdown Crh expression in the PL and examine the effects on stress-enhanced alcohol consumption using the CIE-FSS model that models chronic stress effects on drinking.

Results: Expression of CRH is found nearly evenly distributed throughout layers 2-5 of the PL and infralimbic (IL) cortices of the mPFC, with slightly (but not significantly) denser expression in PL compared to the IL ($p = 0.12$), and an increasing gradient from anterior to posterior PL. Interestingly we also characterized expression in the paraventricular thalamus (PVT), and saw an increase in expression of CRH across the anterior to posterior axis, and thus will be the focus of future studies in the lab. While, CRH has been reported to be co-expressed in both GABAergic and glutamatergic neurons in the PL, we found that approximately 92% of neurons were GABAergic. The GABAergic-CRH subtype in the PL shows a fast-spiking phenotype and are modestly sensitive to going into depolarization block at higher current injection steps (~33% of neurons recorded). Interestingly, when exposed to the CIE-FSS paradigm, GABAergic-CRH neurons become hyperexcitable, showing decreases in rheobase ($p < 0.01$) and significant leftward shifts in the dose response curve for current-evoked action potential firing ($p < 0.05$). Unexpectedly, ~96% of GABAergic-CRH neurons in PL are sensitive to depolarization block at current steps higher than 100pA, which represents a significant shift in excitability in this population. Further, we knocked down Crh in the PL through Cre-Lox excision in the Crh-Flox mice and, as expected, we saw greater than 55% knockdown in functional CRH protein product as confirmed by western blot. Using this knockdown approach, we show a complete blockade of the stress-enhanced escalation of alcohol consumption in Crh-knockdown mice exposed to CIE-FSS compared to Crh-intact CIE-FSS mice and unstressed controls ($p < 0.05$, and $p < 0.01$, respectively).

Conclusions: Taken together, we have identified the PL mPFC as a critical hub for CRH effects on stress and alcohol interactions. Chronic alcohol and stress recruit CRF in many regions throughout the brain, but GABAergic-CRH neurons in the PL mPFC are particularly sensitive to these effects becoming hyperexcitable in response to chronic stress and alcohol exposure. Hyperexcitability and activity in local CRH circuitry in the PL are crucial for driving stress-enhanced alcohol consumption, as knockdown of Crh in the PL completely blocks this effect. While intact CRH signaling in the PL mPFC is crucial, future experiments will examine synaptic changes induced by excessive CRH signaling in response to chronic stress and alcohol to identify additional druggable targets for treatment of this sensitive population. Additionally, it might be worthwhile to examine CRH antagonists again as potential treatments for AUD with an emphasis on individuals suffering from co-morbid exposure to extreme stress.

Keywords: Alcohol Dependence, Acute and Chronic Stress, Corticotropin-Releasing Hormone, Corticotropin-Releasing Factor

Disclosure: Nothing to disclose.

P748. Alcohol and Orexin Effects on VTA-CeA Circuitry and Anxiety-Like Behavior in Rats

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Background: Humans with alcohol use disorder (AUD) often experience negative affect during withdrawal, 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 circuit between the ventral tegmental area (VTA) to the central nucleus of the amygdala (CeA), regions 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 withdrawal. The mechanism by which the VTA-CeA circuit becomes activated is unknown, but may occur via orexin-mediated disinhibition. Here, we explored the role of the VTA-CeA circuit in mediating aversive or anxiety-like behavior in alcohol-dependent and naïve rats, as well as the role of orexin signaling in mediating circuit activation and behavior. We hypothesized that (1) VTA-CeA activation is aversive and (2) contributes to increased anxiety-like behavior during alcohol withdrawal, (3) that VTA-CeA circuit activation occurs via an orexin-mediated mechanism, and (4) that driving the VTA orexin system alone is sufficient to produce an anxiety-like phenotype.

Methods: Adult male and female Long-Evans rats were used in all experiments. To evaluate the role of VTA-CeA 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 transfect cells with a cre-dependent excitatory DREADD (pAAV-hSyn-DIO-hM3D(Gq)-mCherry), inhibitory DREADD (pAAV-hSyn-DIO-hM4D(Gi)-mCherry), or inactive control (pAAV-hSyn-DIO-mCherry; $n = 3$-4/group). To characterize the role of intra-VTA orexin on behavior, orexin A (50 nM) was site-specifically administered in the VTA prior to anxiety-like behavioral assays ($n = 7$-8/group).

Alcohol dependence was induced using a chronic ethanol vapor exposure model, and rats were tested during acute withdrawal. Activity of CeA-projecting VTA rat neurons was measured using retrograde tracing and slice electrophysiology, and anxiety-like behavior was assessed using light/dark box and elevated plus maze behavioral assays. To investigate the mechanism behind altered VTA-CeA physiology in alcohol dependence, we manipulated VTA orexin 1 receptor activity pharmacologically in the above experiments.

Results: Preliminary data suggest a role for the VTA-CeA circuit in mediating aversive and anxiety-like behavior in rats. Using a chemogenetic approach to activate the VTA-CeA circuit in otherwise experimentally naïve rats, we demonstrate that circuit activation alone may be aversive, as tested in a conditioned place aversion assay. Additionally, chemogenetic inhibition of VTA-CeA projections may rescue increased anxiety-like behavior during alcohol withdrawal, as measured in a light/dark box behavioral assay.

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 ($p = 0.0236$; two-tailed t-test), and preliminary electrophysiological data suggest that VTA-CeA neurons may become activated via an orexin receptor-dependent mechanism.

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 aversive and anxiety-like behavior, as well as informing therapeutic strategies for individuals with AUD.

Keywords: Alcohol and Substance Use Disorders, Orexin, Ventral Tegmenal Area, Central Amygdala

Disclosure: Nothing to disclose.

P749. Circuit- And Subregion-Specific Effects of Chronic Ethanol Exposure on Medial Prefrontal Cortex Inputs to the Rostromedial Tegmental Nucleus

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Background: Chronic ethanol induces physiological neuroadaptations in the medial prefrontal cortex (mPFC) that are thought to play an important role in driving maladaptive behaviors that impede recovery. Previous research has focused primarily on mPFC neurons arising from the prelimbic (PL) subregion of the mPFC despite the fact that the infralimbic (IL) subregion is also heavily implicated in addiction. Moreover, the circuit-specificity of ethanol's effects on the mPFC remain relatively unexplored. The rostromedial tegmental nucleus (RMTg) plays an important role in alcohol-related behaviors and recent work from our lab reveals the presence of dense input from layer V PL and IL mPFC neurons. The present study was designed to investigate the subregion- and circuit-specific effects of chronic ethanol exposure on projection-undefined vs RMTg-projecting mPFC neurons.

Methods: Adult male Long-Evans rats were stereotaxically injected with fluorescent retrobeads into the RMTg. After one week of recovery, rats were rendered dependent using a standard 14-day chronic intermittent ethanol (CIE) vapor exposure paradigm. Rats were euthanized and slices prepared for whole-cell patch-clamp recordings approximately one week after CIE exposure. Recordings were made from both tracer-labeled (RMTg-projecting) and unlabeled (projection-undefined) PL and IL neurons. Injection sites were confirmed after recordings by microscopic inspection of slices containing the RMTg.

Results: Current clamp recordings in the PL mPFC revealed that projection-undefined neurons were significantly more excitable than their RMTg-projecting counterparts ($p < 0.05$). Interestingly, CIE exposure produced a significant increase in excitability in RMTg-projecting PL mPFC neurons but not projection-undefined population ($p < 0.05$). CIE exposure also significantly increased sEPSC frequency in RMTg-projecting but not projection-undefined PL mPFC neurons ($p < 0.001$). In contrast, we observed no significant differences in excitability in either RMTg-projecting or projection-undefined IL mPFC neurons between CIE and AIR controls. While CIE exposure had no effect on sEPSCs, it significantly increased sIPSC frequency in RMTg-projecting neurons but not projection-undefined IL mPFC neurons ($p < 0.05$).

Conclusions: Taken together, our data indicate that CIE exposure produces distinct subregion- and circuit-specific effects.

These CIE-induced alterations in RMTg-projecting PL and IL mPFC neuron physiology suggest that deficits in mPFC-dependent behaviors due to chronic ethanol may be linked to functional changes in RMTg-projecting PL and IL mPFC neurons.

Keywords: Prelimbic Cortex, Infralimbic Cortex, Reward And Aversion, Alcohol and Substance Use Disorders

Disclosure: Nothing to disclose.

P750. Physiological Changes in Noradrenergic Neurons in a Mouse Model of Opioid Use Disorder

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Background: Noradrenergic systems are implicated in playing a role in both the aversion to opioid withdrawal and in opioid reward learning. Previously we have shown that an opioid withdrawal model of opioid use disorder (OUD) enhances norepinephrine (NE) release dynamics in the bed nucleus of the stria terminalis (BNST), a brain region playing important roles in both the rewarding and aversive properties of reinforcing drugs, receiving the densest innervation of NE in the brain, mainly from the medullary A2/Nucleus of the solitary tract (NST). Changes in noradrenergic neurons have been studied in models of opioid use disorder, but have mainly been the pontine A6/locus coeruleus neurons and not those found in the medulla. To begin to explore how opioid exposure and withdrawal may alter the physiology of A2/NST neurons we used a genetic reporter mouse that expresses EGFP in noradrenergic neurons.

Methods: Methods were approved by the University of North Carolina IACUC. Female adult (at least 10 weeks in age) dopamine-beta-hydroxylase (DBH)-cre mice were crossed to L10a-EGFP mice (L10a) to label NE neurons.

Mice were administered one of 3 different treatment paradigms consisting of 2 injections (s.c.) timed 2 hours apart (first injection between 10-11 AM) across 3 days. The first group was administered a saline injection followed by a naloxone injection (SN). The second group was administered a morphine (10 mg/kg) injection followed by a saline injection (MS). The third group was administered a morphine (10 mg/kg) injection followed by a naloxone injection (1 mg/kg). On day 4, 24 hours following the last injection, mice were euthanized, brains were sliced, and recordings were performed (as; Torruella Suarez et al., 2020; Downs et al., 2022).

Results: To explore the impact of opioid withdrawal on noradrenergic function in the A2/NSS noradrenergic neurons. DBH-cre mice were crossed to the L10a line so that noradrenergic (A2) neurons could be differentiated from neighboring neurons in the NTS.

To explore adaptations in glutamatergic transmission on to the A2/NST neurons, we recorded spontaneous excitatory postsynaptic currents (sEPSCs) at -80 mV in the presence of picrotoxin (25 uM). While we did not see a change in the amplitude of the events across the groups (one-way ANOVA), there was a trend for a decrease in the frequency of events in the animals that had been exposed to morphine ($p = 0.0730$). When the area of the currents was further examined (total charge transfer), there was a significant reduction (one-way ANOVA, $F(2,28) = 11.84$, $p < 0.001$) in the current area in the MN group as compared to the SN and MS groups (Tukey's multiple comparisons $p < 0.01$ and $p < 0.001$ respectively.) Correspondingly, there was a significant reduction in the decay of these currents in the MN group (one-way ANOVA, $F(2,28) = 13.79$, $p < 0.0001$) as compared to the SN and MS groups (Tukey's multiple comparisons $p < 0.0001$ and $p < 0.01$ respectively).

We next examined miniature excitatory post synaptic currents (mEPSCs) both at baseline and in the presence of NASPM (50 uM) a blocker of calcium-permeable AMPA receptors (CP-AMPARs). While there was a main effect of the group ($F(2,14) = 4.804$, $p < 0.05$) on the amplitude, we found no differences in the average amplitude of the mEPSCs between the groups both in the presence and the absence of NASPM. When we examined event frequency however, there was a main effect of NASPM treatment (two-way repeated measures ANOVA, $F(1,28) = 4.441$, $p < 0.05$), however the only group with a significant difference between the baseline frequency and post-NASPM frequency was the MN group (Sidak's multiple comparisons test, $p < 0.05$). When we examined decay kinetics we found that there was a significant interaction (two-way repeated measured ANOVA, $F(1,14) = 9.225$, $p < 0.01$) between the groups before and after NASPM application such that there was a significant difference only in the MN group following NASPM (Sidak's multiple comparisons test, $p < 0.01$).

Finally, we examined properties of excitability in the A2/NST neurons. We first examined spontaneous discharge from these neurons and found that there was a significant effect of treatment on the frequency of A2/NST action potentials (one-way ANOVA, $F(2, 17) = 4.467$, $p < 0.05$), where the MN group was significantly higher than the SN group (Tukey's multiple comparisons, $p < 0.05$) and a trend for a difference between with the MS group (Tukey's multiple comparisons, $p = 0.0818$). Cells were then injected with current to maintain them at -75 mV, and a increasing steps of current (delta 20 pA, to a total of 400 pA) were examined to determine the number of action potentials that fire at each current step. Here we found a current by treatment group interaction ($F(40, 220) = 3.101$, $p < 0.0001$), and a main effect of treatment group ($F(2, 11) = 8.866$, $p < 0.01$), such that MN neurons fired more action potentials compared to the other groups and there were no differences between the MS and SN animals.

Conclusions: Together, these data suggest that there are adaptations to morphine exposure and withdrawal that occur in the A2/NST neurons, and that the effects are most dramatic in our model of repeated opioid withdrawal, as compared to mice exposed to morphine without naloxone, or naloxone alone. Our data here suggest that the MN model results in profound plasticity on glutamatergic synapses promotion the insertion of CP-AMPA receptors, and regulates the intrinsic excitability of these neurons. Future studies will explore these phenomena in male mice, and examine how long lasting and/or transient these changes are.

Keywords: Norepinephrine, Opioid Addiction, Opioid Dependence, Pharmacotherapy, Animal Model, Withdrawal

Disclosure: Nothing to disclose.

P751. Altered Cognitive Control and Neurophysiological Signaling Following Rodent Prenatal Alcohol Exposure

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Background: It is well established that alcohol consumption during pregnancy can lead to poor outcomes in offspring. However, Fetal Alcohol Spectrum Disorders (FASD) are still the most common type of neurodevelopmental syndrome. Based the National Institutes of Mental Health (NIMH) recent focus on examining Research Domain Criteria (RDoC) via quantification of behavior more likely to reflect a specific neurophysiological circuit. Individuals with FASD have consistently been found to have difficulties with the domain of cognitive control. We have recently shown that the touchscreen 5 Choice Continuous Performance Task (5C-CPT) can measure both attention and cognitive control in rodents, but also demonstrates similar cross-species neural signatures when coupled with EEG-recording. Here we examined the impact of a moderate prenatal

alcohol exposure model on 5C-CPT, as well as measures of effort and reward learning, performance and EEG signatures in frontal, parietal and motor cortices.

Methods: Male and female mice (12-16 per sex/trt) were obtained from the New Mexico Alcohol Research Center where PAE is established via a drinking-in-the-dark model (10% EtOH with 0.066% saccharine for 4 h/day throughout gestation; BAC: ~90 mg/dL; controls=0.066% sweetened water, "SAC"). Beginning at 8-10 weeks, mice were trained to touch white square target stimuli. Once responding rapidly and accurately mice underwent stereotactic surgery and fitted with dura-resting skull screws targeting medial prefrontal, parietal, and motor cortices. After recovery and reminder to criterion, non-target trials (5 stimuli presented; withholding of response was rewarded) were added at a 2:1 ratio for 5 days followed by recording at a 5:1 ratio for 12 days. ANOVA was used to examine main effect of treatment, sex, and interaction effects for dependent variables including behavioral measures of accuracy, impulsivity and false alarm rates as well as EEG measures such as power.

Results: During training to target trials, all mice required more sessions to reach criteria as stimulus presentation decreased [Session: $p < 0.0001$]. However, there was no significant differences on sessions to reach criterion between sex or treatment with no interaction [Sex: $p = 0.79$; Trt: $p = 0.97$; Sex x Trt x Session: $p = 0.89$]. When nontarget trials were introduced for the 5C-CPT at a 2:1 ratio for 5 sessions there was a significant difference for sex and treatment, but not for session, and no interaction for hit rate [Session: $p = 0.96$, Sex: $p = 0.04$; Trt: $p = 0.002$; Sex x Trt:, $p = 0.06$]. The false alarm rate was significantly different for sex and treatment with an interaction, with no main effect of session [Session: $p = 0.74$, Sex: $F(1,140)=17.49$, $p < 0.0001$; Trt: $F(1,140)=32.07$ $p < 0.0001$; Sex x Trt: $F(1,140)=4.49$, $p = 0.04$]. During EEG recording on a difficult the 5:1 variant, PAE mice were significantly more likely to respond to nontargets than SAC controls [Session: $p < 0.0001$, Sex: $p = 0.59$; Trt: $p = 0.001$] with no interaction [Sex x Trt: $p = 0.25$], indicative of poor response inhibition to these unpredictable stimuli. EEG Time-frequency analysis revealed strong high frequency enhancement in correct rejection trials compared to target correct trials with an interaction of sex x treatment [ROI: $p = 0.20$; Sex: $p = 0.54$; Trt: $p = 0.65$; Sex x Trt: $p = 0.04$]. In addition, we found a strong low frequency enhancement in target-post-error response trials compared to target-post-target [ROI: $p = 0.005$] with no effect of sex, treatment, nor an interaction [Sex: $p = 0.31$; Trt: $p = 0.22$ Sex x Trt: $p = 0.11$].

Conclusions: Utilizing a PAE model that impairs cognitive control, we found that no alterations attention to target-only trials. However, PAE consistently impaired performance when animals were required to withhold responding to rare nontarget trials. Importantly, EEG results showed an increase in frontal theta in both PAE and SAC animals regardless of sex for post-error correction as well as an increase in posterior beta during choice conflict indicative of increased mental effort. Analysis of posterior beta revealed a sex x treatment effect with increased power during correct rejection trials in female PAE mice as compared to target correct trials indicative of increased mental effort required to differentiate trial types. These results demonstrate 1) that 5C-CPT can detect post-error signal changes consistent with findings in human EEG studies and 2) that alterations in cortical activity via EEG may be used as a potential biomarker of PAE.

Keywords: Cognitive Control, EEG Biomarkers, Touchscreen

Disclosure: Nothing to disclose.

P752. Prelimbic Neuron Calcium Activity Predicts Perceived Hedonic Value Across Drinking Solutions and Alcohol Dependent States in Mice

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Background: Binge alcohol (ethanol) drinking and per capita alcohol consumption are increasing, as are alcohol-related hospitalization rates and deaths. The prefrontal cortex (PFC) encodes anticipatory and goal-directed behaviors, and prolonged alcohol misuse impairs function of the PFC and shifts behaviors toward those that bias alcohol-seeking over natural rewards. Because of the importance of neural activity in the prelimbic (PrL) cortex during active ethanol drinking and adaptations in the PrL produced by alcohol dependence that drive excessive and compulsive ethanol-seeking behaviors, the first goal of this study was to determine population activity in PrL glutamatergic projection neurons surrounding drinking for different rewarding solutions and how alcohol dependence alters PrL activity during voluntary alcohol drinking and alcohol-bias for natural rewards. A second focus of this study was to determine how adulterating alcohol with quinine changes the neural activity pattern of PrL neurons before and after induction of dependence.

Methods: To achieve these goals, we used fiber photometry recordings of GCaMP6f calcium transients to define PrL neural activity patterns surrounding licking bouts while C57BL/6 J mice were actively drinking water, alcohol (20%, v/v), and sucrose (1%, w/v) in their home cages. Next, we determine how chronic intermittent ethanol (CIE) exposure altered alcohol drinking and choice behaviors for alcohol over natural rewards in male and female mice. We also assessed neural activity patterns surrounding binge alcohol drinking in the presence of quinine. Because machine learning (ML) can predict neural activity from behavior, we applied two different ML algorithms – support vector machine (SVM) and extreme gradient boost (XGBoost) classifiers – to determine if the signature of neural activity preceding drinking behavior can predict the hedonic value across three different solutions and the alcohol dependence-induced change in the PrL signal. All data were analyzed using mixed linear models.

Results: Mice consumed more sucrose than water and alcohol, and licking bouts occurred during prolonged up-states in the GCaMP6f signal that were higher for sucrose and alcohol compared to water. Consistent with evidence of increased neural activity during goal-directed behaviors, we observed an increase in the PrL GCaMP6f population signal preceding drinking bouts. The GCaMP6f signal for sucrose was significantly higher than alcohol and water, and the signal for alcohol was significantly higher than water. Contrary to our hypothesis that PrL→NAcore circuitry would encode for alcohol over water, the GCaMP6f signal surrounding licking bouts for alcohol were similar to those for water. Using the population signal, the SVM significantly predicted when mice were drinking water, alcohol, and sucrose with high accuracy ($p < 0.001$) within individual drinking sessions, and the XGBoost classifier demonstrated modest, but highly significant ($p < 0.001$) accuracy for predicting the three drinking solution from one another across drinking sessions. CIE exposure increased alcohol drinking and produced a bias toward alcohol over sucrose. Over time, the signal preceding water, alcohol, and sucrose drinking bouts decreased in non-dependent mice, but the signal preceding alcohol bouts remained elevated in dependent mice. The addition of quinine to the alcohol drinking solution also decreased the signal in controls, but not in CIE exposed mice, and the SVM significantly predicted the quinine-induced change in the calcium signal prior to, but not after CIE exposure.

Conclusions: In this study, we provide experimental and computational evidence from supervised machine learning classifiers that PrL population activity patterns track the perceived hedonic value of rewarding solutions in C57BL/6 J mice voluntarily drinking in a home cage model. One striking finding from these studies is that ramping of PrL activity predicts drinking behavior

for three solutions with differing hedonic value. Moreover, we showed that mice predominantly consumed fluid when the PrL population signal was in a prolonged upstate, and features of the pre-bout ramping signal could accurately discriminate between the three drinking solutions. Consistent with a declining VTA signaling for sucrose licking within a single drinking session, we observed a decrease in the peak calcium signal for all solutions across time. Another main finding from our study is that the pre-bout PrL signal parallels escalated and compulsive drinking behavior in a mouse model for the study of AUD. Our results provide evidence for a functional signature in the PrL cortex that aligns with the salience of reward-related behaviors likely signaling the intention to drink, even when mice are rendered dependent upon alcohol. Finally, we identified an aberrant intention signal in the PrL cortex of dependent mice that likely serves as a mechanism driving compulsive-like alcohol drinking and alcohol-biased choice behaviors.

Keywords: Alcohol, Medial Prefrontal Cortex, Compulsive Behavior, Fiber Photometry, Machine Learning

Disclosure: Nothing to disclose.

P753. Heroin-Seeking Behavior is 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 in the nucleus accumbens (NAc). BDNF mRNA levels are oppositely regulated following chronic heroin exposure vs withdrawal, suggesting it may play a role in opioid-related behavior during both these phases. Here we show that endogenous PLCgamma1 in the rat nucleus accumbens (NAc) can both limit and enhance the development of relapse-like heroin seeking depending on the phase of the self-administration paradigm when PLCgamma1 is manipulated.

Methods: We first infused a shRNA expression viral vector that reduces PLCgamma1 levels (AAV-shPLCgamma1) 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 first allowed rats to acquire heroin self-administration for 12 days, then rats underwent a stereotaxic surgery to infuse AAV-shPLCgamma1. After 3 weeks of abstinence, we again measured context-associated heroin seeking during extinction conditions. Finally, we also examined the effects of AAV-shPLCgamma1 infused before the acquisition of sucrose-taking to determine if the effects on heroin-seeking were generalizable to non-drug rewards. 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 found that reducing PLCgamma1 in the NAc prior to the start of aquiring self-administration behavior led to an eventual increase in heroin-seeking behavior. Since we reduced PLCgamma1 levels in the NAc prior to the initiation of heroin self-administration, we next examined whether reducing PLCgamma1 after cessation of self-administration would also increase heroin-seeking behavior. Surprisingly, reducing PLCgamma1 levels after the self-administration phase suppressed context-associated heroin seeking, an effect opposite to when PLCgamma1 was reduced prior to the acquisition of heroin self-administration. In contrast to the significant effects on heroin-seeking, no obvious effects were observed for sucrose-seeking behavior. In addition, no obvious sex differences were observed in these experiments.

Conclusions: These results strongly suggest that PLCgamma1 plays different roles during different phases of heroin self-administration: acquisition, abstinence, and context-associated heroin seeking. Moreover, our findings suggest that therapeutics targeting PLCgamma1 function might be helpful for treating relapse vulnerability in individuals suffering from opioid use disorder.

Keywords: Heroin Self-Administration, Extinction and Reinstatement, Nucleus Accumbens

Disclosure: NeuroEpigenix, LLC: Founder (Self).

P754. Parabrachial Extended Amygdala Circuit Activity is Heightened Following Repeated Stress: Implications for Alcohol Abstinence

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Background: Forced abstinence in a mouse preclinical model of chronic ethanol vapor exposure (CIE) increases anxiety-like behavior and produces neuronal adaptations in the bed nucleus of the stria terminalis (BNST), a region critical for affective behavior. Lateral parabrachial nucleus (LPBN) projections expressing calcitonin gene related protein (CGRP, Calca gene) drive bed nucleus of the stria terminalis (BNST) in vivo activity in synchrony with anxiety-like behavior. We hypothesize an anxiogenic role for LPBN(CGRP) in alcohol abstinence and stress, associated with heightened BNST activity.

Methods: All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and institutional guidelines approved by the Institutional Animal Care and Use Committee at Vanderbilt University. Male and female CalcaCRE mice ($n = 5$-10/sex/ virus group) received bilateral injections of CRE-dependent hM3D(Gq) DREADDs or CRE-dependent mCherry fluorophore in the LPBN. Anxiety-like behavior was measured with the elevated plus maze (EPM) following LPBN(CGRP) hM3D(Gq) activation by CNO (clozapine-n-oxide, 3 mg/kg, IP; 30 min pretreatment). To investigate the role of LPBN(CGRP) activation on stress-induced anxiety and BNST activity, CalcaCRE male mice ($n = 3$/exposure/treatment) received bilateral injections of CRE-dependent hM3D(Gq) DREADDs in the LPBN and the calcium indicator GcAMP7 in the BNST. Following 4 days of repeated forced swim test stress paired with LPBN(CGRP) hM3D(Gq) activation, anxiety-like behavior was measured with NSFT. To identify cell-specific changes in LPBN(CGRP)-- > BNST neurotransmission BNST-containing brain slices were bath applied CNO (10uM) and changes in GCaMP fluorescence were measured with slice photometry recordings. Post hoc thick slice immunohistochemistry was performed to characterize LPBN(CGRP) projections innervating BNST cells. Lastly, to investigate LPBN(CGRP) inhibition on anxiety-like behavior CalcaCRE male and female mice received bilateral injections of CRE-dependent hM4D(Gi) DREADDs in the LPBN and were exposed to chronic intermittent ethanol vapor exposure (CIE; $n = 4$-8/sex/ vapor exposure). In early CIE withdrawal (4-6 hr) mice were given CNO to activate LPBN(CGRP) inhibitory hM4D and tested in EPM. To investigate stress and anxiety during prolonged abstinence (2 wks), mice underwent

4 days of (FSS) and anxiety-like behavior measured by novelty suppressed feeding task (NSFT).

Results: LPBN(CGRP) activation increased EPM time immobile in hM3D(Gq) relative to mCherry group (2-way ANOVA virus $p = 0.03$). LPBN(CGRP) activation paired with FSS increased body rotations across time on day 3 (2-way ANOVA time x treatment $p < 0.02$) with no changes in NSFT behavior. Ex vivo slice recordings demonstrate LPBN(CGRP) activation decreased global BNST spike frequency through recruitment of inhibitory and excitatory neuronal populations. This LPBN(CGRP)-induced decrease in BNST activity was potentiated in FSS relative to no stress controls (3-way ANOVA, wash, stress, treatment $p < 0.02$). Post hoc analysis demonstrates colocalization of pituitary adenylate cyclase activating (PACAP)-expressing LPBN(CGRP) projections in the BNST. In early withdrawal LPBN(CGRP) inhibition increased EPM immobility in CIE relative to air vapor exposure (2-way ANOVA vapor $p = 0.05$; treatment $p = 0.01$). In prolonged withdrawal, LPBN(CGRP) inhibition increased total body rotations relative to saline controls on FSS day 2 (2-way ANOVA, $p = 0.004$). A history of LPBN(CGRP) inhibition increased NSFT immobility relative to saline controls (2-way ANOVA treatment $p = 0.05$).

Conclusions: These data demonstrate LPBN(CGRP) modulates stress and anxiety-like behavioral responses associated, in part, with heightened BNST activity. Additionally, the data demonstrate a history of repeated LPBN CGRP neuron inhibition is anxiogenic in prolonged abstinence suggesting long lasting LPBN(CGRP)-induced changes in behavior. Given pharmaceutical treatments for migraines targeting CGRP inhibition are bioavailable, these studies inform a potential role for CGRP treatments in alcohol-abstinence.

Keywords: Alcohol Abstinence, Anxiety and Stress, Anxiety Circuitry, BNST, Parabrachial Nucleus

Disclosure: Nothing to disclose.

P755. Cell-Type Specific Ca2 + Dynamics in the Nucleus Accumbens Underlying Natural Reward Seeking Behavior

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Background: The Nucleus Accumbens core (NAc) plays a central role in reward seeking and cue-reward associations. Its constituents Dopamine Receptor type 1 and 2 expressing GABAergic medium spiny neurons (D1- and D2- MSNs) integrate cortical and limbic inputs to regulate reward seeking behavior. The downstream effects of these adaptations on D1- and D2- MSNs, and the information by these neuronal populations during reward seeking, remains largely unknown.

Methods: We recorded single-cell calcium Ca2+ dynamics in D1- and D2- MSNs in freely behaving Drd1- and Drd2-cre transgenic mice using a miniature microscope (nVista) and virally expressed Cre-dependent Ca2+ indicator (syn- GCaMP6f). Mice were trained to self-administer sucrose in daily 2 h-sessions during which a single nose-poke (FR1) resulted in an intravenous cocaine injection and presentation of drug-contingent cue. Natural reward seeking was assessed after 10 days of abstinence, during which cues associated with the natural reward were present, but the reward was omitted. The same behavioral paradigm was also used to assess the effect of direct time-locked optogenetic stimulation of D1- and D2- MSNs (20 Hz, 1 s) expressing ChR2(H134r) on reward seeking behavior. All recordings were motion corrected and normalized to background, and PCA/ICA algorithm was used to extract individual cell locations and traces. Individual neuronal traces were aligned to relevant behavioral events, and further analyzed using custom MATLAB codes.

Results: While time-locked optogenetic stimulation of Dl- or D2-MSNs ($n = 4$ per group, $p < 0.05$) respectively potentiated or halted reward seeking activity, overall recorded calcium activity shows an increase in D2-MSNs activity, compared to D1-MSNs following reward seeking (ANOVA, $n = 150$-200 cells, $p < 0.01$). At a single cell level, both Dl- ($n = 5$, ~200-250 cells) and D2- ($n = 4$, ~180-200 cells) MSNs showed a highly heterogenous response around a seeking epoch (5 seconds before to 10 seconds after a seeking nosepoke). 30-40% of D1- or D2-MSNs exhibit a stable divergent (equally distributed between excitatory and inhibitory ensembles) time-locked response associated with cued-seeking across trials. This divergent activity profile around seeking epochs is absent early in training and develops progressively over training in both population of neurons.

Conclusions: This data challenges the functional dichotomy of the direct/indirect pathways hypothesis and suggest a dynamic interaction between both cell types to regulate reward seeking.

Keywords: Reward, Nucleus Accumbens, in Vivo Calcium Imaging

Disclosure: Nothing to disclose.

P756. Paternal Cocaine Self-Administration Enhances Fear-Related Memory and Inhibits Amygdala Synaptic Activity Only in Male Offspring

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Background: Cocaine self-administration by male rats induces epigenetic modifications in sperm resulting in neuronal and behavioral alterations in offspring including decreases in cocaine taking, enhanced anxiety, spatial memory deficits, and reductions in hippocampal synaptic activity. Given the high degree of overlap between the brain systems resulting in pathological responses to cocaine and stress, we examined whether sire cocaine taking would influence fear-associated behavioral and physiological effects in drug-naïve male and female progeny.

Methods: Adult male Sprague-Dawley rats were trained to self-administer cocaine for 60 days while a second group of male rats received yoked saline infusions. Rats then mated with drug-naïve female rats to create an F1 generation of saline- or cocaine-sired offspring. Both male and female F1 rats were then subjected to amygdala-dependent auditory fear conditioning and cue extinction. Separate groups of rats underwent electrophysiological analyses of amygdala synaptic activity.

Fear-associated memories were generated by exposing rats ($n = 8$-9 per group) to 3 presentations of an audio tone (conditioned stimulus) that co-terminated with a 2 second, 0.75 mA footshock. Each presentation was separated by a 1-minute intertrial interval, during which we recorded the animal's freezing response. 24 hours later, rats were placed in a novel context (different floor, odor, and visual cues) and underwent cue extinction, which consisted of 18 tone-only presentations. Freezing was measured in response to each tone presentation, averaged across 3 consecutive trials. On Day 3, rats received 18 more tone-only presentations in the extinction context.

For electrophysiology experiments, we performed whole-cell patch clamp recordings in lateral amygdala principal neurons ($n = 15$-21 neurons, 5-7 rats per group) and compared baseline measures of synaptic activity (spontaneous activity, AMPA:NMDA, paired-pulse ratio [PPR]) by stimulating the external capsule, which carries information from cortical sources such as the medial prefrontal cortex and auditory cortex. We used a spike-timing dependent plasticity protocol ([0.1 ms presynaptic pulse followed

by a 1 ms postsynaptic spike, separated by 10 ms] delivered at 1 Hz for 100 sec) to induce long-term potentiation at these synapses.

Fear conditioning experiments were analyzed using two-way repeated measures analysis of variance (RM-ANOVA) with the within-subjects factor being time (CS trial) and the between-subjects factor being sire. Electrophysiological comparisons were made with either unpaired t-test or RM-ANOVA.

Results: Similar to other studies, these experiments revealed a sex-dependent effect of sire, whereby cocaine-sired males but not female rats generated stronger fear-associated memories. There were no differences in the acquisition of auditory fear, with equivalent levels of freezing observed in both saline-sired and cocaine-sired offspring. On Day 2, both cocaine- and saline-sired male rats exhibited a high level of initial freezing that was subsequently extinguished across the session. However, cocaine-sired male rats spent a significantly higher percentage of time freezing relative to saline-sired rats. The total Day 2 extinction data, analyzed by an unpaired t-test, indicated a significant difference between groups [$t(100)=4.537$, $p < 0.0001$]. On Day 3, there was again slightly higher initial freezing in the cocaine-sired offspring, however by the end of the session both groups exhibited similar degree of extinction. Taken together, these data are indicative of a stronger cue-evoked fear memory in cocaine-sired male rats that persists longer and is therefore more resistant to extinction. These phenotypic differences were absent in female offspring; saline- and cocaine-sired females had equivalent levels of freezing across all phases of behavioral testing.

In male rats that had not been subjected to fear conditioning, we found no differences in baseline measures of synaptic activity, but did observe differences in LTP induction. In saline-sired rats, there was a robust LTP induction as indicated by an increase in EPSC amplitude compared to baseline recordings, that persisted for more than 45 minutes post-induction. However, there was no increase in EPSC amplitude in amygdala synapses from cocaine-sired animals. 2 way ANOVA indicates a main effect of sire [$F(1,12)=13.90$, $p < 0.0029$], suggesting a deficit in LTP induction in cocaine-sired animals. Similar to our behavioral findings, there were no differences in LTP induction in saline vs. cocaine-sired female offspring.

Conclusions: In summary, male, but not female, cocaine-sired rats exhibit resistance to extinction of cue-conditioned fear memories and this resilience may be related to deficits associated with cortical-amygdala synaptic LTP. Ongoing experiments are working to further delineate the molecular and physiological mechanisms responsible for the deficits in fear extinction and LTP in cocaine-sired male rats.

Keywords: Slice Electrophysiology, Fear Conditioning and Extinction, Cocaine Self-Administration, Amygdala, Transgenerational

Disclosure: Nothing to disclose.

P757. Activation of Thalamic Inputs to the Dorsomedial Striatum Drives Reinforcement, Place Preference, and Locomotor Activity

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Background: Glutamatergic thalamic inputs to the striatum produce dopamine release via actions on dopaminergic terminals, and this form of dopamine release is independent of somatic firing of dopamine neurons. There is increasing evidence that dopamine release evoked by somatic firing vs. terminal regulation mechanisms produces unique contributions to behavior. Striatal dopamine release plays important roles in the acute and chronic effects of several classes of psychoactive drugs, but whether psychoactive drugs can differentially impact dopamine-mediated behaviors driven by dopamine neuron firing vs. terminal mechanisms such as thalamically-evoked dopamine release has not been elucidated. Our previous work demonstrated that closed-loop optogenetic stimulation of thalamic inputs to the dorsomedial striatum (DMS) is sufficient to reinforce operant responding in mice. We further assessed reward, reinforcement, and locomotor stimulation driven by this pathway using optogenetic stimulation.

Methods: Adult male and female C57BL/6 J mice were injected with AAV9-hSyn-hChR2(H134R)-eYPF (or control virus expressing eYFP) targeted to the anterior intralaminar nuclei of the thalamus to allow optogenetic stimulation of thalamic neurons using channelrhodopsin-2 (ChR2). Optical fibers were implanted in the DMS to selectively activate terminals of DMS-projecting thalamic neurons. In experiment 1, mice were trained to press a lever to receive a one-second train of optogenetic stimulation (455 nm, ~7 mW, 20 Hz, 5 ms pulse width) of thalamostriatal terminals on a continuous reinforcement schedule. Following ten 30-minute training sessions, the effect of within-session changes in stimulation frequency was assessed using 4 stimulation frequencies (presented in ascending order) for 10 minutes each. In experiment 2, we determined whether mice would seek optogenetic stimulation in a real-time place preference test. Optogenetic stimulation (20 pulses, 20 Hz, delivered every 5 s) was delivered in one chamber of a place preference apparatus and the amount of time mice spent in the stimulation-paired side was measured. In experiment 3, we assessed the effects of thalamostriatal stimulation on locomotor activity in an open field. Data were analyzed using unpaired t test or one-way, two-way, or three-way repeated measures ANOVA.

Results: In experiment 1, all mice ($n = 6$) readily acquired lever-pressing behavior when lever presses were reinforced with 20 Hz optical stimulation of thalamic terminals in the DMS. Across ten training sessions, mice escalated lever pressing of the active lever but not the inactive lever (two-way repeated measures ANOVA, lever x session interaction: $F(9, 45) = 9.40$, $p < 0.001$). In response to within-session changes in stimulation frequency, mice updated their response rates, with lower stimulation frequencies producing lower rates of responding (one-way repeated measures ANOVA, main effect of stimulation frequency: $F(3, 12) = 6.735$, $p = 0.0065$). In experiment 2, mice expressing ChR2 ($n = 18$) spent more time in the chamber paired with optogenetic stimulation of thalamostriatal terminals during 3 consecutive sessions, but did not exhibit conditioned place preference when the stimulation was discontinued. In contrast, mice expressing YFP ($n = 9$) did not exhibit real-time place preference for optogenetic stimulation (three way RM ANOVA, virus x stimulation side interaction, $F(1,25) = 5.087$, $p = 0.0331$). We also assessed whether thalamostriatal activation can impact locomotor activity in an open field. Compared with mice expressing YFP, optogenetic stimulation in ChR2-expressing mice enhanced locomotor activity in a 20-min open field test by increasing both velocity and distance traveled (distance traveled ChR2 vs. YFP, unpaired t test: $p = 0.0263$; velocity: $p = 0.036$; $n = 9$/group). Effects on locomotion were rapidly modulated during repeated light-on versus light-off epochs in a single open field session (distance traveled, two-way RM ANOVA virus x stimulation interaction: $F(1,16) = 11.40$, $p = 0.0039$; velocity, virus x stimulation interaction: $F(1,16) = 11.43$, $p = 0.0038$). Thalamostriatal activation selectively enhanced horizontal locomotion, as we did not observe changes in the performance of other natural behaviors (i.e., rearing and grooming). Optogenetic stimulation of thalamostriatal terminals also did not affect avoidance of the center of the open field (ChR2 vs. YFP, unpaired t test: $p = 0.69$).

Conclusions: Together with our previous findings, these results suggest that activation of thalamostriatal neurons can support

both reward and reinforcement learning, while also promoting locomotion. We have also established a model of optogenetic intracranial self-stimulation of thalamostriatal terminals that will allow investigation of the effects of psychoactive drugs on reward and behavioral reinforcement driven by this pathway. Future studies will investigate the potential roles of this circuit in reward, reinforcement, and psychomotor stimulation produced by psychoactive drugs.

Keywords: Dorsomedial Striatum, Thalamus, Reward Circuitry, Reinforcement Learning, Locomotor Activity

Disclosure: Nothing to disclose.

P758. Activity in the Lateral Habenula Predicts Avoidance of Fentanyl Withdrawal Associated Contexts and is Modulated During Oral Fentanyl Self-Administration

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Background: Opioid abuse has reached epidemic proportions in the United States, with the synthetic opioid fentanyl involved in almost half of the 40,000 opioid related deaths each year. Fentanyl produces rapid and potent euphoria while withdrawal is severe, causing significant physical and emotional turmoil. Both the positive and negative emotional experiences associated with fentanyl use are strong motivators of relapse. Though many brain structures and circuits are involved in opioid seeking, a compelling node for study is the Lateral Habenula (LHb), a small brain structure with an outsize impact on decision making. The LHb receives convergent input from across the basal ganglia and the limbic system and projects strongly to several key midbrain nuclei involved in decision making. This circuitry makes the LHb poised to integrate cognitive and emotional stimuli with both positive and negative valance, and to influence behavior. Using fiber photometry, this study aims to explore the role of the LHb in two unique components of relapse, 1) avoidance of negative emotional states associated with withdrawal modeled by conditioned place aversion to naloxone precipitated withdrawal, and 2) drug seeking in response fentanyl associated cues, modeled by oral fentanyl self-administration and cued reinstatement testing.

Methods: Fiber Photometry: 500nL of AAV1-Syn-jGCaMP7f was injected into the LHb and an optical fiber (400 µm, NA0.66) was placed directly above the injection. An isosbestic 405 nm LED as well as the signal generating 465 nm LED were used to eliminate artifacts. Event related calcium transients (ΔF/F) were analyzed around behavioral events including chamber transitions, lever presses, head entries, etc.

Conditioned Place Aversion (CPA): On day 0, rats were allowed free access to both sides of a CPA test chamber for 15 minutes. On days 1-5, rats underwent rapidly dose-escalating fentanyl (or vehicle) injections (2x daily; 200, 400, 600, 800, 1000 µg/kg ip). On day 6, animals received their morning fentanyl injection, then 2 hours later received a saline injection and were confined to one side of the CPA chamber for 1 hour. After returning to their home cage for 3 hours, animals received an injection of naloxone (0.3 mg/kg ip) and were confined to the other side of the chamber for 1 hour. On day 7, animals were allowed free access to both sides of the CPA chamber for a 15-minute test session.

Oral Fentanyl Self-Administration (SA): Three days prior to SA, rats were pre-exposed to 0.1% saccharin solution overnight in their home cage. Male and female rats ($n = 8,8$) were trained to lever press (FR1) for liquid fentanyl (70 µg/mL) in Hydropac H20 (0.15 ml/kg/delivery) for 3 hours a day, 5 days a week, over 3 weeks. The first 5 days of SA employed a "saccharin fade" where saccharin concentration quickly fell (0.1%, 0.08%, 0.06% 0.04%, 0.02%). Upon active lever press, liquid was delivered paired with a compound stimulus (CS) consisting of illumination of a lever light (1 s) coincident with a 4.5 kHz tone (10 s) and extinction of the house light (10 s), during which a time-out was imposed. Beginning week 4, rats underwent extinction training for 7 days. After extinction, animals underwent cued reinstatement testing signaled by a CS presentation. Repeated measures ANOVA were used to assess change in behavior across SA, and ANOVA were used to compare extinction to reinstatement.

Results: Conditioned Place Aversion: Naloxone reliably produces withdrawal in both male and female rats that have undergone escalating fentanyl injections ($f(1,29)=7.71$, $p < 0.01$). During CPA testing, LHb activity increases during trials where approaches from the saline to the naloxone paired chamber are halted, and trials where rats successfully exit the naloxone paired chamber.

Oral Fentanyl Self Administration: Both male and female rats self-administer escalating quantities of fentanyl across 3-weeks of self-administration ($F(14,154)=12.76$, $P < 0.01$). The latency between lever pressing and head entry also decreased significantly over weeks ($F(14,154)=4.96$, $P < 0.01$). Conditioned cues were sufficient to induce reinstatement of fentanyl seeking in males and females following 7 days of extinction training, as evidenced by increased lever pressing ($F(1,24)=16.81$, $P < 0.01$) and head entries ($F(1,24)=15.1$, $P < 0.01$), and decreased head entry latency ($F(1,24)=-7.15$, $P = 0.013$). LHb activity increases after lever presses and decreases during rewarded head entries. If head entries go unrewarded, LHb activity rebounds rapidly.

Conclusions: The lateral habenula is engaged during conditioned place aversion testing and oral fentanyl self-administration, though the exact nature of that activity and whether it is necessary for the expression of these behaviors is still unclear. While recording from the LHb holistically, we noticed some heterogeneous responses across animals that may be due to subtle variations in GCaMP7f expression profiles, or a mix of increases and decreases in activity. In the future we aim to record from individual neurons in the LHb while optically tagging their projections to the midbrain so we can capture circuit specific increases and decreases in activity, and ultimately manipulate those circuits to try to reduce conditioned place aversion and reinstatement to fentanyl seeking.

Keywords: Fentanyl, Oral Self-Administration, Fiber Photometry, Lateral Habenula, Opioid Withdrawal

Disclosure: Nothing to disclose.

P759. Amygdala-Cortical Circuit Determinants of Social Isolation-Induced Alcohol Consumption

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Background: Although there are correlations between social isolation and increased alcohol consumption, as further supported by the surge in alcohol sales and use during the COVID-19 pandemic, little is known about the neurobiological mechanisms underlying these phenomena. What brain changes are induced by social that trigger alcohol drinking?

Methods: To address this question, we used a combination of behavior, ex vivo patch electrophysiology, optogenetics, cellular resolution calcium imaging, and machine learning.

Results: We found that social rank predicts alcohol drinking ($r2 = 0.25$; $^{**}p = 0.01$; $n = 26$), where subordinates drink more than dominants ($^{**}p < 0.01$; $n = 3$/group), and that social isolation further increases alcohol drinking in all mice ($^{***}p < 0.001$; $n = 14$),

while decreasing sucrose drinking (*$p < 0.05$; $n = 6$). We then found that social isolation increases neural excitability in the basolateral amygdala (BLA) (*$p < 0.05$; $n = 13$-14 cells), and stimulating BLA terminals in the medial prefrontal cortex (mPFC) is sufficient to increase consumption of alcohol (***$p < 0.001$; $n = 6$/group). To reveal how social isolation modifies BLA representations of alcohol, we used longitudinal cellular-resolution calcium imaging and machine learning. We found that alcohol responsive amygdala functional clusters turnover following social isolation ($n = 309$ neurons/ 6 mice). To determine the impact of amygdala functional turnover on representation of alcohol, we used a generalized linear model (GLM) and found population-level amygdala dynamics was sufficient to decode alcohol verses water consumption, and social isolation increases GLM decoding performance. In contrast, social isolation decreases GLM decoding performance of sucrose verses water consumption, consistent with the diametrically opposing effects of social isolation on alcohol and sucrose consumption. To then determine how amygdala inputs can modify mPFC representations of alcohol, we combined optogenetics and imaging and found that amygdala-cortical terminal activation abolishes positive valence responses to sucrose without altering negative valence responses to shock ($n = 708$ neurons/ 6 mice), suggesting that the amygdala-cortical circuit induces a negative affective or loneliness-like state by inhibiting positive encoding cortical neurons which may motivate alcohol use.

Conclusions: Together, we identified a cellular substrate of social isolation and resolved a role for the amygdala-cortical circuit in social isolation-induced escalated alcohol drinking.

Keywords: Basolateral Amygdala, Social Isolation, Alcohol, Prefrontal Cortex

Disclosure: Nothing to disclose.

P760. Cocaine Induced Neurophysiological Alterations in Corticostriatal Circuits to Reward Predictive Cues Following Outcome Devaluation

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Background: The ability to alter behavior in response to changes in consequences is necessary for navigating an ever-changing environment. Substance use disorders (SUDs) are characterized by a continuation of maladaptive behavior despite negative consequences. Thus, characterizing the underlying processes that modulate the ability to change or stop behavior in response to updated expected outcomes is critical for understanding the neurobiological alterations in SUDs. A history of cocaine exposure leads to impaired ability to adjust behavioral responding to a decrease in expected outcome value following a devaluation procedure in rats. Here, we investigated how cocaine exposure followed by withdrawal leads to aberrant, differential patterns of neural activity in subregions of the rat frontal cortex (prelimbic, PrL vs infralimbic, IL) and striatum (ventral vs dorsal striatum) to reward predictive cues following a decrease in expected outcome value.

Methods: Mildly water deprived Long-Evans rats either underwent self-administration for cocaine (i.v., 1 mg/kg/press, cocaine group) or self-administration for water (0.2 ml into a water trough) and saline (i.v.) as a control group. Rats were either implanted with unilateral microwire arrays in the PrL and nucleus accumbens core (NAc) subregion of the ventral striatum ($n = 13$ cocaine, $n = 8$ control) or unilateral microwire arrays in the IL and dorsolateral striatum (DLS) subregion of the dorsal striatum ($n = 6$ cocaine, $n = 7$ control). Rats underwent self-administration daily for 2-hour sessions over 14 days (~22 mg of cocaine per session). After 3 weeks of abstinence, rats underwent Pavlovian conditioning for 10 days. They were presented with two distinct cue light patterns as conditioned stimuli (CS+), each predicting a different reward (grain or sucrose pellet). One CS + predicted a sugar pellet and the other CS + predicted a grain pellet (10 trials each). Two other light patterns did not predict a reward (CS-). After 10 days of conditioning, rats underwent a devaluation procedure for the sugar pellets to induce a conditioned taste aversion (LiCl, i.p., 0.3 M; 7.5 ml/kg). Post-devaluation, rats were tested on the same Pavlovian task to evaluate their ability to avoid the CS + previously paired with the devalued outcome vs nondevalued outcome in the absence of the rewards (under extinction).

Results: PrL, IL, NAc and DLS electrophysiological recordings showed neuronal populations that were phasic to CS + [excited or inhibited] or nonphasic (no response to CS+). In the PrL, there were no differences in the overall responding to NonDevalued and Devalued cues in cocaine and control rats. In the NAc, rats with a history of cocaine showed more phasic neurons to the devalued CS + (29% inhibited and 23% excited) compared to NonDevalued CS + (22% inhibited and 13% excited). In contrast, control rats show similar % of phasic to both Devalued (10% inhibited and 7% excited) and NonDevalued (18% inhibited and 7% excited) CS + . In the DLS, we found a higher % of excited phasic neurons compared the control group in the DLS to both Devalued CS + (cocaine 37% and control 14%) and NonDevalued CS + (cocaine 26% and control 14%). In the IL, we found similar pattern as the DLS in that the cocaine group showed higher % of excited phasic neurons compared to the control group in response to both Devalued CS + : (cocaine 18% and control 3%) and NonDevalued CS + (cocaine 18% and control 7%).

Conclusions: Here, we show that the NAc neurons respond more to the devalued CS + compared to NonDevalued CS + in cocaine rats compared to control rats, while we found elevated neural activity, particularly excited neurons, in the IL and DLS after a history cocaine to reward predictive cues. In contrast, we did not observe differences in PrL neural activity to NonDevalued and Devalued CS + after a history of cocaine. Together these findings suggest overactive neural encoding, particularly to the devalued CS + in the NAc, DLS, and IL. One possibility is this overactive neural responding to reward predictive cues post-devaluation contributes to the inability to disengage from the cues that predict the devalued outcome. Future studies will aim to determine if these changes in neural activity in these regions after history of cocaine are causally linked to impaired ability to shift behavior following outcome devaluation.

Keywords: Dorsolateral Striatum, Infralimbic Cortex, Prelimbic Cortex, Nucleus Accumbens, Pavlovian Conditioning

Disclosure: Nothing to disclose.

P761. Stress-Induced Escalation of Cocaine Intake is Modulated by Sex and Endocannabinoid Signaling

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Background: Clinical evidence has identified stress as an important contributing factor to substance use disorder (SUD). This is particularly problematic as stress is unavoidable in daily life. Therefore, understanding the neurobiological mechanisms that underlie the contribution of stress to SUD is critical. One characteristic of SUD is a loss of control over drug intake that is modeled, in the rat, by conditions that result in escalating patterns of drug self-administration (SA). Repeated daily stress at the time of SA induces an escalation of cocaine intake in a glucocorticoid-

dependent manner. This stress-induced escalation of SA is likely influenced by sex, as there are notable sex differences in both SUD and stress reactivity, and likely involves neurobiological mediators 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 induces a persistent increase in eCB signaling, particularly in regions critical for both cocaine taking and seeking, that results in escalation of cocaine use and increased susceptibility to 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 ×30 min SA sessions separated by 5-min drug-free periods. Some rats received intermittent electric footshock stress in the SA chamber during the 5 min drug-free period over 14 days. Rats underwent extinction training and were tested for increased susceptibility for reinstatement to various stimuli. We examined the involvement of endocannabinoid signaling in stress-escalated cocaine intake by administration of a cannabinoid receptor type 1 (CB1R) antagonist systemically or directly into the nucleus accumbens (NAc) shell or ventral tegmental area (VTA) prior to a SA session. Changes in CB1R binding and density were examined in the NAc shell and VTA 24 h after the last SA session in rats that underwent cocaine or saline SA under stress and non-stress conditions. 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 test for involvement of endocannabinoid signaling in augmented reinstatement following stress-induced escalation of cocaine intake. Rats are currently being tested for the role of eCB signaling on changes in cocaine-induced dopamine dependent upon drug or stress history by using in vivo fiber photometry measurement of a dopamine biosensor.

Results: Electric footshock stress administered daily at the time of self-administration induced an emergent escalation of cocaine intake in both male and female rats ($n = 16$-18/group). However, female rats tend to escalate faster and to a greater degree. 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 ($n = 6$-10/group). In male rats, this effect is localized to both the NAc shell and VTA as direct administration of the antagonist attenuates cocaine intake in stress-escalated rats ($n = 6$-9/group). A history of stress at the time of cocaine SA increases the time to reach extinction criterion in both sexes though the effect is greater in female rats. Rats with a history of stress at the time of SA show augmented reinstatement to a priming injection of cocaine ($n = 11$-14/group), re-introduction of the footshock stress during the 5-min drug-free period ($n = 9$-12/group), and to an injection of the alpha-2 adrenergic receptor antagonist yohimbine ($n = 14$-17/group). 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 endocannabinoid system as repeated stress recruits endocannabinoid signaling in mesolimbic regions to drive drug use. Additionally, these stress-induced neuroadaptations are long-lasting and also occur in prefrontal cortical regions critical for drug-seeking behavior. All of these behaviors are also influenced by sex as females appear to be more sensitive to both the effects of stress of drug use and seeking and the role of endocannabinoid signaling in these effects. Understanding the unique mechanisms by which stress can drive drug use has implications for identifying and treating patients with SUD in whom stress is a contributing factor.

Keywords: Acute and Chronic Stress, Endocannabinoids, Cocaine Self-Administration and Reinstatement, Sex Differences

Disclosure: Nothing to disclose.

P762. Methamphetamine Effects are Altered by Alcohol Bingeing: Sex Differences in Behavior and Neurochemical Measures

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Background: Methamphetamine (MA) a highly addictive psychostimulant is frequently abused in combination with alcohol. More than 75% of those diagnosed with amphetamine dependence also have an alcohol use disorder (AUD). Despite different acute effects, both are associated with changes in key neurochemical in several brain regions associated with motivation and learning. Despite the co-morbidity little is known of the neurochemical and behavioral consequences of co-abuse. Moreover, little is known of their co-abuse in male and female populations. Using voluntary oral methamphetamine administration (VOMA) in both male and female mice, we have found major differences in behavioral and neurochemical systems in male and female mice. Using a modified escalating dose-access VOMA paradigm female mice exhibited greater behavioral and neurochemical deficits than males. [Previous studies have identified acute and chronic deficits in dopamine related markers following binge and VOMA models (Avila JA, 2018; Braren, Drapala, Tulloch, and Serrano, 2014; Xu, Zhu, and Angulo, 2005). Thus, we examined the effects of the dopamine transporter (DAT), the dopamine precursor tyrosine hydroxylase (TH) and the the dopamine receptor 1 and 2 (D1, D2) in hippocampus following chronic MA. The effect of neurotoxic or binge dosing of MA shows increased susceptibility in males than females as observed by more extensive striatal DA reduction and larger decreases in DAT (Bourque, Liu, Dluzen, and Di Paolo, 2011; Dluzen, Anderson, and Pilati, 2002; Wagner, Tekirian, and Cheo, 1993; Yu and Liao, 2000).] In addition, alcohol has been shown alter these systems with chronic self-administration. Previous research exploring the interaction of alcohol with the psychomotor stimulant cocaine has shown agonistic effects brain reward systems and activity (Lewis and June, 1994) and low dose alcohol and amphetamine effects on food intake. Effects of the combinations of alcohol with MA or other psychomotor stimulants on male and female mice is unknown.

Methods: We used an animal model of voluntary oral methamphetamine administration (VOMA) that consisted of an acquisition phase from days 1-14 characterized by escalating doses of MA, and a binge phase from days 14-28 characterized by static doses of MA. Female VOMA mice displayed increased MA consumption during the binge phase of VOMA, demonstrating sex specific vulnerabilities to the maintenance of MA addiction. Mice were tested for spatial working memory performance on a radial 8-arm task following abstinence from VOMA. Other groups of mice were exposed to a binge model of alcohol self-administration prior to VOMA. Animals were trained to self-administration alcohol using the drinking in the dark (DID) paradigm. Male and female mice were given access to 15% (W/V) alcohol for two hours per day starting one hour into the dark portion of a 12/12 light dark cycle.

Results: Results indicate that female VOMA mice had more working memory deficits correlated to higher MA consumption, a

result not observed in male VOMA mice. Hippocampal and accumbal tissue were collected and analyzed using western blotting. Female VOMA mice had decreased GluA1, but not GluA2, in the hippocampus which may perpetuate synaptic destabilization and may underlie the observed increase in working memory deficits as well as increased GluA1 expression in the nucleus accumbens suggesting a female specific vulnerability toward abstinence induced drug craving and drug seeking. Furthermore, p-GSK3β signaling was decreased in the nucleus accumbens, hinting at enhanced downstream neurotoxicity and inflammation that is female specific. The effects of alcohol binge drinking prior to VOMA are expected to alter the intake of MA in both male and female mice in complex ways indicating enhancement of drug seeking behaviors. Effects on brain neurochemistry will follow.

Conclusions: Our study reveals female specific neurochemical shifts in AMPA receptor signaling in the hippocampus and nucleus accumbens following abstinence from chronic MA consumption suggesting that these molecular modulations may underlie female susceptibility to MA-induced dysfunction. These data demonstrate a novel molecular signaling pathway that may be involved in enhanced vulnerability to drug craving and drug seeking that exacerbates memory deficits in vulnerable female populations following MA abuse. These data also validate VOMA as a model sensitive to sex differences in behavior and hippocampal neurochemistry following chronic MA exposure. We anticipate that prior bingeing on alcohol affects MA intake and neurochemical effect and that they differ in males and females.

Keywords: Methamphetamine, Alcohol and Drugs, Nucleus Accumbens Glutamatergic Afferents, Hippocampal Function, Sex Differences

Disclosure: Nothing to disclose.

P763. VTA Dopamine Neurons Engage Subregion-Specific Striatal Dopamine Signals During Pavlovian Learning

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Background: Environmental cues, through Pavlovian learning, become conditioned stimuli that guide animals toward the acquisition of rewards (for example, food) by invigorating and directing seeking behavior. We have previously shown that brief optogenetic excitation of dopamine neurons, in temporal association with visual sensory cues, can instantiate those cues as conditioned stimuli that evoke conditioned movements. It remains unclear 1) how dopamine-neuron mediated, cue-evoked behavior is signaled by dopamine release downstream in striatal subregions and 2) how subregional signals evolve across stages of learning.

Methods: Here, we made use of a genetically encoded dopamine biosensor (dLight) to monitor dopamine signaling in the nucleus accumbens core (NAC), dorsomedial striatum (DMS), and dorsolateral striatum (DLS) with fiber photometry, while tracking detailed movement features during optogenetic Pavlovian cue conditioning of VTA dopamine neurons.

Results: Our results demonstrate a progressive recruitment of cue-evoked dopamine signaling across striatal subregions that correlates with different features of behavior. Cues paired with optogenetic activation of VTA dopamine neurons evoked dopamine release preferentially in the NAC early in training when behavioral responses were slower and directed toward the cue. Critically, these NAC signals got larger, rather than diminishing with extended training. As conditioning progressed, cue evoked signals also emerged in the DMS and DLS, when movement patterns became more vigorous and not directed at the cue. We found subregion specific heterogeneity in the relationship between cue-evoked signals and movement vigor. DMS cue-evoked signals predicted slower movements, while DLS signals predicted faster movements, and NAC signals were not correlated with movement. In additional studies, we found that brief optogenetic inhibition of DLS dopamine terminals at cue onset late, but not early, in training decreased the probability of cue-evoked locomotion and slowed the onset of movement initiation.

Conclusions: Together our studies show dissociable, parallel functions for ventral and dorsal striatal dopamine signaling in guiding versus invigorating behaviors. Further, they suggest that large-scale plasticity across the striatal dopamine network emerges during Pavlovian learning to coordinate behavior.

Keywords: Dopamine, Striatum, Fiber Photometry, Motivation, Cortico-Striatal-Thalamo-Cortical Circuits

Disclosure: Nothing to disclose.

P764. A Novel μ-Opioid Receptor Transgenic Cre Rat: Cellular and Behavioral Characterization

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Background: To specifically manipulate MOR-expressing neurons, we developed a transgenic rat to co-express Cre-recombinase and MOR under the endogenous Oprm1 gene promoter. We performed validation experiments to show expression patterns of both Oprm1 and Cre-recombinase and assess impact of targeting Cre to the Oprm1 gene on opioid-mediated pain responses and heroin self-administration (SA).

Methods: We used RNAscope, fluorescence in situ hybridization chain reaction (HCR RNA-FISH), and autoradiography to verify that the knock-in manipulation had no effect on Oprm1 mRNA expression, and that iCre co-expresses with Oprm1. We test basal response to pain, morphine analgesia and tolerance. We trained male and female heterozygote (HET) rats and wildtype (WT) littermates to self-administer heroin and tested them in three relapse measures. We also tested the effect of nucleus accumbens (NAc) AAV1-EF1a-Flex-taCasp3-TEVP (Caspase3) injections on initiation and maintenance of heroin SA.

Results: There were no differences between HET and WT rats in NAc MOR expression and function. Preliminary results showed co-localization of Oprm1 with iCre in HET rats. There were no differences in pain sensitivity or response to morphine, and no genotype-related differences for heroin SA, extinction responding, context-induced reinstatement, and heroin reacquisition. NAc Caspase3 lesions decreased MOR expression and function in HET but not WT. Additionally, the lesions had sex-specific effects on initiation and maintenance of heroin SA maintained by different drug doses and different fixed-ratio reinforcement schedules.

Conclusions: The novel Oprm1-Cre transgenic rat can be used to study the role of brain Oprm1-expressing cells in opioid addiction- and pain-related behaviors, as well as other opioid-mediated learned and innate behaviors.

Keywords: Opioid Abuse, Self-Administration, Pain Analgesia

Disclosure: Nothing to disclose.

P765. Nicotine Normalizes the Dynorphin-Dependent Expression of Negative Emotional States During Nicotine Abstinence

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Background: Tobacco use disorder is the leading preventable cause of death worldwide. Negative emotional states during nicotine abstinence significantly contribute to the subsequent escalation of nicotine intake, but the neurobiological mechanisms that underlie this phenomenon are unclear. We hypothesized that the upregulation of dynorphin in the central nucleus of the amygdala (CeA) mediates the negative emotional states of abstinence, relapse, and escalation of nicotine intake through the inhibition γ-aminobutyric acid (GABA) transmission.

Methods: We used intracellular recordings, immunohistochemistry, and viral-mediated downregulation of dynorphin in the CeA to evaluate the role of dynorphin in GABA transmission, hyperalgesia, conditioned place aversion, stress-induced reinstatement, and escalation of nicotine intake in male rats. The data were analyzed using one-way analysis of variance (ANOVA) for between-subject comparisons or repeated-measures ANOVA for within-subject comparisons, followed by the Newman-Keuls post hoc test as appropriate. For individual means comparisons, Student's t-test was used.

Results: Abstinence from nicotine increased dynorphin levels and dysregulated GABA transmission in the CeA, and nicotine (0.03 mg/kg/infusion) reversed these responses, normalizing dynorphin levels and GABA transmission back to control (pre-abstinence) level. Viral-mediated downregulation of dynorphin mRNA (by 75%) in the CeA prevented abstinence-dependent behaviors (hyperalgesia, aversion to withdrawal, and yohimbine-induced reinstatement of nicotine seeking) but not nicotine-dependent behavior (escalation of nicotine intake).

Conclusions: These results demonstrate that nicotine normalizes dynorphin levels and dynorphin-dependent changes in GABA transmission during abstinence, and the upregulation of dynorphin in the CeA during abstinence is critical for the expression of the negative emotional states and stress-induced relapse but not nicotine intake. These results suggest that tobacco smoking may be partly driven by the ability of nicotine to normalize aberrant dynorphin/GABA transmission in the CeA during abstinence. Targeting the dynorphin system may represent a novel approach to reduce the negative emotional states of nicotine abstinence and reduce stress-induced relapse.

Keywords: Dynorphin, Nicotine Addiction, GABA Transmission

Disclosure: Nothing to disclose.

P766. Context- and Lever Access-Dependent IEG Expression During Heroin Seeking After Forced Abstinence in Rats: Insights Into Functional Network Connectivity

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Background: Opioid relapse after detoxification reflects the interplay of neural circuits that control goal-directed and habitual responses, attentional processing, and negative affect. While several relevant brain regions have been identified, functional connectivity among these brain regions during opioid-seeking behaviors has not been evaluated. The aim of this study was to map correlated activity-related protein/plasticity-related protein (ARP/PRP) expression across 43 brain regions as a function of exposure to heroin-paired contextual stimuli and lever access.

Methods: Male Sprague-Dawley rats were trained to lever press for heroin infusions in a distinct environment (heroin-paired context) over 10 days. The rats were then exposed to a different environment without access to operant levers or heroin (unpaired context) over 14 days. On post-drug day 15, the rats ($n = 7$/group) received a 2-h drug-free test session in the heroin-paired context or the unpaired context, either with or without lever access. ARP/PRP expression (i.e., c-Fos and Zif268 immunoreactivity) was quantified in 43 brain regions in brain tissue collected immediately after the test session. Lever responses, cFos immunoreactivity, and Zif268 immunoreactivity were analyzed using analyses of variance with context and lever access as factors. Correlational and principal component analyses were used to evaluate relationships between normalized ARP/PRP expression values within and across brain regions after Fisher's transformation. Functional network analyses were restricted to brain regions that exhibited significant ARP/PRP expression. Alpha was set at 0.05.

Results: Heroin-seeking behavior was more persistent in the heroin-paired context than in the unpaired context at test ($p < 0.05$), and ARP/PRP protein expression varied as a function of testing context and lever access, with interaction between these variables observed in only eight brain regions. Furthermore, heroin-seeking behavior positively correlated with cFos expression in the medial septum and dorsal cornu ammonis 3 subregion of the hippocampus.

Network-connectivity analyses revealed that exposure to the heroin-paired context augmented positively correlated ARP/PRP expression ($p < 0.05$), or coupling, among subregions of the frontal cortex, amygdala, and dorsal striatum compared to unpaired context exposure. The specific pattern of coupling suggested that subcircuits involved in executive, limbic, and motor function independently influence focused motivated behavioral output at multiple levels, including at the level of the M1 motor cortex. In contrast, unpaired context exposure generated coupling among a more diffuse set of brain regions, including subregions of the hippocampal formation and septum, which might reflect cognitive processes related to a wider response repertoire in the unpaired context.

Lever access versus no-lever access elicited distinct correlated ARP/PRP expression ($p < 0.05$) among a smaller number of brain regions compared to context exposure. In particular, lever access resulted in coupling among brain regions that mediate affective, attentional, and stress responses, including the septum, lateral habenula, and central amygdala, possibly due to the emergence of negative affective states when lever responses were not reinforced during the test session. While lever access also elicited ventral pallidum-M1 coupling, no-lever access elicited negatively correlated ARP/PRP expression ($p < 0.05$), or decoupling, between these and other brain regions, suggesting functional connectivity that can bidirectionally control the initiation of operant behavior.

Conclusions: These findings expand upon existing literature and provide impetus for future research investigating the causal contributions of several novel brain regions and circuits to heroin relapse.

Keywords: Opioid, Self-Administration, cFos, Zif268, Heroin Seeking

Disclosure: Nothing to disclose.

P767. Dynorphinergic Control of Amygdalo-Striatal Circuits for Goal-Directed Behavior

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Background: Goal-directed action-outcome behavior is essential for survival and co-opted during pathological drug-seeking. Recent work suggests that neurons from the basolateral amygdala (BLA) projecting to the dorsomedial striatum (DMS) may contribute to goal-directed action; yet, how BLA-DMS activity instantiates goal-directed action, or how it is regulated during action-outcome behavior is unknown. Furthermore, ~50% of the neurons in the DMS express the endogenous opioid peptide dynorphin (dyn); however, how dyn in the DMS regulates action-outcome behavior is unknown. Here, we hypothesize that BLA-DMS projections coordinate action-outcome behavior, and their activity and subsequent behavior is refined by dyn-KOR signaling.

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. Ex-vivo viral tracing and in situ hybridization of BLA projections to DMS (WT/Ai14/vglut1-cre/KOR-cre, $n = 2$-3 mice, 6 slices).

2. Ex-vivo electrophysiology of DMS neurons during optogenetic activation of BLA terminals (D1-tdtmto, $n = 5$ mice, 16 cells); KOR activation (U69,593 – 1uM) at BLA terminals during optogenetic activation (D1-tdtmto, $n = 4$ mice, 9-16 cells).

3. In-vivo fiber photometry during operant behavior of BLA-DMS terminalls (vglut1-Cre, $n = 4$ mice).

4. Time-locked optogenetic manipulations of BLA-DMS terminals in-vivo via activation using ChR2 (vglut1-cre or WT, $n = 9$ mice) or inhibition using PPO (vglut1-cre or WT, $n = 9$ mice).

5. In-vivo 2-photon imaging of BLA-DMS ensembles during head-fixed operant behavior (Ai14, $n = 4$ mice).

6. In-vivo fiber photometry during operant behavior of BLA-DMS terminals multiplexed with conditional dyn deletion (WT/pdyn-cKO, $n = 7$ mice) or dyn neuron stimulation (pdyn-cre, $n = 4$ mice).

7. In-vivo fiber photometry during operant behavior of dyn release in the DMS (WT/pdyn-cre, $n = 4$ mice).

Results: We show that ~20% of BLA neurons project to the DMS ($n = 2$-3 mice, 6 slices) and are glutamatergic (vglut1+) using a combination of anterograde and retrograde viral tracing, and in situ hybridization. Additionally, BLA terminals preferentially activate DMS D1 + /dyn neurons ($n = 16$ cells, 2 Way ANOVA; $p = 0.0014$ – D1(+) vs. D1(-), $F(1,203) = 10.50$). During operant behavior where animals perform a nosepoke (action) to obtain a sucrose reward (outcome) multiplexed with fiber photometry, we find that as animals learn goal-directed action-outcome behavior ($n = 4$ mice, operant, Simple Linear Regression; $p < 0.005$, $R2 = 0.48$), BLA-DMS terminal activity emerges during action-outcome learning. We show that this activity is increased during action ($n = 4$ mice, z-score, t test; $p < 0.0005$ – untrained vs. trained), and inhibited during outcome ($n = 4$ mice, z-score, t test; $p < 0.01$ – untrained vs. trained). To determine whether BLA-DMS activity is causal to action-outcome behavior, we perform optogenetic manipulation of BLA-DMS terminals during operant behavior. We observe that photoactivation during outcome disrupts action ($n = 9$ mice, operant, t test; $p < 0.05$, $t(8)=3.14$) and photoinhibition enhances action ($n = 9$ mice, operant, t test; $p < 0.005$, $t(8)=4.92$). Furthermore, to determine if this pattern of activity is reflected at the BLA soma or is unique to BLA-DMS axon terminals, we use in vivo 2-photon Ca2+ imaging of BLA-DMS ensembles during head-fixed operant behavior. We observe that as mice learn operant behavior ($n = 4$ mice, operant, t test; $p < 0.05$, $t(3)=3.4$), distinct BLA ensembles (512 cells) are engaged during action vs. outcome, and that BLA-DMS ensembles (50/512 cells) are predominantly only action-activated. This suggests that the inhibition in BLA-DMS terminal activity during outcome occurs locally at BLA-DMS terminals. Using retrograde viral tracing and in situ hybridization, we find that ~60% of BLA-DMS neurons express KOR. We also observe that optogenetically-evoked activity via BLA terminals at DMS D1 + /dyn neurons is sensitive to KOR activity ($n = 9$-16 cells, t test; $p < 0.001$, $t(21)=3.038$). To delineate how BLA-DMS terminal activity may be regulated in the DMS by dyn-KOR signaling, we use conditional deletions of dyn or photoactivation of dyn release during in vivo photometry of BLA-DMS terminals and action-outcome behavior. We find that deletion of dyn in the DMS or KOR in the BLA, negatively impacts action-outcome learning and maintenance ($n = 7$ mice, operant, 2 Way ANOVA; $p < 0.0005$ – day x genotype, $F(4,40) = 8.99$ - WT vs. dyncKO) and BLA-DMS activity ($n = 7$ mice, z-score, t test; $p < 0.0005$ – WT vs. dyncKO). Conversely, we show that stimulating dyn release during outcome enhances action ($n = 4$ mice, operant, t test; $p < 0.05$, $t(3)=3.2$ – stim vs. no stim), and BLA-DMS activity ($n = 4$ mice, z-score, t test; $p < 0.05$ – stim vs. no stim). Finally, we show via in vivo photometry during action-outcome behavior using a novel dyn sensor (KLight1.3) that significant dyn release occurs during outcome retrieval and consumption ($n = 4$ mice, z-score, t test; $p < 0.05$ – baseline vs. outcome).

Conclusions: Altogether, we show that BLA-DMS activity engenders goal-directed action-outcome learning and maintenance and that BLA-DMS terminal activity is distinct to the activity of DMS-projecting BLA soma. Furthermore, DMS dyn release during outcome, resulting in retrograde dyn transmission from the DMS onto KOR at BLA terminals enables dynamic changes in BLA-DMS activity and promotes action-outcome behavior.

Keywords: Goal-Directed Behaviors, Dorsal Striatum, Basolateral Amygdala, Dynorphin, Kappa Opioid Receptor

Disclosure: Nothing to disclose.

P768. Time Varying Functional Connectivity Signatures of Acute Psychostimulant Administration

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Background: Psychostimulants, like cocaine and bath salts, activate the mesolimbic and mesocortical pathways via dopaminergic activity, leading to extended functional brain circuit alterations that can spread to the entire brain. We can explore the characteristics of acute psychostimulant activation and spread over the whole brain using computational neuroscience approaches and resting state fMRI. One approach that stands out is time-varying functional connectivity (tvfc) to determine the spatiotemporal characteristics of the brain under psychostimulants administration. To this end, we sought to explore the features of a single administration of a psychostimulant in the rat brain using resting state fMRI and tvfc.

Methods: Rats were imaged under combined dexmedetomidine (0.02 mg/kg intraperitoneal)/isoflurane (0.5%) sedation (delivered in 70%N2/30%O2 at 0.1 L/min). Resting-state fMRI datasets were collected in a 4.7 Tesla Agilent system (Magnex Scientific) at 1 hour after i.p. administration of 3.0 mg/kg MDPV ($n = 8$), 15 mg/kg cocaine ($n = 8$), or saline ($n = 7$). Resting fMRI data were acquired with a 2-shot spin-echo echo planar imaging with 210 repetitions for a total acquisition time of ~7.5 mins (an image was acquired every 2 s).

The resting state fMRI datasets were analyzed using time window approaches to estimate graph-theoretical biomarkers of network node flexibility. Notably, we began our efforts focusing on providing robust statistical benchmarks (i.e., surrogates) to

provide convincing standards to improve the sensitivity and the interpretation of dynamical properties. We analyzed the global resting state using windows of 10 s, 20, 40, and 1 min, and overlapping between windows of n-4 time points, n-3, n-5, n-7, and n-10 time points per window, where n is the size of the time window. Then, we focused on the differences between drug and saline groups using a time window of 1 min and an overlap of n-1.

The network metric assessed was flexibility, a node's ability to switch modules between time windows. A module is defined by the modularity index, which measures the propensity of separating the network into modules with inter-module connectivity.

Results: Comparison between drug and saline administered shows that the resting state data consistently shows a distinct pattern of flexible behavior in the brain. We compared three surrogates as null hypothesis times series, and they all showed similar behavior and different to the brain data. The surrogates were: phase scrambled fMRI time series without preserving static network metrics, phase scrambled fMRI time series preserving static features, and a limited bandpass series of random numbers resembling the temporal characteristics of fMRI time series. The global features between all experimental groups showed similar dynamical features indicative that the drug doses and studied time points preserve the dynamical features of the control group. However, individual node features showed a consistent, distinct profile among a subset of nodes in the temporal lobes, somatosensory cortices, and frontal cortices. Cocaine groups showed reduced flexibility; meanwhile, MDP showed increased flexibility compared to saline. These differences were significant between psychostimulants but not between individual psychostimulants and saline.

Conclusions: This work shows that tvfc can offer unique features of dynamical function indicative of psychostimulant-induced activity. Despite the surrogates' widely different time series features, they all showed similar properties. They were distinct from the fMRI data, indicating that the flexibility is quantifying an organized dynamical feature unique to brain function. Further studies are needed to establish the neural relevance of these flexible transitions and the mathematical formulation to interpret these results concerning brain function.

Keywords: fMRI, MDPV, Time Varying Functional Connectivity, Network Analysis

Disclosure: Nothing to disclose.

P769. Contextual Opiate Tolerance: Brain-Wide Changes in Neuronal Activity and Control by Mediodorsal Thalamus and Anterior Cingulate Cortex

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Background: Opioid analgesics are one of the most commonly prescribed medications in the U.S. However, opioids have considerable abuse liability due to their non-analgesic effects. As users increase their opioid intake in a particular context, they build tolerance against the effects of these drugs within the context. This phenomenon is known as associative analgesic tolerance. Since associative analgesic tolerance can lead to dose and use escalation, it increases opioid use disorder (OUD) risk. Therefore, a deeper understanding of the factors controlling associative tolerance may inform the development of treatments aimed at decreasing OUD risk in patient populations. While associative tolerance and its reversal have been described in animal studies, the biological mechanisms underlying this process are not understood. Here, we examined how associative opioid tolerance changes activity patterns throughout the entire brain and identified two target regions that orchestrate associative tolerance in male mice.

Methods: In experiment 1, we established a training regimen that induces associative analgesic tolerance. Male and female C57BL/6 J mice ($n = 20$/ group) underwent associative tolerance training for 14 days. Each day, mice received injections of either fentanyl (25 ug/kg, s.c.) or saline before being placed in a context with distinct multi-sensory features. After 15 minutes within the context, basal nociception and fentanyl-induced analgesia were measured using the hotplate assay (latency to nociceptive response, max time = 30 seconds). Following this training phase, associative tolerance was measured by administering fentanyl in the fentanyl-paired, saline-paired, and a novel context before the hotplate test. In experiment 2, we identified which neuronal populations are engaged during associative tolerance across the entire brain. In this experiment we used male Ai14xArc-TRAP mice ($n = 4$/group), which allow for brain-wide, temporally defined tagging of active neuronal populations with the fluorescent marker tdTomato, were administered the TRAP activator tamoxifen on the last day of tolerance training to tag tolerance-active neurons. 4 weeks later, mice were injected with fentanyl and exposed to either the fentanyl- or saline-paired environment. Brains were extracted and processed using SHIELD to clear the brains and preserve endogenous fluorescence and then imaged using a custom- built high-speed selective plane illumination microscope (SPIM) to detect tagged neurons using an automatic cell counting algorithm. In experiment 3, we determined how context and/or fentanyl changed neuronal activity in specific areas implicated in contextual memory and pain processing in tolerant mice. Male mice ($n = 3$-4/group) underwent tolerance training before being exposed to one of the following conditions: saline-context and saline, saline context-and fentanyl, fentanyl-context and saline, or fentanyl context and fentanyl. Brains were extracted and analyzed via immunohistochemistry by staining for cFOS. Based on anatomical experiments, Based on anatomical experiments, in experiment 4, viral vectors encoding Designer Receptors Exclusively Activated by Designer Drugs (DREADDs; AAV5-hSyn-hM4D(Gi)-mCherry or AAV5-hSyn-mCherry, 300nL) were infused into the anterior cingulate (ACC) or dorsomedial thalamus (MDT) of male mice ($n = 6$-9/group). Mice underwent tolerance training and were injected with the DREADD activator CNO (3 mg/kg i.p.) before receiving fentanyl in the fentanyl context or saline in the saline context. Data were analyzed using one-way, two-way and three-way repeated measures ANOVA with post-hoc Sidak's multiple comparisons tests when appropriate.

Results: In experiment 1, we found that male and female mice that received fentanyl in the fentanyl-paired context showed tolerance, defined as a decrease in analgesic responses in the hotplate assay over the course of 7 daily exposures to fentanyl (male change = 11 seconds, F1, 133 = 753.7 $p < 0.0001$ female change = 10.5 seconds, 1, 19 = 110.3, $p < 0.0001$). However, tolerance was reversed in male, but not female, mice exposed to fentanyl in the saline-paired context or a novel environment (males $p < 0.001$, $p > 0.05$ females). In experiment 2, we found that exposure to fentanyl in the fentanyl-paired context increases activity in 80 identified brain regions. Post hoc literature analyses revealed that the activated areas cluster by function primarily into motor, sensory, learning, pain, and reward-associated groups. In experiment 3, we confirmed that the combination of fentanyl and the fentanyl context increased the number of cFOS-expressing neurons in the ACC and its upstream projection area, the MDT. Finally, chemogenetic silencing of either the MDT or ACC in the fentanyl-paired context suppressed fentanyl analgesic tolerance (ACC F1,14 = 5.279, $P < 0.05$ MDT F1,14 = 7.146 $p < 0.05$) but did not alter nociception when mice received saline in the saline-paired context (ACC $p > 0.05$, MDT $p > 0.05$).

Conclusions: These findings establish a robust behavioral procedure for the study of associative opioid analgesic tolerance

in mice. The findings also reveal how associative tolerance changes activity throughout the brain. Among the identified regions, the MDT and ACC appear to be important targets for the modulation of associative analgesic tolerance.

Keywords: Opioid Tolerance, Environment, Whole-Brain Rodent Imaging, Anterior Cingulate Cortex (ACC), Thalamus

Disclosure: Nothing to disclose.

P770. Basolateral Amygdala Parvalbumin Expressing Interneurons Critically Regulate Reward Seeking Behaviors

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Background: The basolateral nucleus of the amygdala (BLA) is essential for both fear and reward learning alike. Recent advances have shown that BLA microcircuitry contributes to fear conditioning, particularly that parvalbumin-expressing interneurons (PV) are able to regulate both fear expression and fear-relevant BLA oscillatory states. However, despite BLA's known role in regulating appetitive reward seeking, little is known about BLA oscillatory states during reward learning and even less is known about how BLA PV interneurons contribute to reward-related behaviors. Therefore, we aimed to fill this gap in knowledge by probing the role of PV interneurons during operant conditioning and, separately, characterizing BLA field potentials during reward learning to better understand population-level activity changes in BLA as reward is encoded and used.

Methods: Subjects:

Experiment 1: 24 adult heterozygous PV-Cre+ mice (12 female, 12 male)

Experiment 2: 12 adult wild-type C57 mice (6 female, 6 male)

Surgical Procedures:

Basolateral amygdala (AP: -1.5 mm; ML: + /- 3.3 mm; DV: 4.5 mm) was targeted in both Experiments. Animals in Experiment 1 received bilateral infusions of a Cre-dependent inhibitory designer receptor exclusively activated by a designer drug (AAV9-DIO-hM4Di-mCherry) to target PV interneurons. Animals in Experiment 2 underwent unilateral implantation of a depth electrode targeting BLA (same coordinates). Following surgery, animals were allowed to recover 2 weeks prior to commencing behavioral training and testing.

Behavioral Procedures:

Behavioral procedures were carried out in 4 identical Med-Associates conditioning chambers enclosed in sound-attenuating chambers. Training began with a free-reward (0.1 mL 20% sucrose solution) magazine training session. Then, animals underwent operant conditioning where one of two nose-poke ports were reinforced (left or right, counterbalanced). Training sessions progressed from FR1 (3 sessions) to RR2 (2 sessions) to RR3 (2 sessions) before advancing to behavioral testing. Testing included a 5-min baseline test session in extinction followed immediately by a fully-rewarded RR3 session to re-establish responding levels. The following day, animals underwent satiety-induced devaluation where they were given free access to the reward then tested afterwards, in extinction, to assess whether responding changed as a result of the reward being reduced in value. Then, the next 3 days, the animals underwent a reversal where the identity of the reinforced operandum was changed. In Experiment 1, PV interneurons were inhibited during the test sessions only (Devaluation and Reversals). In Experiment 2, animals were split into behavioral groups for comparison (Deval/non-Deval; Reversal/non-Reversal) and local field potentials in BLA were recorded.

Statistical analyses:

Behavioral measures collected include nose-poke responses and magazine entries. Individual linear mixed models were used to analyze effects of dependent variables by fixed effects of group and session while accounting for random effects of differences in starting points for the variable in Session 1. Reported statistics include parameter estimates, 95% confidence intervals, and p-values. Behavioral analyses and data visualization were carried out in R ("lme4", "ggplot2"). For Experiment 2, spectral analysis was performed on the recorded field potentials to characterize BLA network activity. Analyses and visualization were performed using custom Python scripts.

Results: We demonstrated that BLA PV interneurons are necessary for goal-directed instrumental behavior following outcome devaluation. Further, we provided evidence that suppressing BLA PV interneuron activity is sufficient to drive improved performance after contingency reversals. Lastly, we characterize BLA field potential changes as a product of reward experience and operant conditioning.

Conclusions: Taken together, these experiments investigate how local BLA activity governs appetitive reward seeking, both at the microcircuit and population activity levels. Specifically, showing that PV interneurons are necessary for flexible reward seeking is a first step towards a better understanding of how microcircuits within the amygdala mediate appetitive reward behaviors. We also examine BLA local field potential changes to characterize population-level dynamics as reward learning takes place. Given that PV interneurons can modulate BLA oscillatory states relevant to fear behaviors, it is possible that PV interneurons could perform a similar function for reward behavior. Thus, future studies will seek to identify a causal mechanism linking PV interneuron activity and BLA field potentials during appetitive reward seeking.

Keywords: Parvalbumin Interneurons, Operant Behavior, Local Field Potentials, Amygdala-Based Networks, Reward

Disclosure: Nothing to disclose.

P771. Regional Differences in Striatal Dopamine Dynamics in Pavlovian Training With a Natural Reinforcer

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Background: Dopamine is a critical neuromodulator for several neurobiological processes including learning and memory. Within the striatum, dopamine dynamics in the nucleus accumbens have been studied extensively and been classically associated with reward and reinforcement. In contrast, few studies have examined dopamine dynamics in the dorsal striatum in vivo. This is likely due to the difficulty in obtaining dorsal striatal dopamine signals in vivo with fast-scan cyclic voltammetry. Recently, genetically-encoded fluorescent biosensors for dopamine were developed and have facilitated monitoring of real-time dopamine dynamics in the dorsal striatum and other brain regions where detection was previously difficult.

Methods: Male C57BL/6 J mice were stereotaxically infused with AAV encoding dLight1.3b into the nucleus accumbens, dorsomedial striatum, or dorsolateral striatum (4-6 mice per region). Three weeks later a fiber optic ferrule was implanted into the brain region of interest with fluorescence monitored

intraoperatively to ensure placement in a location with adequate dLight expression. One month later, mice began a Pavlovian conditioning protocol with either a tone or white noise (CS+) being paired with a food reinforcer delivery (US+). Fiber photometry was performed during Pavlovian conditioning using a custom-built system and analysis was completed using custom MATLAB and python scripts. All experimental procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: Similar to previous work with fast-scan cyclic voltammetry, we found that in the nucleus accumbens, phasic dopamine transients were elicited by the US + early in training. As training progressed, the phasic dopamine transient became time-locked to the CS + and no longer responded to the US + . In the dorsomedial and dorsolateral striatum, we observed phasic dopamine increases in response to US + , and with extended Pavlovian conditioning phasic release increased in response to the CS + , but not the CS-, albeit to a lesser degree than in the nucleus accumbens. Interestingly, and unlike dopamine dynamics in the nucleus accumbens, in both dorsal striatal regions, the phasic dopamine increase to the US + persisted. This US + dopamine response was not present in unexpected omission trials suggesting that this response was not tied to motor responses but rather reflects a reinforcer-associated signal. Additionally, we found that dopamine transient decay kinetics differed between striatal subregions, with faster responses in dorsolateral striatum relative the other two regions ($p < 0.01$).

Conclusions: This work suggests a role for dopamine in dorsal striatum subregions in processing of reinforcer-associated cues as well as reinforcer delivery, independent of motor activity.

Keywords: Dopamine, Pavlovian Conditioning, Striatal Dopamine Signaling, Optical Biosensors

Disclosure: Nothing to disclose.

P772. Perirhinal Projections in Methamphetamine-Induced Recognition Memory Deficits and Relapse

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Background: Methamphetamine (meth) self-administration reliably produces object recognition memory deficits due drug-induced plasticity within the perirhinal cortex (Prh). Prh projections are numerous and include the prefrontal cortex (PFC) and nucleus accumbens (NA)- two key nodes involved in relapse biology. We have previously shown that meth exposed animals (long access; LgA) perseverate responding to meth associated cues with little regard for a novel cue presented in the same environment, and the salience of the novel cue is enhanced with stimulation (mGluR5 positive allosteric modulator, GqDREADDs) of the Prh. This Prh stimulation restored meth induced deficits in recognition memory. We hypothesize that the Prh->PFC projection mediates recognition memory while the Prh->NA drives responding for novel cues.

Methods: To evaluate the function of the Prh->PFC and Prh->NA we used a dual viral approach consisting of a retrograde adeno-associated virus encoding GFP-tagged Cre recombinase (rgAAV-GFP-Cre) in the projection area combined with excitatory or inhibitory cre-dependent DREADD in the cell body region [rAAV-hSyn-DIO-hM3D(Gq)-mCherry or rAAV-hSyn-DIO-hM4D(Gi)-mCherry (hM3Dq or hM4Di, respectively)]. First, we inhibited the Prh->PFC circuit in drug naive rats to induce a deficit in recognition memory. After three weeks for viral expression rats were tested for OR memory. Second, we activated the Prh->PFC to restore meth-induced deficits in recognition memory. Rats went through long access meth self-administration and object recognition memory tests. Brain tissue was processed for confocal microscopy to visualize mCherry and c-fos expression in the Prh. Similar experiments were conducted in the Prh->NA circuit in short (ShA) and LgA access meth animals in which relapse tests were conducted in the presence of the meth associated lever and an additional novel lever in the chamber as well as a novel cue light (novel cue group).

Results: Inhibition of the Prh-PFC circuit prevented expression of OR memory, whereas activation of this circuit with an excitatory Cre-dependent DREADD in animals with a history of long access meth self-administration reversed the meth-induced OR deficit. Inhibition of the Prh-NAc circuit with CNO increased responding on the meth associated lever relative to the novel, whereas Veh resulted in similar responding on both levers. Unexpectedly, activation of the circuit following long access meth was without an effect.

Conclusions: These data suggest a more complex circuitry governing OR memory than previously indicated with anatomical or lesion studies and informs future work aimed at understanding the role of the Prh and PFC and their connectivity in meth addiction. These findings indicate inhibition of PRH-NA in a ShA procedure is sufficient to shift behavior, however, activating the pathway in LgA "addicted" procedure is not sufficient to shift focus from meth associated cues to novel stimuli.

Keywords: Methampheatmine Use Disorder, Drug Seeking, Episodic Memory Rodents, Novelty Seeking, Novel Object Recognition

Disclosure: Nothing to disclose.

P773. Striatonigral Mediated 2-Arachidonoylglycerol Mobilization Contributes to Ethanol Mediated Signaling and Behaviors

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Background: Alcohol Use Disorder (AUD) is a major public concern and deaths involving AUD have increased during the pandemic. Striatal endocannabinoids (eCBs) and cannabinoid type 1 (CB1) receptors are implicated in ethanol's actions and may contribute to AUD. The principal striatal cells, striatopallidal and striatonigral medium spiny neurons (MSNs) can produce and release eCBs as needed in response to synaptic activity. Ethanol can alter 2-arachidonoylglycerol (2-AG) levels, which in turn may contribute to the reinforcing effects of ethanol. Furthermore, ethanol exposure preferentially affects striatonigral mediated synaptic plasticity and behaviors. Despite this, the role of striatonigral 2-AG mobilization in mediating alcohol effects remains unclear.

Methods: In this study, we used targeted deletion of the 2-AG-synthesizing enzyme, diacylglycerol lipase α (DAGLα), in striatonigral MSNs to examine the role of striatonigral 2-AG mediated signaling in ethanol-induced eCB-CB1 inhibition, sedative effects of ethanol and voluntary ethanol consumption. To study ethanol effects on presynaptic eCB signaling dynamics in rodent brain slices in real-time with high-fidelity, we used acute brain slices expressing a genetically encoded fluorescent eCB biosensor (GRABeCB2.0) in primary sensorimotor cortex combined with slice photometry.

Results: Mice lacking the 2-AG synthesizing enzyme in striatonigral MSNs (DAGLα D1 Cre+ mice) exhibited dramatically reduced evoked eCB release detected at corticostriatal terminals

in the dorsolateral striatum compared to controls. Evoked eCB transients were blocked by a CB1 receptor antagonist, confirming the specificity of eCB mediated signal as measured by the fluorescent signal of GRABeCB2.0. Although evoked eCB release was altered in the DAGLα D1 Cre+ mice, the induction of eCB-dependent long-term depression was intact. Ethanol application inhibited eCB release, and this inhibition was also reduced in the DAGLα D1 Cre+ mice. Compared to control mice, male and female DAGLα D1 Cre+ mice showed decreased sensitivity to the sedative effects of ethanol. This decreased sensitivity was not accompanied by differences in blood ethanol content. DAGLα D1 Cre+ male mice showed decreased ethanol preference compared to controls in an intermittent two-bottle free choice escalating ethanol concentration consumption procedure. This decrease in ethanol preference was not accompanied by a decrease in ethanol intake, but an increase in ethanol-induced water consumption and total volume of fluid consumption. Female mice consumed more ethanol than male mice both for control and DAGLα D1 Cre+ mice. Also, this paradigm induced an escalation in ethanol consumption in both sexes and genotypes. Furthermore, there were no differences in sucrose and quinine intake and preference in a two-bottle free choice paradigm following ethanol consumption in the sexes and genotypes tested.

Conclusions: Our findings show that selective deletion of 2-AG-mediated signaling in striatonigral MSNs is sufficient to alter ethanol-induced modulation of presynaptic eCB signaling dynamics and sensitivity to ethanol. My work aims to reveal a more accurate and detailed picture of eCB-CB1 signaling following ethanol exposure and the contribution of this signaling to behavioral effects of ethanol and AUD.

Keywords: Endocannabinoids, Alcohol Use Disorder, Optical Biosensors

Disclosure: Nothing to disclose.

P774. Multi-Site Recordings of Neural Activity in the Infralimbic Cortex, Insular Cortex, and Basolateral Amygdala Across Heroin-Seeking Behavior

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Background: Evidence indicates that infralimbic cortex (IL) promotes the extinction and inhibition of cocaine seeking, though its role in the extinction of opioid seeking is less clear. It is likely, however, that the IL interacts with other regions known to regulate drug seeking during this process. Indeed, anatomical findings reveal dense connections between the IL, the basolateral amygdala (BLA), and rostral agranular insular cortex (RAIC). Moreover, evidence from both human and rodent work indicates that the BLA and RAIC play strong roles in mediating drug craving and controlling drug-seeking behavior. Yet, how these regions interact with each other on a neurophysiological level across self-administration, extinction, and reinstatement for heroin seeking is unknown. Therefore, we sought to simultaneously record from this network in this behavioral paradigm to gain an understanding of the neural interactions among these regions during such behavior.

Methods: Adult Sprague-Dawley rats were catheterized for intravenous heroin self-administration. The self-administration procedures used standard operant conditioning chambers with dual retractable levers. Self-administration began with a high-to-low dose tapered schedule, decreasing heroin dosage every two sessions until arriving at 0.083 mg/kg/infusion. Self-administration began under an FR1 schedule, where an active lever press produced a 50 uL infusion of heroin, onset of cue light and a 20 s timeout period. After rats achieved >100 presses in an FR1 session, self-administration transitioned to trial-based sessions in which the levers were only available in specific signaled trials during the session. When rats pressed on at least 80% of the trials, they were fitted with a three-site, 96-channel microdrive array (9 tetrodes/site), aimed at the IL, RAIC, and BLA. Microdrives were custom-designed and 3D-printed with stereolithography using standard and biocompatible photopolymer resins. Single-unit and local field potential (LFP) data was collected from each tetrode. Non-parametric, Wilcoxon signed-rank analysis was conducted on neural data (both spikes and LFPs) for the three recording sites comparing neuronal activity 2 s before/after 1) trial start and 2) lever press. We analyzed spike and LFP data from the IL, BLA, and RAIC during self-administration, the 1st and 5th extinction session, and heroin-primed reinstatement.

Results: SINGLE-UNIT DATA: IL single-units had heterogeneous response profiles to the lever press during self-administration and extinction. During self-administration, some units had a short latency burst after the lever press, while others decreased firing. A third type of unit response profile was a ramping down of activity 500 ms before the lever press, then a recovery 500 ms after the lever press. This response profile was the most common during the first extinction session, but the number of units showing this diminished as extinction progressed. At reinstatement, IL single units showed a decreased in firing upon lever pressing. During self-administration, BLA single-units responses increased upon trial start and at reinforced lever press. However, these responses diminished during extinction and were not identified in reinstatement. During self-administration, RAIC single units exhibited heterogeneous responses at lever press. A nearly equal proportion of units ramped up or down within 1 s of the lever press. During early extinction, half of RAIC single-units' activity significantly decreased upon the unreinforced lever press, but these responses were absent by the end extinction.

LFP DATA: Spectral analyses revealed that RAIC alpha band (10-13 Hz) power significantly increased upon lever press during self-administration. During extinction sessions 1 and 5, theta band (4-10 Hz) power significantly dropped at the trial start signal, which was not observed during self-administration. However, significant increases in theta power occurred at the lever press during reinstatement. Throughout extinction, dynamic changes were observed in the IL. Upon unreinforced lever press, there was a significant decrease in the alpha band, followed by increases in the theta band (consistent with our previous findings). These band changes became more prominent as extinction progressed. IL theta power significantly increased upon lever press during the reinstatement session (also, consistent with our previous findings). During self-administration, BLA theta increased at trial onset, followed by an increase in power in the alpha band upon lever pressing. During extinction BLA theta increased after the unreinforced lever press and became more prominent throughout extinction and was maintained in reinstatement.

Conclusions: Although this work is still being conducted, there appears to be a dynamic network in the IL-RAIC-BLA circuitry during drug seeking behavior. Subpopulations of IL and RAIC single units ramp their activity during goal-directed behavior, whereas BLA appears to encode predictive cues relevant to drug-seeking behavior. Oscillations in the theta and alpha bands were sensitive to changes in contingencies from self-administration to extinction in the IL and RAIC. Further data collection and in-depth connectivity analyses may reveal how the current corticolimbic circuit forms a functional network during the extinction of heroin seeking.

Keywords: Recordings, Anterior Insula, Heroin Self-Administration, Basolateral Amygdala, Infralimbic Cortex

Disclosure: Nothing to disclose.

P775. Conditional Inducible Deletion of CB1 Receptors on Ventral Tegmental Area Astrocytes Maladaptively Potentiates Calcium Mobilization and Curtails Motivation for Food Reward

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Background: Cannabinoid type I receptor (CB1R) is a critical regulator for dopamine (DA) neuron activity in the ventral tegmental area (VTA). In addition to the retrograde inhibition of neurotransmitter release, CB1R has been found to profoundly modulate neuronal plasticity by promoting astrocytic calcium (Ca2+) transients and gliotransmission in the hippocampus and the nucleus accumbens (NAc). However, whether VTA astrocytic CB1R modulate Ca2+ mobilization has not yet been explored. Furthermore, the consequences of ventral tegmental astrocytic CB1R deletion on mesolimbic dopamine release and motivated behaviors remain undetermined.

Methods: In this study, we applied conditional mutagenesis to delete VTA astrocytic CB1R transcripts in adult mice to eliminate the confounding and potentially adverse effect of region-specific constitutive CB1R knockdown on neural development. And using fiber-photometry in freely behaving mice, we measured VTA astrocytic Ca2+ transients and NAc dopamine release in a progressive ratio task.

Results: Conditional, inducible CB1R deletion in VTA astrocytes maladaptively elevated astrocytic Ca2+ transients, reduced motivation for food reward and inhibited reward-evoked DA release in the NAc.

Conclusions: These findings establish CB1R on astrocytes in the VTA as critical regulators of mesolimbic dopaminergic projections recruited during the motivated pursuit of reward.

Keywords: Astrocyte, CB1 Receptor, Ventral Tegmental Area (VTA), Dopamine, Incentive Motivation

Disclosure: Nothing to disclose.

P776. Chronic Stress Alters the Intrinsic Properties of Parvalbumin-Positive Interneurons in the Ventral Hippocampus

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Background: The ventral hippocampus (vHipp) regulates a diverse set of behaviors associated with motivation and emotion. Functional abnormalities in this region are observed in a variety of psychiatric disorders, including mood and anxiety disorders. We previously demonstrated that restoring the function of parvalbumin (PV)-positive inhibitory interneurons in the vHipp of rodents alleviates physiological and behavioral deficits, including alterations in dopamine cell activity, reduced social interaction, and cognitive inflexibility. Chronic stress is a major risk factor for mood and anxiety disorders. Therefore, the goal of the current experiments is to determine how chronic stress affects the synaptic connectivity and intrinsic membrane properties of PV-positive interneurons in the vHipp.

Methods: To induce chronic stress, mice underwent chronic unpredictable stress (CUS), in which they were exposed to a total of 14 varied stressors, administered twice per day for 21 days. We used the mammalian GFP reconstitution across synaptic partners (mGRASP) technique to measure the synaptic connectivity between PV positive-interneurons and pyramidal cells in the vHipp. In a separate cohort of animals, we used whole-cell current clamp to measure the intrinsic properties of PV-positive neurons in the vHipp.

Results: Our preliminary results suggest that CUS does not alter the number of synaptic contacts between PV neurons and vHipp pyramidal cells. However, PV neurons from CUS mice had a significantly lower input resistance compared to control mice. Additionally, CUS PV neurons fired fewer action potentials and had a smaller afterhyperpolarization compared to control PV neurons.

Conclusions: These results suggest that the activity of PV-positive interneurons is altered by chronic stress. Understanding how chronic stress affects PV-positive interneurons in the vHipp may lead to the development of new cellular targets for the treatment of psychiatric disorders associated with vHipp dysfunction.

Keywords: Parvalbumin Neurons, Ventral Hippocampus, Chronic Stress, Slice Electrophysiology, Synaptic Connections

Disclosure: Nothing to disclose.

P777. Corticotropin Releasing Factor (CRF) in Primate Extended Amygdala and Ventral Pallidum: Let's Discuss Co-Transmission!

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Background: The central extended amygdala (CEA) and ventral pallidum are involved in diverse motivated behaviors, and encompass a large region of the basal forebrain in primates. Corticotropin releasing factor (CRF), a canonical 'stress molecule' is enriched in the CEA, and is dynamically regulated. CRF's role in behavior--particularly in higher species--has been hard to parse, in part because it modulates primary 'fast' transmitters in neural circuits. As a first step in clarifying CRF's role in the CEA and ventral pallidum in a species that is anatomically close to the human, we delineated CRF's distribution and co-expression with 'fast' transmitters' in this complex region.

Methods: To clarify CRF's role in the context of its primary transmitters, we used a combination of immunocytochemical and spatial transcriptomics (RNAScope) approaches to 1) survey the distribution of CRF-positive neurons in subregions of the CEA and ventral pallidum, 2) examine the overall proportions of GABAergic and glutamatergic neurons by subregion, and 3) determine the proportion of CRF-mRNA neurons that co-express glutamate (Vglut2 mRNA) and GABA (Vgat mRNA) by subregion. Three young male macaques of similar age (3 years old) and housing were used.

Results: Despite a dense cluster of CRF labeled cells in the BSTLcn, the majority of CRF-IR neurons were broadly dispersed throughout the rest of the CEA, including in the BSTLP, SLEA, CeLcn and CeM. This picture may be somewhat at odds with rodent models in which CRF-positive neurons are depicted as largely confined to dense clusters in BSTL or CeN. Surprisingly, the VP—both at classic rostral levels and its continuation into the ventromedial GPi-- also contained CRF-expressing cell bodies, detected both with protein and mRNA assays. We first examined VGAT and VGluT2 expression without respect to CRF expression. Overall, single-labeled VGAT-mRNA positive cells were the most prevalent cell type in the CEA and ventral pallidum (80%); however, VGluT2 mRNA was expressed in 20% of all neurons, 10% of which were VGAT/VGluT2 positive. Regional differences in the distribution of VGAT- and VGluT2- mRNA positive cells across CEA and ventral pallidal subregions were striking. With respect to the

CRF neuronal population, CRF/VGAT-only neurons were found in highest proportions in lateral central bed nucleus, lateral central amygdala nucleus, and medial central amygdala nucleus (74%, 73%, and 85%, respectively). Lower percentages of CRF/VGAT-only labeled cells (53%, 54%, 17%, respectively) characterized the sublenticular extended amygdala, ventrolateral bed nucleus, and ventral pallidum. These regions had higher complements of CRF/VGAT/VGluT2 labeled neurons (33%, 29%, 67%, respectively). Across all subregions, relatively stable, low proportions of CRF/VGluT2 and CRF-mRNA single-labeled cells comprised the balance of the CRF labeled cells.

Conclusions: CRF expression in the CEA and VP was in general widely dispersed, although a distinct CRF-positive cell clusters was appreciated in the BSTLcn. This was appreciated in both protein and transcript assays. CRF co-expression with its primary transmitters was heterogeneous, depending on subregion. The dorsal bed nucleus and central nucleus (the 'poles' of the CEA) have relatively homogeneous populations of CRF- GABAergic neurons, mirroring their relatively restricted efferent systems. In contrast, the ventral lateral bed nucleus and sublenticular extended amygdala, and the ventral pallidum, have heterogeneous CRF cell types, including mixed GABA/glutamatergic subpopulations, and broader efferent paths.

Keywords: Glutamate GABA Co-Release, Corticotropin-Releasing Factor (CRF), Nonhuman Primate

Disclosure: Nothing to disclose.

P778. Midfrontal Theta-Band Activity During Response Monitoring in Pediatric Patients With Attention-Deficit/Hyperactivity Disorder and Other Psychiatric Conditions

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Background: The error-related negativity (ERN) is an event-related potentials (ERPs) characterized by a negative deflection in midfrontal electroencephalogram (EEG) recording after making an error in speeded response tasks. It is one of the most commonly studied ERP components and has been investigated as a potential physiological indicator of underlying transdiagnostic mechanisms, particularly in individuals with obsessive-compulsive disorder (OCD; abnormally large ERN) and attention-deficit/hyperactivity disorder (ADHD; abnormally small ERN). However, the ERN has been shown to reflect the combination of increased total power and partial increased intertrial phase coherence (ITPC) in theta-band (4-8 Hz) activity. Our group previously demonstrated that theta-band power was abnormally enhanced in OCD pediatric patients, but that the ITPC was comparable to HC. This study extends this knowledge on theta-band power and ITPC to other forms of psychopathology during response monitoring to enable a richer understanding into the transdiagnostic neurophysiological mechanism of various psychiatric conditions.

Methods: EEG was recorded from 461 pediatric participants (Age $M = 14.20$, $SD = 3.16$; 57% female) with ADHD ($N = 107$), non-OCD forms of Anxiety disorders (AD; $N = 185$), and major depressive disorder (MDD; $N = 67$), as well as healthy control participants (HC; $N = 196$), while they completed the arrow flanker task. To extract theta-band power and ITPC from a midfrontal channel (Cz), time-frequency analyses using complex Morlet wavelet convolutions were conducted. Preliminary ANCOVAs, including sex, age, and accuracy as covariates, comparing each psychiatric condition group to HC were conducted separately. For each psychiatric group, two mixed ANCOVAs were conducted to investigate the effects of diagnostic status (vs. HC) and response type (error vs. correct) on post-response theta-band (1) power and (2) ITPC (total of 6 ANCOVAs).

Results: Theta-band total power and ITPC were larger on error responses than on correct responses in all four groups (All F statistics larger than 208 [$p < 0.001$] for power and larger than 18 [$p < 0.001$] for ITPC). In the ANCOVAs of power, there was a Group x Response Type interaction in ADHD patients ($F[1,301] = 10.46$, $p = 0.001$), such that correct response power was comparable to HC, but ADHD patients had lower error response power than HC participants. There was no other interaction effect of Group x Response Type and there was no main effect for group differences in total power among AD, ADHD, and MDD pediatric patients. For ITPC, there was no significant Group x Response Type interaction for any ANCOVA. However, there was a main effect of group in the ANCOVA comparing ADHD and HC (smaller ITPC in ADHD patients; $F[1, 298] = 10.34$, $p = 0.001$). There was no other main effect of group.

Conclusions: This project investigated the potential theta-band power and ITPC abnormalities in pediatric patients with ADHD, AD, and MDD. AD and MDD patients exhibited comparable theta-band power and ITPC to HC. However, ADHD patients showed a complex theta-band power and ITPC pattern. They had comparable correct response power to HC, but abnormally smaller power on error responses. They also showed lower overall ITPC regardless of response type compared to HC participants. These data suggest that pediatric patients with ADHD have difficulty specifically in monitoring error responses, which could underlie their difficulty in noticing and correcting mistakes. At the same time, they also appear to have general response monitoring deficit as reflected by the generally lower ITPC, which might underlie the general impulsive tendencies of individuals with ADHD. The findings also underscore the utility of time-frequency analyses in investigating similarities and differences in neural mechanisms across various psychiatric conditions and in informing future neuromodulation treatment research.

Keywords: EEG Electrophysiology, Time-Frequency, Attention Deficit Hyperactivity Disorder, Major Depressive Disorder, Anxiety Disorders

Disclosure: Nothing to disclose.

P779. Serotonin Receptors 2 A in the Rat MPFC are Necessary for Retrieval Induced Forgetting

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Background: Forgetting is a ubiquitous phenomenon that is actively promoted in many species. The very act of remembering some experiences can cause forgetting of others, in both humans and rats. We previously found that when rats need to retrieve a memory to guide exploration, it reduces later retention of other competing memories encoded in that environment. As with humans, this retrieval-induced forgetting (RIF) relies on prefrontal control processes, is competition-dependent (only occurs when memories compete) and is cue-independent (forgetting generalizes to a variety of cues). RIF is thought to be driven by inhibitory control signals from the prefrontal cortex that target areas where the memories are stored. Here we started disentangling the neurochemical signals in the prefrontal cortex that are essential to retrieval-induced forgetting. Specifically, this work aims to explore if and how the serotonergic system, participates in RIF and if the βarr2 signaling pathway is recruited when competition between memories takes place.

Methods: Ethics statement: All experimental procedures were conducted in accordance with institutional regulations (Institutional Animal Care and Use Committee of the School of Medicine, University of Buenos Aires, ASP #49527/15) and government regulations (SENASAARS617.2002). All efforts were made to minimize the number of animals used and their suffering.

Subjects: Male and female adult Wistar rats (weight range, 200–300 g) were used for the different experiments. When possible, we conducted within-subject or mixed experiments to reduce the number of total animals required. For each treatment, we reached a sample size of between 8-11 rats.

Behavioral task: We modified the spontaneous object recognition procedure to include three phases equivalent to the ones present in human studies of retrieval-induced forgetting: encoding, retrieval practice, and test. In addition to this retrieval practice condition, there were two control conditions in which the intervening retrieval practice phase was replaced either by returning the rat to its home cage (time control) or by giving the rat the same number of exploration trials on entirely new objects (the interference control).

Pharmacology: We used a pharmacological approach to manipulate the serotonin receptor 2 A (5-HT2aR) activity in the medial prefrontal cortex (mPFC) of cannulated rats, specifically during the phase when memories compete (retrieval practice). In different experiments, we infused an antagonist of the 5-HT2aR (MDL 11,939), specific inhibitors for members of the βarr2 signaling pathway, and an agonist of the 5-HT2aR (TCB-2).

Quantification of behavior and statistical analysis: exploratory behavior was measured offline. We calculated a discrimination index as a proxy of memory and perform one-way ANOVA followed by Bonferroni's post hoc comparisons in the experiments without

intracranial infusion and two-way repeated-measures ANOVA followed by Bonferroni's post hoc comparisons in the experiments with drugs or vehicle infusions.

Results: We found that retrieval-induced forgetting of competing memories in rats requires prefrontal serotonin signaling through 2 A receptors. Blockade of medial prefrontal cortex 2 A receptors as animals encountered a familiar object impaired active forgetting of competing object memories as measured on a later long-term memory test ($p < 0{,}0001$). Moreover, infusion of a PI3K inhibitor, which is part of the Barr2 pathway, impaired RIF but did not affect memory in other ways (forgetting did not occur in any of the control conditions, $p < 0{,}001$). Consistently, injection of an agonist of the 5-HT2aR promoted RIF in animals that would not normally forget ($p < 0{,}0001$).

Conclusions: We observed a bidirectional modulation of retrieval-induced forgetting by agonists and antagonists of 5-HT2aR in the medial prefrontal cortex. These findings establish the essential role of prefrontal serotonin in the active forgetting of competing memories, contributing to the shaping of retention in response to the behavioral goals of an organism. Future experiments will be designed to elucidate if this serotonin signaling targets the hippocampus to address RIF and if it is through the nucleus reuniens as the intermediate structure.

Keywords: Retrieval Induced Forgetting, 5-HT2A Receptors, Behavioral Tasks, Inhibitory Control, Medial Prefrontal Cortex

Disclosure: Nothing to disclose.

P780. Inhibitory Circuit Correlates of Suicidal Ideation in Veterans

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Background: Background: Problems with emotional regulation are associated with greater risk for suicidal ideation and behavior. Studies using functional magnetic resonance imaging (fMRI) and the stop signal task, an inhibitory control task, have linked lower activation in inhibitory brain circuits to poor emotional regulation. Lowered inhibitory circuit function may influence how ideation escalates and evolves over time, though few fMRI studies have examined this directly. Here, we examined the relationship between inhibitory function and ideation in a transdiagnostic sample of Veterans.

Methods: We collected fMRI blood oxygen level-dependent (BOLD) activation during the stop signal task from Veterans ($N = 16$) with suicidal ideation referred from in-patient psychiatry or outpatient clinics at the Providence VA Health System. Self-reported ideation and behavior was collected prior to MRIs using the Columbia-Suicide Severity Rating Scale (C-SSRS). We identified inhibitory regions by contrasting BOLD during trials when participants successfully inhibited motor responses (stop trials) with non-stop trials (Go trials). Results were dual threshold multiple comparisons corrected (voxel $p < 0.001$, cluster p-FDR < 0.05).

Results: Successful stopping evoked stronger fMRI activation in regions within circuits supporting inhibitory control (left supplementary motor area [SMA], left rostral anterior cingulate cortex [ACC], and right opercularis) and in right orbitalis. In all regions, recent ideation was negatively correlated with differences in stop and go trial activation (all r < -0.31).

Conclusions: Our results support inhibitory function as a potential transdiagnostic correlate of ideation in Veterans. Notably, two regions (ACC, orbitalis) have also been linked to negative valance processing by previous fMRI studies examining correlates of ideation. Future studies should consider evaluating how interactions between inhibitory and valence circuits influence suicidal ideation.

Keywords: Suicidal Ideation, Inhibitory Control, Veterans, fMRI

Disclosure: Nothing to disclose.

P781. The Mediating Role of the Addictions Neuroclinical Assessment Domain Factors in the Relationship Between Childhood Trauma and Alcohol Use Disorder With and Without Comorbid Mood Disorder

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Background: The Addictions Neuroclinical Assessment (ANA) is a neuroscience-based clinical framework to better understand the etiology and heterogeneity of alcohol use disorder (AUD). ANA consists of three neurofunctional domains which are thought to underlie AUD: Executive Function (EF), Negative Emotionality (NE), and Incentive Salience (IS). History of childhood trauma (CT) is associated with disruptions in EF development, mood and affect, and reward processing in adulthood, as well as higher risk of AUD and comorbid psychiatric conditions. However, the mechanisms by which CT confers risk to AUD and other comorbidities is unclear. The present study seeks to examine the mediating role of the ANA domains in the relationship between CT and AUD with and without a comorbid mood disorder (MD).

Methods: Participants ($N = 300$; 41.0% female; 74.0% with history of AUD; 40.7% with a history of MD) who were enrolled in the NIAAA Natural History Protocol completed the ANA battery, which consist of neurocognitive and self-report assessments of the

three ANA domains. EF measures were response inhibition, short-term memory, inferential reasoning, task switching, mental rotation, attention, metacognition, and interoception. NE measures were distress tolerance, ostracism, amotivation, anhedonia, resilience, affect and alexithymia. IS measures were approach-avoidance bias, implicit alcohol associations, and alcohol demand. Ancillary measures assessed in the Natural History Protocol pertinent to the three domains were included in the analyses (alcohol sensitivity, anxiety, depression, aggression, impulsivity, craving, personality, delay discounting). CT was measured using the Childhood Trauma Questionnaire (CTQ). Psychiatric diagnoses were assessed using the Structured Clinical Interview for DSM-5 Disorders. Participants were classified based on their history of AUD and mood disorder: Healthy controls (HC; $n = 68$, 23.9%), AUD only ($n = 108$, 36.0%), and AUD with comorbid MD (AUD + MD; $n = 113$, 37.7%). Those with MD only (i.e., without comorbid AUD) were excluded due to the small group size ($n = 8$, 2.7%). Factor analyses were used to identify the latent factors underlying each domain. Analysis of variance were used to compare CTQ scores and factor scores across the three diagnostic groupings. Structural equation models (SEM) were used to evaluate the relationships between CT, domain factors, and psychiatric diagnoses. Age, sex and race were included as covariates. Bonferroni correction was used to correct for multiple testing.

Results: Five factors of EF were identified: Response Inhibition, Working Memory, Interoception, Rumination, and Impulsivity (TLI/CFI > 0.91, RMSEA = 0.05). Two factors of IS were found: Alcohol Sensitivity and Alcohol Motivation (TLI/CFI > 0.99, RMSEA = 0.03). Three factors of NE were elucidated: Internalizing, Externalizing, and Resilience (TLI/CFI > 0.94, RMSEA = 0.07). CTQ scores were associated with all factors (standardized estimates = 0.11 to 0.57, p's < 0.02), except for alcohol sensitivity (p = 0.10) and response inhibition (p = 0.052). Compared to HC, individuals with a history of AUD (with and without comorbid MD) showed elevated dysfunction in all domain factors (Cohen's d = 0.09 to 3.34; p's < 0.01) except for resilience and response inhibition (p's > 0.17), and endorsed greater levels of CT (Cohen's d = 0.61, $p < 0.001$). Those with AUD + MD also showed greater levels of dysfunction in all domain factors (Cohen's d = 0.17 to 3.53; p's < 0.01) except for response inhibition and working memory (p's > 0.82), and exhibited greater CT compared to those with AUD only (Cohen's d = 0.46; $p < 0.01$). After controlling for all identified factors and covariates, only alcohol motivation mediated the relationship between CT and membership to the AUD only group (indirect effect = 0.31, $p < 0.001$), but not the AUD + MD group (indirect effect = 0.10, p > 0.20).

Conclusions: We identified unique factors underlying each ANA domain. Individuals with AUD with and without comorbid MD showed elevated levels of dysfunction on most of these domain factors relative to HC, and individuals with AUD + MD showed additional levels of dysfunction compared to AUD only. CT was associated with most of these identified factors, but only alcohol motivation was a significant mediator between CT and risk of developing AUD without MD. This suggests that addressing heightened alcohol motivation in individuals with a history of CT may be especially pertinent in improving AUD treatment outcomes. Future analysis will focus on subtypes of CT (e.g., abuse, neglect) as well as additional psychiatric comorbidities commonly associated with AUD.

Keywords: Childhood Trauma, Alcohol Use Disorder, Mood Disorder, Addictions Neuroclinical Assessment, RDoC

Disclosure: Nothing to disclose.

P782. Transdiagnostic Neural Correlates of Fear and Anxiety Sensitivity in a Focal Fear Sample

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Background: Fear and anxiety sensitivity (the belief that anxiety symptoms or arousal can be harmful/"fear of anxiety-related sensations"), are transdiagnostic constructs that may help parse heterogeneous diagnostic categories into more homogeneous, neurobiologically-based constituents, laying the groundwork for more targeted classification of the anxiety disorders. Both constructs have both been linked to aberrant processing of negative stimuli; as distinct contributors to psychopathology, fear and anxiety sensitivity were expected to show specific associations with neurocircuit response.

Methods: Fifty-two adults (37 female; M = 23.65 years, SD = 9.69) who all shared a common "focal fear" diagnosis (i.e., specific phobia or performance-only social anxiety disorder), but varied in extent of additional comorbid anxiety and mood disorders, passively viewed negative and neutral pictures while fMRI BOLD was recorded. Analyses focused on identifying the unique neural correlates of fear and anxiety sensitivity, above and beyond the other dimension.

Results: Greater fear was associated with reduced negative > neutral fMRI BOLD in the thalamus (t = 3.85, $p < 0.05$ FWE), suggesting avoidant processing of negative stimuli. On the other hand, higher levels of anxiety sensitivity were associated with increased negative > neutral fMRI BOLD in the insula (t = 3.93, $p < 0.05$ FWE), a brain region implicated in the representation of interoceptive information.

Conclusions: Fear and anxiety sensitivity show unique associations with threat neurocircuitry, suggesting distinct means of responding to negative stimuli in the environment. These transdiagnostic constructs could serve as candidates for a more fine-grained means of understanding pathological anxiety.

Keywords: Transdiagnostic, Negative Emotionality, fMRI, Adult Clinical Anxiety

Disclosure: Nothing to disclose.

P783. Race, Ethnicity, Education, Sex and Gender Effects on Neuropsychological Test Scores: Limitations of Current Evidence and Impact on Clinical Trials and Clinical Practice

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Background: Interpretation of neuropsychological (NP) tests depends on the quality of the normative standards available for the tests. The precision of measurement for each test variable depends on the psychometric properties of the test and how many people were used to standardize that test. Co-norming across tests is necessary when interpreting differences between scores on different tests at one time point (i.e., profile or discrepancy score analysis), or differences between scores on the same test repeated over time. The relevance of specific norms for an individual examinee further depends on multiple design features of the standardization studies, including: when the studies were conducted, sampling strategy, inclusion/exclusion criteria, age, sex/gender, education, race and ethnicity, socioeconomic status, and region. This paper examines the standardization studies of the most widely used NP tests, identifies their strengths and weaknesses, and makes recommendations for interpretive caveats based on these analyses.

Methods: We reviewed the standardization strategies and coded information about the sampling frames, inclusion/exclusion criteria, stratification methods, sample sizes overall and within each stratum where relevant, methods for representing or

analyzing race, ethnicity and other demographic characteristics. These methods were applied to the WAIS-IV, WMS-IV, CVLT3, D-KEFS, Pearson Advanced Clinical Solutions (ACS), Rey Complex Figure Test, WCST, Symbol Digit Modalities Test, RBANS, BVMT-R, HVLT, Halstead-Reitan ("Heaton et al.") Norms for Boston Naming, Finger Tapping, Grooved Pegboard), MOANS, and MOAANS (Boston Naming, Trail Making Test, Judgement of Line Orientation). We calculated multiple indexes for each test, including standard errors and confidence intervals for scaled scores, and standard errors of measurement for repeated measures based on reported test reliabilities.

Results: Most tests used age only as a stratification factor, providing "age corrected" scores for selected age bands. The sample sizes for the age strata range from 1 to ~200 but are usually less than 100 participants/stratum. Sex differences were rarely reported, and while larger studies estimated sex distributions from census statistics, some studies had markedly uneven distributions of sex. Education was not used as a stratification factor in any study, and only the ACS, Heaton and MOANS/MOAANS norms attempt corrections for education. The possible interactions of age and education on test scores are seldom reported and cell sizes for combinations of age and education may be too small to enable robust estimates of scores especially at lower levels of education and older ages. The possible impact of race and ethnicity are rarely interrogated except in ACS, Heaton and MOAANS norms, which all focus on "African American" participants. Discrepancies in scores across ACS, Heaton and MOAANS suggest marked sampling differences, and show the same raw scores may yield clinically meaningful differences in scaled scores depending on which norms are used. Most of the norms studied are at least 15 years old, and poorly represent current racial and ethnic characteristics of the United States.

Conclusions: Existing norms have major limitations and may impact the clinical assessment of individuals and result in inappropriate treatment recommendations as well as inappropriate classification in clinical trials, which may include score "cutoffs" based on widely used normative standards. Particularly, race and ethnicity are poorly represented and existing norms present major conflicts for African American groups, with the same raw scores differing by a full standard deviation depending only on the source of normative data. Furthermore, these norms often fail to reflect demographic shifts in the United States, making underrepresentation of racial and ethnic minority groups more marked than before and leading to questions about whether results from these measures can be generalized. Additionally, sex differences are examined infrequently and it remains unclear to what extent sex or gender differences may affect some scores. Co-norming was done for only selected measures and those sample sizes are smaller so the precision of measurement of difference scores is often low; this is unfortunate because interpretation of clinical results and findings from clinical trials often involves examining differences between tests. Most norms use only age as a stratification factor, despite robust impacts of education on scores. Lack of standardization by educational background and selection of "representative" samples means that those of higher education will be given inappropriately higher standard scores and those of lower educational opportunity will be given inappropriately lower standard scores relative to their true abilities. There is an urgent need for new, preferably "dynamic" normative standards, that include sampling by socially and demographically meaningful metrics, to provide greater precision in assessment of neuropsychological scores and score discrepancies, and for evaluating the inclusion/exclusion criteria, and criteria for efficacy in clinical trials that use neurocognitive endpoints

Keywords: Neuropsychology, Neurocognitive Assessment, Psychometric Properties, CNS Clinical Trials

Disclosure: Atai Life Sciences: Honoraria (Self), Verasci: Honoraria (Self).

P784. Tonic and Phasic Neurophysiological Relationships With Adverse Posttraumatic Outcomes Vary by Racial/Ethnic Group

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Background: Racial and ethnic groups experience differing levels of socioeconomic stress that can affect responses to traumatic stress. Emergent work demonstrates that both structural inequities and individual racism-related stressors underlie race-related differences in neural and physiological responses. In particular, core threat neurocircuitry such as the amygdala may be a key region impact by structural racism. Greater levels of negative life events throughout development appear to contribute to both lowered threat-elicited amygdala responses and subsequent skin conductance responses (SCRs) in racially minoritized individuals. Disparate levels of stressor exposure between groups may also partially explain race-related differences in posttraumatic symptoms after trauma exposure. However, limited research to date has investigated brain and physiological responses that may predict posttraumatic outcomes and whether these may differ by racial/ethnic group as a result of structural racial inequities. Understanding potential heterogeneity in brain-behavior relationships is crucial for the development of generalizable neural treatments and interventions for trauma and stress-related disorders.

Methods: Participants ($n = 283$) were recruited as part of the AURORA Study, a multisite, longitudinal study of trauma and posttraumatic outcomes. Briefly, trauma-exposed participants were recruited from 22 Emergency Departments (EDs) from across the United States. Initial participant demographic and psychometric data were collected after admission to the ED which included trauma exposure type, participant marital status, income, education level, and employment. Participants' home address was geocoded to derive an area deprivation index (ADI) to reflect neighborhood disadvantage. PTSD, depression, and anxiety symptoms were assessed with the PTSD Symptom Checklist for DSM-5 (PCL-5) and the PROMIS. Participants completed psychophysiological recording and functional magnetic resonance imaging (fMRI) approximately 2-weeks after trauma exposure. Skin conductance level (SCL) and SCR were assessed during a Pavlovian conditioning task. Amygdala reactivity to threat was assessed during passive viewing of fearful and neutral faces during fMRI and was indexed as the BOLD response for the fearful > neutral contrast. Amygdala functional connectivity was assessed using resting-state fMRI using a seed to voxel approach. Multiple comparisons at the voxel level were corrected using a clustering approach (alpha = 0.05) and Benjamini-Hochberg False Discovery Rate adjustments were made for other statistical tests.

Results: Demographic data by racial/ethnic group are reported in Table 1. We observed significant differences in education level [$X2 = 9.90$, $p = 0.007$], income [$X2 = 7.47$. $p = 0.023$], and marital status [$X2 = 6.46$, $p = 0.040$]. No significant difference was observed in employment within the sample [$X2 = 0.59$, $p = 0.745$]. A significant difference in ADI was observed between the groups [$F(2,280) = 31.73$, $p < 0.001$]. There were significant racial/ethnic differences in tonic SCLs [$F(2,126) = 6.41$, $p = 0.002$] with Black participants showing significantly lower tonic SCL than White participants [$t(61.40) = 3.25$, $p = 0.002$, variance inequality adjusted]. Similarly, there were significant racial/ethnic differences in amygdala connectivity to dorsal anterior cingulate cortex, dorsolateral prefrontal cortex (PFC), insula, and cerebellum. However, there was no main effect of racial/ethnic group

($p = 0.932$) or racial/ethnic group by stimulus-type interaction ($p = 0.832$) on SCRs during threat acquisition. Similarly, there were no racial/ethnic differences in amygdala reactivity to threat ($p > 0.05$). Finally, multivariate models showed that racial/ethnic groups differed in the relationship between amygdala to dorsolateral PFC/dACC/insula connectivity and PTSD symptoms 3-months after trauma such that Hispanic individuals showed a negative relationship, white individuals showed an orthogonal relationship, and Black individuals showed a positive relationship between connectivity and symptom severity.

Conclusions: The present findings suggest tonic levels of neurophysiological arousal differ between racial and ethnic groups in the early aftermath of trauma. However, phasic responses to threat remain highly similar. Importantly, race-related tonic differences also appear to influence prediction of subsequent PTSD symptoms. Together, these findings raise two important issues. First, they highlight how differences in exposure to life stressors may augment neural and behavioral reactions to subsequent traumatic stress as well as recovery from exposure. Second, these findings may suggest that neuromodulatory treatments thought to act on amygdala-PFC connectivity may have opposing effects depending on the life history of the patient. Further research is needed to better understand the neural mechanisms underlying the impact of structural racism on neurophysiological tone, and how to leverage such information for effective early interventions after trauma exposure.

Keywords: Race Disparities, PTSD, Amygdala, Psychophysiology, Functional MRI (fMRI)

Disclosure: Wiley Publishing: Honoraria (Self).

P785. Mismatch Negativity Predicts Initial Auditory-Based Targeted Cognitive Training Performance in a Heterogeneous Veteran Population Across Psychiatric Disorders

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Background: Auditory based targeted cognitive training (TCT) is a bottom-up, neuroplasticity-based computerized approach to cognitive remediation in which patients train on progressively more difficult auditory processing exercises designed to improve pitch and temporal acuity of low-level sensory information. This adaptive approach seeks to improve the speed, accuracy and fidelity of auditory information processing to generate upstream gains in cognition and function. Thus far, application of TCT has demonstrated utility in improving cognitive functioning and quality of life in those with schizophrenia spectrum disorders (SSD). Furthermore the event-related potentials mismatch negativity (MMN) and P3a, biomarkers of early auditory information processing known to be abnormal in SSD, predict TCT-associated cognitive gains in SSD populations. However, TCT may be broadly helpful for cognitive deficits associated with other psychiatric disorders as well, as presumably the neural circuits underlying TCT-associated gains in SSD populations are also present in others. In this pilot study we aimed to understand 1) how initial TCT performance is related to cognitive functioning in a heterogeneous population of patients with a range of psychiatric disorders, and 2) the utility of MMN and P3a in predicting initial performance on a TCT exercise which is representative of a typical ~30 h TCT training program.

Methods: Age-matched Veteran participants (SSD $n = 13$, nonSSD $n = 13$, average age=51.9) with a range of psychiatric diagnoses engaged in mental health rehabilitation were recruited, and performance on a 1 hour TCT session was assessed. The SSD cohort was primarily composed of those with schizophrenia and schizoaffective disorder (85%), while the nonSSD group was primarily diagnosed with MDD (77%) and PTSD (69%). The TCT exercise consisted of Sound Sweeps, an auditory frequency discrimination task, in which participants completed "levels," which consisted sets of 35 stimuli presentations. Stimuli presentation length were algorithmically modified based on correct or incorrect responses, in an adaptive manner. Initial TCT performance was operationalized as the score achieved over the first level of training, reported in milliseconds of stimuli presentation. Cognitive functioning was assessed by the MATRICS Consensus Cognitive Battery (MCCB). MMN and P3a were collected prior to completing 1 h of TCT.

Results: On average, participants completed 13.1 levels (st.dev=5.3), with nonSSD participants completing more levels than SSD (nonSSD, average=15.5 levels, st.dev=5.3; SSD, average=10.7 levels, st.dev=4.4, $p = 0.02$). SSD participants scored lower on all MCCB domains compared to nonSSD participants, with significant differences in visual learning, problem solving and neurocognitive composite (all ps < 0.01). MMN amplitude between SSD and nonSSD subjects did not significantly differ in this cohort; P3a amplitude ($p = 0.04$) was significantly more impaired in SSD compared to nonSSD. Initial TCT performance was poorer in SSD compared to nonSSD participants, though did not achieve significance (nonSSD = 144.9 msec, st.dev=260.8; SSD = 276.2 msec, st.dev=349.0, $p = 0.28$). MCCB domain scores in working memory, verbal learning, visual learning, problem solving and neurocognitive composite were related to number of levels completed ($r = 0.44$, 0.60, 0.66, 0.55, 0.55 respectively; all ps<0.01). MCCB domain scores in attention, verbal learning, visual learning, problem solving and neurocognitive composite were related to initial TCT performance ($r = -0.41$, -0.45, -0.44, -0.39, -0.42 respectively; 0.023 < all ps <0.047). MMN was related to initial TCT performance ($r = 0.52$, $p < 0.01$), driven by SSD participants ($r = 0.70$, $p < 0.01$). Multivariable regression modeling revealed MMN and MCCB neurocognitive score were significant predictors of initial TCT performance (MMN, $F = 8.2$, $p = 0.009$; MCCB neurocognitive score, $F = 6.0$, $p = 0.023$) while P3a and diagnosis (SSD vs nonSSD) were not (Fs < 0.71, ps > 0.79).

Conclusions: Neurophysiological biomarkers may have utility in predicting TCT performance not only in those with SSD but also other psychiatric disorders. These results inform future studies that aim to ameliorate cognitive impairment via TCT in a transdiagnostic manner. Further study of biomarker-guided TCT approaches across psychiatric populations is warranted.

Keywords: Auditory Mismatch Negativity, Targeted Cognitive Training, Schizophrenia, PTSD, MDD

Disclosure: Nothing to disclose.

P786. Effects of Incentives on Spatial Working Memory in Patients With Schizophrenia and Patients With OCD

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Background: Motivational influences on cognition are critical for shaping goal-directed behaviors. Cognitive and motivational deficits co-occur across numerous psychiatric illnesses, presenting an opportunity to examine shared, as well as distinct, mechanisms

across disorders. To begin to understand cross-diagnostic effects, we analyzed motivated spatial working memory (sWM) performance in adult patients with schizophrenia (SCZ), adult patients with obsessive-compulsive disorder (OCD) and typical adults (TYP) without psychiatric illness.

Methods: An age-matched cohort of 27 patients with SCZ (m21, f6), 39 patients with OCD (m18, f21) and 34 typical adults (m21, f13) performed a previously published incentivized sWM task. This task was translated from similar tasks used with non-human primates that require spatial locations to be kept in mind. A joystick was used to indicate where participants remembered prior spatial locations. The possibility for monetary reward or loss was presented using cues (cued condition) or as a non-cued, contextual instruction given before a block of sWM trials (non-cued condition). A control condition that did not require working memory, but only motor movements with the joystick, was also included. sWM accuracy was calculated as the angular difference between presented and remembered spatial locations.

Results: The TYP group performed significantly better than patients with SCZ and patients with OCD at manipulating the joystick without memory requirements (control condition) (main effect, $p < 0.001$). All subsequent analyses were covaried for performance in the control condition. Patients with OCD and TYP had significantly better baseline, neutral sWM performance, than patients with SCZ (ANCOVA, $p < 0.001$)). Reflecting the overall effects of incentives, there was a significant incentive (neutral sWM, reward, loss) x group (SCZ, OCD, TYP) interaction ($p < 0.05$). All groups improved sWM performance in response to reward and loss incentives ($p < 0.05$ for all pairwise comparisons). The interaction between incentive presentation (neutral sWM, cued, non-cued) and group trended towards significance ($p = 0.065$). Pairwise comparisons demonstrated that TYP differentiated between cued and non-cued presentation with significantly better sWM performance during cued presentations, compared to non-cued presentations ($p < 0.005$), while SCZ sWM performance did not differentiate between the two presentations ($p > 0.05$), and patients with OCD showed a trend towards differentiating between the two presentations ($p = 0.06$). When the effects of rewards and losses during cued presentation only were examined, all groups improved sWM performance when incentives were cued (main effect of cued incentive (neutral sWM, cued reward, cued loss), $p < 0.01$)) (non-significant interaction (cued incentive x group), $p > 0.05$). There was a significant interaction of non-cued incentive presentation (neutral sWM, non-cued reward, non-cued loss) and group ($F(4,194) = 3.4$, $p < 0.05$). Patients with SCZ had better sWM performance during non-cued loss ($p < 0.005$), but not during non-cued reward ($p > 0.05$). Patient with OCD and TYP had better sWM performance during both non-cued loss and non-cued reward (all $p < 0.05$).

Conclusions: In general, incentives improved sWM performance in all groups. Differential effects of incentives on sWM performance in patients with SCZ, patients with OCD and TYP appeared related to the type of incentive presentation, and incentive valence. Future work may examine cognitive and motivational neural systems to better understand the systems in these groups that support sWM improvements in response to motivation.

Keywords: Cognition, Reward, Schizophrenia (SCZ), OCD, Working Memory

Disclosure: Nothing to disclose.

P787. Willingness to Exert Social Effort is Selectively Related to Avoidance Motivation

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Background: Asociality, impairment in social motivation, is a disabling symptom of multiple neuropsychiatric disorders including schizophrenia, major depression, and autism. However, social motivation and willingness to exert social effort remain relatively understudied compared to motivation for monetary rewards. Choices about whether to exert social effort involve the desire for positive social interactions (social approach), but are also strongly influenced by a desire to avoid negative social experiences (social avoidance). In this study we investigated the relationships between social motivation, approach and avoidance tendencies, and clinical symptom domains associated with anxiety and depression. To quantify behavioral willingness to exert social effort, we developed a novel social effort discounting task (SEDT) and a parallel monetary effort discounting task (MEDT). We hypothesized that SEDT and MEDT would demonstrate similar relationships with general motivational tendencies, but that the social task would selectively relate to individual differences in self-reported avoidance tendencies and to social aspects of anxiety and depression.

Methods: A sample of 500 participants (50% female, age range 18-30 mean 25) were studied via an online crowdsourcing platform (Prolific), yielding analyzable data from 413 individuals. Participants performed both the SEDT and MEDT, with two blocks of each task in counterbalanced order, and involving 200 total hypothetical choice trials. In both SEDT and MEDT, participants chose between a fixed low-effort/low-reward (EASY) and a variable higher-effort/higher-reward option (HARD) on each trial. In the SEDT, the reward unit was a "match" with a potential friend and the effort unit was a 25+ word invitation message; in the MEDT, the reward was a $1 gift card and the effort was a 25+ word request message. Task motivation was quantified using a linear discounting function $SV = A\text{-}B*E$, where subjective value (SV) equals the amount of reward A discounted by the effort E. The free parameter B is estimated from the participant's choices; the higher the B, the greater the negative impact of effort on SV and therefore the lower the motivation. Participants also completed a battery of self-report questionnaires including assessment of general motivation orientations for approach (Behavioral Activation Scale, BAS) and avoidance (Behavioral Inhibition Scale, BIS), as well as state motivation on the Motivation and Pleasure Scale (MAP), Chapman social and physical anhedonia scales, and general and social anxiety measures; their relationship to log-transformed SEDT and MEDT B values was examined.

Results: SEDT and MEDT logB were strongly correlated ($rho = 0.68$), indicating roughly half the variance was shared reflecting general motivation. Higher logB (lower behavioral motivation) in both the SEDT and MEDT was inversely related to self-reported MAP global motivation (SEDT $rho = -0.13$, $p = 0.006$; MEDT $rho = -.10$, $p = 0.04$). Both task measures also correlated with trait approach motivation (BAS Drive, SEDT $rho = -0.15$, $p = 0.002$, MEDT $rho = -0.14$, $p = 0.006$). We also observed hypothesized task specificity. Trait avoidance motivation (BIS) correlated selectively with social task motivation (SEDT $rho = -0.11$, $p = 0.03$; MEDT $rho=0.02$, $p = 0.65$), and the relative strength of social vs. nonsocial task motivation (SEDT logB minus MEDT logB) correlated with avoidance motivation ($rho = 0.10$, $p = 0.04$). This relative strength subtraction measure also correlated significantly with social anxiety ($rho = 0.10$, $p = 0.04$) and trait social motivation ($rho = -0.12$, $p = 0.01$). Neither task related to depression severity.

Conclusions: Consistent with our hypotheses, social and nonsocial task motivation were robustly related to each other and showed similar relationships to self-reported global state motivation and trait approach motivation. We also observed hypothesized differential relationships to avoidance motivation,

trait social motivation and state social anxiety. Overall, our results indicate that the social EDT captures social-specific motivation above and beyond general motivation. These tasks can provide objective parallel behavioral measures of social and nonsocial motivation, facilitating investigation of the common and dissociable neurobehavioral underpinnings of social and nonsocial motivation and their disruption in neuropsychiatric disorders.

Keywords: Social Reward, Motivation, Approach/Avoidance

Disclosure: Nothing to disclose.

P788. Neighborhood Violent Crime and Neural Correlates of Emotion Regulation in Patients With Major Depressive Disorder and Social Anxiety Disorder

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Background: The capacity to regulate emotions in the face of aversive information plays a critical role in mental health. Reappraisal is a well-studied cognitive-linguistic regulation strategy known to engage regions that play a central role in top-down control and semantic processes. Accumulating data indicate the brain pathophysiology of debilitating internalizing conditions such as Major Depressive Disorder (MDD) and Social Anxiety Disorder (SAD) involve impairment in reappraisal mechanisms to varying degrees, highlighting individual differences in regulation capacity. Importantly, most of this work in adults has focused on individual-level factors (e.g., trauma history, symptom severity, problematic sleep). However, it is also important to understand how broader contextual factors (e.g., neighborhood characteristics) impact neurobiological correlates of emotion regulation. Specifically, there is increasing evidence that exposure to community violence or living in a neighborhood characterized by high levels of violent crime is associated with emotion regulation deficits in childhood and adolescence. However, the impact of community-level factors on the neural correlates of regulation in adults with psychopathology has been understudied. Therefore, the current study sought to address this gap in the literature by examining the relation between neighborhood violent crime and neurobiological indices of emotion regulation in patients with MDD and SAD from a large urban area.

Methods: Participants were 63 un-medicated, treatment-seeking patients between the ages of 18 and 55 (Mean Age = 27.62; 63.5% female). Patients were administered the Liebowitz Social Anxiety Scale (LSAS) and Hamilton Depression Rating Scale (HAMD) to assess social anxiety and depression symptoms, respectively. Before treatment, participants completed a validated Emotion Regulation task in the scanner. The task comprised standardized images of general negative content, and regulation was evaluated by comparing the condition in which participants were instructed to decrease their emotional response to negative images via reappraisal (ReappNeg) relative to looking at negative images (LookNeg). Crime data were downloaded from the publicly available Chicago Data Portal and aggregated within census blocks to create indices of neighborhood violent and non-violent crime rates. Patients' neighborhoods were defined by the census block of their home address at study enrollment. Finally, additional measures included the Area Deprivation Index, a geocoded index of neighborhood disadvantage, and the PCL-5, a measure of posttraumatic stress symptoms. Whole-brain regression analyses were conducted to evaluate the neural correlates of ReappNeg>LookNeg in which neighborhood violent crime was the covariate of interest and age and internalizing symptom severity (LSAS and HAMD composite score) were covariates of no interest. 3dClustSim was used to control for multiple comparisons, and clusters exceeding 558 voxels were considered significant ($\alpha < 0.05$, $p < 0.005$).

Results: Whole-brain regression analyses revealed an association between neighborhood violent crime and a cluster comprised largely of left inferior frontal gyrus (IFG; $k = 627$, $z = 4.00$, $p < 0.001$), such that greater violent crime associated with greater IFG engagement during reappraisal of negative stimuli. A similar cluster emerged for right IFG, though it did not survive correction for multiple comparison ($k = 538$, $z = 3.91$, $p < 0.001$). Follow-up tests of robustness indicated the main effect of neighborhood violent crime on left IFG activation was maintained when statistically controlling for patient neighborhood non-violent crime, neighborhood socioeconomic disadvantage, education, racial identity, and posttraumatic stress symptoms (all ps < 0.002).

Conclusions: Current findings indicate that patients with MDD or SAD who live in neighborhoods with higher rates of violent crime exhibit more prefrontal engagement during emotion regulation. This association was, at least in part, independent of current symptomatology, neighborhood disadvantage or non-violent crime, trauma-related symptoms, and demographic characteristics, highlighting the unique contribution of environmental threat. Future research is needed to determine whether associations between neighborhood violent crime and greater IFG engagement during reappraisal also generalize to other internalizing conditions.

Keywords: Functional MRI (fMRI), Emotion Regulation, Neighborhood Crime, Major Depressive Disorder, Social Anxiety Disorder

Disclosure: Nothing to disclose.

P789. Effects of Serotonin in Rats and Humans on Computations Underlying Flexible Decision-Making

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Background: Serotonin is critical for adapting behaviour flexibly to meet changing environmental demands; however, a unifying mechanistic framework accounting for its role in behavioural flexibility has remained elusive. Computational modelling may offer refined measures of behaviour which could additionally enhance comparisons across species and aid translational research.

Methods: Probabilistic reversal learning, a well-established laboratory paradigm for studying behavioural flexibility, was tested in rats and humans following modulation of serotonin (5-hydroxytryptamine; 5-HT). Male rats were administered acutely either 1 mg/kg ($n = 12$) or 10 mg/kg ($n = 18$) of the selective serotonin reuptake inhibitor (SSRI) citalopram, or the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) which produces profound depletion of forebrain serotonin.

In addition, rats ($n = 7$) were administered repeated 5 mg/kg citalopram for seven consecutive days compared with a vehicle group ($n = 7$). After seven days, the citalopram group received 10 mg/kg of citalopram twice per day for five consecutive days. Furthermore, healthy human male and female volunteers were administered an SSRI acutely (20 mg escitalopram; $n = 65$) in a double-blind randomised placebo-control design. Data were reanalysed using Bayesian hierarchical reinforcement learning models. Model parameters of interest were learning rates, for rewarded or punished outcomes, and "stickiness" which indexes a

basic perseverative tendency, or choice repetition regardless of reinforcing feedback. In rats, stickiness was tied to a location (side or location of the cage) whereas in humans, stickiness was tied to a particular visual stimulus on a computer screen (stimulus stickiness).

Results: Model comparison demonstrated the best fitting models included parameters for "stickiness," learning rates (based on rewards and punishments separately), and reinforcement sensitivity which indexed the extent to which behaviour is driven by acquired value. We found that 5-HT depletion via 5,7-DHT significantly decreased side (location) stickiness (95% HDI: [-0.4635, -0.1134]) and the reward learning rate (85% HDI: [-0.0757, -0.0033]). There was no significant effect of 5,7-DHT on the reward learning rate, punishment learning rate, or reinforcement sensitivity [no group differences, all 0 $\in$ 75% HDI]. A single dose of 1 mg/kg citalopram in rats also diminished the side (location) stickiness parameter (95% HDI: [-0.3330, -0.0441]). The reward learning rate was enhanced by the 1 mg/kg dose in rats (95% HDI: [0.0184, 0.3959]). There was no significant effect of 1 mg/kg on the punishment learning rate or reinforcement sensitivity (both 0 $\in$ 75% HDI). A single high dose of citalopram in rats (10 mg/kg) decreased the reward learning rate (85% HDI: [-0.2888, -0.0009]) and enhanced reinforcement sensitivity (85% HDI: [0.0346, 0.5590]). There was no significant effect of 10 mg/kg on the punishment learning rate or side (location) stickiness. Seven-day administration of 5 mg/kg citalopram enhanced both the punishment learning rate (95% HDI: [0.0432, 0.6404]) and side (location) stickiness (75% HDI: [0.0135, 0.3054]). There was no significant effect of seven-day administration of 5 mg/kg citalopram on the other parameters (0 $\in$ 75% HDI). Augmenting the preceding regimen with 10 mg/kg of citalopram twice per day, for five consecutive days, enhanced both the reward (95% HDI: [0.2699, 0.6780]) and the punishment (95% HDI: [0.2172, 0.7323]) learning rates, increased side (location) stickiness (90% HDI: [0.0075, 0.3414]), and decreased the reinforcement sensitivity (95% HDI: [-1.7233, -0.2540]). Administration of a single 20 mg dose of escitalopram to healthy humans decreased the reward learning rate (95% HDI: [-0.3612, -0.0392]), stimulus stickiness (85% HDI: [-0.3476, -0.0045]) and reinforcement sensitivity (80% HDI: [-3.1501, -0.1553]), and had no significant effect on the punishment learning rate (0 $\in$ 75% HDI).

Conclusions: Computational modelling of choices during probabilistic reversal learning revealed common effects of manipulating serotonin function across two species (rats and humans). These computational models revealed that the tendency to repeat behaviours irrespective of their outcome (stickiness) was decreased or increased by manipulations known to reduce (serotonin depletion in rats and acute low dose SSRI in both rats and humans) or boost (high repeated dose SSRI in rats) central serotonin function, respectively. Meanwhile, reward learning rates were also modulated bidirectionally across species and SSRI dose, while the punishment learning rates were altered (increased) only in instances of repeated SSRI dosing in rats. Collectively, these results support a role of serotonin in behavioural flexibility and plasticity across species that can be studied using computational measures that dissect choice, and has transdiagnostic relevance for neuropsychiatric disorders in which related subprocesses are perturbed.

Keywords: Serotonin, Probabilistic Reversal Learning, Computational Reinforcement Learning Model

Disclosure: Nothing to disclose.

P790. Distinct Roles for Dopaminergic and Noradrenergic Signaling in Exploration During Decision Making

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Background: The distinct roles of dopamine versus norepinephrine in catecholamine modulation of executive functions, especially decision making, have been surprisingly challenging to tease apart. In particular, whether there are distinct or shared roles for each neuromodulator in the explore-exploit tradeoff remains unclear. As differences in these decision making parameters are identified in numerous neuropsychiatric conditions, identifying neural mechanisms by which these parameters are altered will be important in guiding mechanistic hypotheses emerging from computational psychiatric approaches.

Methods: Using the strengths of rodent models for repeated pharmacological testing, combined with our novel restless two-armed bandit task design, we tested the role of dopaminergic and noradrenergic transmission in decision making. We ran 32 mice (B6129SF1/J, 16 m/16 f) in a restless two-armed bandit task, which encourages both exploration and exploitation. We systemically administered a NE beta-receptor antagonist (propranolol), NE beta-receptor agonist (isoproterenol), a nonselective DA receptor antagonist (flupenthixol), and a nonselective DA receptor agonist (apomorphine) within subjects across sessions and examined changes in exploration as defined by a Hidden Markov Model approach, as well as analysis of overall performance, win-stay/lose-shift, and reinforcement learning model fits.

Results: We found a bidirectional modulatory effect of dopamine receptor activities on the level of exploration. The dopamine receptor agonist apomorphine decreased exploration, while the dopamine receptor antagonist flupenthixol increased exploration. Beta-noradrenergic receptor activities also modulated exploration, but the effect is mediated by sex, possibly reflecting sex-dependent ceiling/floor effects of noradrenergic signaling.

Conclusions: Overall, dopaminergic neuromodulation appears to exert more influence on exploration in decision making than does noradrenergic modulation, but sex differences played a stronger role in what effects are seen due to noradrenergic influences. This suggests that dopamine is a central modulator of the explore-exploit tradeoff, but sex differences in these effects may be due in part to underexplored noradrenergic influences.

Keywords: Decision Making, Catecholamine, Explore-Exploit Dilemma, Sex Differences

Disclosure: Nothing to disclose.

P791. Instructed Motivational States Bias Reinforcement Learning and Memory Formation

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Background: Motivation influences goals, decisions, and memory formation. Imperative motivation links urgent goals to actions, whereas interrogative motivation integrates goals over time and space, thus supporting broader learning (Murty and Adcock, 2017; Dickerson and Adcock, 2018; Chiew and Adcock, 2019.) Whereas interrogative motivational states engage ventral tegmental area-hippocampus circuitry to support learning details and associations for flexible future behavior, imperative motivational states engage amygdala-cortical-medial temporal lobe circuitry to form sparse, decontextualized memories restricted to goal-relevant information. We posit that interrogative and imperative motivational states would have diverging consequences for reinforcement learning versus episodic, relational memory formation due to these underlying neural mechanisms.

Methods: For two randomly assigned groups, we induced motivational states by manipulating only the cover stories for a reinforcement learning task: The Imperative group imagined

executing a museum heist, whereas the Interrogative group imagined planning a future heist. Participants repeatedly chose from four "doors" to sample trial-unique paintings with variable rewards. The next day, participants returned to complete a surprise memory test. Participants performed old/new recognition of paintings and recalled painting-value and painting-door associations. We collected an initial sample ($N = 100$, 50% male, 50% female) and a pre-registered replication sample ($N = 100$, 50% male, 50% female). We analyzed reinforcement learning behavior using hierarchical Bayesian modeling, then classified trial-by-trial choices as either exploitation (choosing the door with the highest expected value), directed exploration (choosing the door with the highest estimated uncertainty), or random exploration (choosing one of the other two doors). Memory performance was assessed with linear models and mixed-effects logistic regression models.

Results: Participants in the Imperative group made more exploitative choices (Sample 1: $W = 1573.5$, $p = 0.026$, Cohen's $d = 0.46$, 95% CI [0.06, 0.85]; Sample 2: 1596, $p = 0.017$, Cohen's $d = 0.49$, 95% CI [0.09, 0.89]) and earned more points during reinforcement learning (Sample 1: $\beta = 0.13$, 95% CI [-0.02, 0.28], $t(96) = 1.74$, $p = 0.086$; Sample 2: $\beta = 0.28$, 95% CI [0.11, 0.45], $t(96) = 3.26$, $p = 0.002$), demonstrating potential performance benefits during reward learning. Conversely, Interrogative motivation increased directed exploration during reinforcement learning (Sample 1: $W = 872.5$, $p = 0.014$, Cohen's $d = -0.71$, 95% CI [-1.11, -0.30]; Sample 2: $W = 961.5$, $p = 0.065$, Cohen's $d = -0.46$, 95% CI = [-0.85, -0.05]). There was no difference in random exploration between groups. At test, participants in the Interrogative group showed superior recognition memory for the incidentally-encoded paintings (Sample 1: $\beta = -0.29$, 95% CI [-0.51, -0.07], $t(73) = -2.62$, $p = 0.011$; Sample 2: $\beta = -0.27$, 95% CI [-0.51, -0.03], $t(75) = -2.26$, $p = 0.027$). Furthermore, we showed that reward modulated memory in the Interrogative group; high-value paintings were prioritized in memory (Sample 1: $\beta = 0.13$, $z = 2.66$, $p = 0.008$; Sample 2: $\beta = 0.12$, $z = 2.96$, $p = 0.003$). In the Imperative group, there was no effect of reward on memory (Sample 1: $\beta = -0.01$, $z = -0.22$, $p = 0.825$; Sample 2: $\beta = -0.004$, $z = -0.10$, $p = 0.924$).

Conclusions: Overall, we found that a subtle instructional manipulation biased participants towards either an imperative or interrogative motivational state, thereby influencing both choice behavior and memory outcomes. The imperative/interrogative motivational framework unifies and reconciles findings from other proposed dichotomies in memory and decision-making research. Manipulating motivational states may enhance context-appropriate performance and memory formation, aligning cognitive processes and memory representations with current goals. Our results offer practical implications for educational practices, interventions for behavior change, and clinical treatments that aim to balance immediate performance goals with long-term learning.

Keywords: Motivation, Reward, Decision Making, Memory, Reinforcement Learning

Disclosure: Nothing to disclose.

P792. A New Tool for Measuring Social Rejection Elicited Aggression Reveals Unique Relations Between Irritability and Aggression in Adolescents and Young Adults

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Background: Interpersonal violence is a leading cause of death for adolescents and young adults. One contributing factor may be an increased sensitivity to social rejection during this critical phase of development. Despite the strong connection between rejection and aggression in youth, little is known about the neurocognitive mechanisms that promote this relation. Mapping these mechanisms is a critical first step towards developing much needed interventions to curb aggressive behavior. Progress towards this goal has been hindered by a lack of ecologically-valid tasks that enable the measurement of in-the-moment social rejection elicited aggression. Here, we describe an initial study that demonstrates the efficacy of a novel task designed to evoke rejection elicited aggression. We replicate promising results in a second study that tests the task's capacity to probe differences in rejection elicited aggression in adolescents and young adults who vary in their level of irritability, a transdiagnostic symptom associated with a proneness towards anger.

Methods: Male and female participants (S1: $N = 55$, 20.36 ± 1.41 years; S2: $N = 83$, 12.39 ± 1.67 years, $N = 90$, 19.06 ± 1.64 years) completed the novel Virtual School With Aggression (VS-WA) task. Participants were told that they would be a new student who interacts with six age- and gender-matched peers online. They then completed a personal profile and avatar they believed would be sent to purported peers. They next learned the reputation of the peers (two nice, mean, unpredictable) via "Yelp-like" reviews left by prior purported participants. To maintain experimental control, participants actually interacted with a computer program that pre-determined the responses of the peers. During the first part of the task participants interacted with each peer 12 times (24 interactions per reputation). On each trial the participant received positive or negative feedback (nice: 100% positive; mean: 100% negative; unpredictable: 50% positive and negative), and responded with pre-specified text options. In the second part of the task, participants were given the opportunity to aggress against each purported peer in the context of a button pressing game. Participants learned that whoever pressed a button the most times in 5 sec would have the opportunity to send a noise blast at a volume of their choosing to each of the other peers. Noise blast options ranged from 1 (0 decibels) to 24 (120 decibel). Participants selected the volume for each peer 12 times (24 per reputation). Aggression was defined as the average volume selected for each peer type. Overall aggression was defined as the average volume selected for all peers.

A repeated measures ANOVA, and follow-up paired samples t-tests, tested the hypothesis that the VS-WA task would elicit aggressive behavior that differed by peer reputation type (nice, mean, unpredictable). In Study 2, bivariate Pearson's correlations tested the relation between age and aggressive behavior towards each peer type and overall aggression. The same analyses were performed for irritability using the Affective Reactivity Index (ARI) total score. A moderation analysis was conducted to test the effect of age on the relation between irritability and aggressive behavior towards mean peers. We focused on mean peers as they provided 100% negative feedback and therefore purely evoked social rejection elicited aggression. Johnson-Neyman analyses more precisely isolated regions of significance in moderation effects.

Results: Across both studies, the VS-WA successfully evoked rejection-elicited aggression that differed depending whether peers were nice (S1: 7.06 ± 4.87; S2: 7.15 ± 5.10), mean (S1: 12.66 ± 7.14; S2: 14.92 ± 6.16), or unpredictable (S1: 10.72 ± 5.46; S2: 12.39 ± 5.13; ANOVAs S1: $F(2, 108) = 20.57$, $p < 0.001$, $\varepsilon 2 = 0.276$; S2: $F(2, 344) = 152.13$, p.< .001, $\varepsilon 2 = 0.469$). Participants were most aggressive to mean vs. unpredictable peers (S1: $t(54) = 4.55$, $p < 0.001$; S2: $t(172) = 8.06$, $p < 0.001$), and less aggressive towards nice vs. mean (S1: $t(54) = 13.18$, $p < 0.001$; S2: $t(172) = 13.18$, $p < 0.001$) and unpredictable peers (S1: $t(54) = -4.12$, $p < 0.001$; S2: $t(172) = -12.70$, $p < 0.001$). In Study 2, age was negatively correlated with aggression for each peer type (r's < -.200, p's < 0.01) and overall aggression ($r = -.285$, $p < 0.001$), whereas irritability was positive correlated with aggression for each peer type (r's > 0.230, p's < 0.01) and overall

aggression ($r = 0.280$, $p < 0.001$). Age moderated the relation between irritability and aggression towards mean peers ($R = 0.335$, $R2 = 0.113$, $F(3, 167) = 7.07$, $p < 0.001$; interaction: $\Delta R2 = 0.032$, $b = 0.159$, $p = 0.015$). While there was no relation between irritability and aggression in younger adolescents, starting at 13.55 years of age, there was a positive relation between irritability and aggression that strengthened as age increased.

Conclusions: We introduced and validated a novel task for studying rejection-elicited aggression that is optimized for studying neural mechanisms that promote this behavior. Across two studies we demonstrate the efficacy of the VS-WA, with level of aggression being sensitive to degree of peer-based rejection. Moreover, we showed the task can be used to probe age- and irritability-related differences in aggression. To that end we isolated unique age-based relations between irritability and rejection-elicited aggression. This effect has important implications for the development and implementation of interventions for irritable adolescents and young adults.

Keywords: Agression, Social Rejection, Irritability, Adolescent

Disclosure: Nothing to disclose.

P793. A Transdiagnostic Assessment of Delay Discounting and Family History of Psychopathology

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Background: Delay discounting is a behavior conserved across species in which individuals prefer smaller immediate versus larger delayed rewards. It has been hypothesized that greater degrees of delay discounting are associated with psychiatric disorders in a transdiagnostic fashion. However, it is unclear if delay discounting behavior is a risk factor for these disorders, with individuals at higher risk demonstrating steeper delay discounting prior to the development of psychopathology. It remains critical to consider confounding variables such as socioeconomic status.

Methods: The data is part of the Adolescent Brain and Cognitive Development (ABCD) study, which is a large, longitudinal study of 11,880 children. First, we looked at Spearman's correlations between family history density of different psychiatric disorders and delay discounting behavior measured as the area under the curve. We then conducted mixed effects models to examine associations between family history density of different psychiatric disorders and delay discounting while accounting for sociodemographic factors.

Results: Correlations between family history of psychopathology and delay discounting behavior were small in magnitude, ranging from $\rho = -0.04$ to 0.03. Without controlling for demographics, the model for family history of alcohol use problems had an estimate of -0.63 (95% CI -1.06, -0.20, $p = 0.004$), and this was one of the larger estimates. When adjusting for covariates, the effect of family history was reduced in the full sample ($p = 0.019$). In contrast, race was significant with an estimated effect of -6.72 for black relative to white individuals (95% CI -8.22, -5.23, $p < 0.001$). Hispanic relative to Non-Hispanic has a similar estimate (-3.69, $p < 0.001$). Males had steeper discounting than females (estimate $= -2.68$, $p < 0.001$). Parental education was significant, where having less than high school education was associated with steeper discounting than having a bachelor's degree or higher (estimate $= -4.61$, $p < 0.001$). Household income was not significant.

Conclusions: These results do not support the hypothesis that individuals at greater risk for psychopathology display steeper delay discounting behavior at ages 10-11. Sociodemographic factors played a larger role in predicting delay discounting behavior than family history of psychopathology.

Keywords: Impulsivity, Delay Discounting, Adolescence, Heritability of Depression

Disclosure: Nothing to disclose.

P794. Prevalence and Correlates of Defense Mechanisms: A National Study

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Background: Despite the clinical relevance of mechanisms of defense, there are no published studies of their prevalence, correlates and association with psychosocial functioning. The goal of this study was to estimate the prevalence and correlates of 12 defense mechanisms in the general adult population.

Methods: Defense mechanisms were approximated from items used to assess personality traits in the National Epidemiological Survey on Alcohol and Related Conditions (NESARC), a representative sample of US adults ($N = 36{,}653$). We examined the associations between sociodemographic characteristics and prevalence of 3 types of defenses mechanisms (pathological, immature and neurotic). For each defense mechanism, we used the Short-Form 12 (SF-12) to compare psychosocial functioning among 3 groups: those who 1) endorsed the mechanism with impairment, 2) endorsed the mechanism without impairment, and 3) did not endorse the defense mechanism.

Results: The prevalence of defense mechanisms ranged from 13.2% (splitting) to 44.5% (obsessive/controlling behavior). Pathological defenses were more strongly associated with immature defenses (OR = 5.4, 95% CI = 5.2-5.6) than with neurotic defenses (OR = 2.0, 95% CI = 1.9-2.0), whereas the association between immature and neurotic defenses had an intermediate value between the other two (OR = 2.2, 95% CI = 2.1-2.2). Pathological and immature defenses were associated with younger age, having been never married, lower educational attainment, and lower income. After adjusting the crude results for age and sex, individuals who did not endorse a given defense generally had higher scores on the mental health component of the SF-12 than those who endorsed the defense without self-recognized impairment who, in turn, had on average higher scores than those with self-recognized impairment.

Conclusions: These results suggest that neurotic, immature, and pathological defense mechanisms are prevalent in the general population and associated with psychosocial impairment. Recognizing defense mechanisms may be important in clinical practice regardless of treatment modality.

Keywords: Defense Mechanism, Psychopathology, Transdiagnostic

Disclosure: Nothing to disclose.

P795. Pleiotropic Influences of Neuropsychiatric Polygenic Risk on Common Laboratory Values in 660,000 US Veterans

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Background: Severe mental illness, including schizophrenia, bipolar, and major depression disorder are heritable, highly multifactorial disorders which cause significant disability, worldwide. Better understanding the pathophysiological mechanisms underlying disease risk and progression will be essential for alleviating the burden of these illnesses and comorbidities. The Million Veteran Program links genomic data, self-report survey data, and electronic health records from the Veterans Health Administration, which is the largest integrated health care system in the United States.

Methods: We constructed and tested polygenic risk scores for schizophrenia, bipolar disorder, and major depression for association with median values for 70 laboratory tests in 660,000 participants. We applied genomic structural equation modeling to derive novel scores indexing shared and disorder-specific latent genetic factors.

Results: Schizophrenia polygenic scores were robustly associated (P value < 10-8) with increased lipid levels, electrolyte imbalances, decreased liver and kidney function, and increased white blood cell count. Many associations were replicable among 125,000 African Americans, albeit with lesser statistical significance (P value < 10-5). We found that a cross-disorder latent genetic factor accounted for many findings, rather than any diagnosis-specific factors. These findings remained significant (P value < 10-6) when restricting analyses to individuals without diagnosed psychotic or affective illness, suggesting that these relationships are not simply consequences of medication side-effects.

Conclusions: By applying a 'reverse genetics' approach to large-scale electronic health records, we add to a growing literature demonstrating the broad pleiotropic effects of currently indexable polygenic risk. Ongoing Mendelian randomization analyses seek to further parse biological from mediated pleiotropy.

Keywords: Schizophrenia (SCZ), Bipolar Disorder (BD), Major Depressive Disorder, Genome-Wide Association Studies

Disclosure: Nothing to disclose.

P796. Novel PET Radioligand [18 F]PF-06445974 Can Measure LPS-Induced Phosphodiesterase 4B in Rat Brain

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Background: Phosphodiesterase-4B (PDE4B) is an enzyme that metabolizes cAMP, thereby terminating the actions of this second messenger. Inhibition of PDE4B has antidepressant-like effects in animals, and inhibition of this enzyme in humans is anti-inflammatory in peripheral disorders (e.g., psoriasis and COPD). We developed [18 F]PF-06445974 as a radioligand that preferentially binds to the PDE4B subtype to study it in humans with major depressive disorder and in animals as a potential biomarker of neuroinflammation. In this study, we sought to determine whether intrastriatal injection of the inflammogen lipopolysaccharide (LPS) increases radioligand binding, which is an indirect measure of activation of the enzyme. That is, phosphorylation of PDE4 has been shown to markedly increase both its enzymatic activity and its affinity to binding of radiolabeled inhibitors.

Methods: A total of 6 male Wild-type Sprague-Dawley rats were implanted with bilateral intrastriatal cannulas. After a week of recovery, 50 mg LPS in 5 µL of saline was infused into one hemisphere with the same volume of sterile saline on the contralateral side. At one and eight days after LPS, animals were injected with [18 F]PF-06445974 (20 MBq iv) and scanned for 90 min.

Results: At one day after LPS injection, binding of [18 F]PF-06445974 in the lesioned striatum increased about 50% compared to the contralateral region. Areas distant from the lesion (e.g., cerebellum) were not affected by LPS injection. To confirm the specificity of the radioligand binding, rolipram (1 mg/kg iv) decreased binding to background levels. Finally, rescanning the animals one week after the lesion showed that radioligand binding had returned to baseline levels.

Conclusions: We found that local injection of LPS into rat striatum increased radioligand binding about 50% compared to the contralateral side one day after the lesioning. The binding was selective for PDE4, as it was blocked by the PDE4-specific antagonist rolipram. In addition, the increase was transient and returned to baseline by one week after LPS injection. The increased binding may be caused by the phosphorylation/activation of PDE4B and suggest that PDE4B imaging may be a biomarker of neuroinflammation in the brain.

Keywords: Neuroinflammation, Brain Based Markers for Depression, Positron Emission Tomography (PET), F-18 PET Imaging

Disclosure: Nothing to disclose.

P797. Exploring Relationships of Peripheral Inflammation With Negative and Positive Valence Systems

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Background: Alterations in both negative (NVS) and positive valence (PVS) systems are characteristics of mood disorders and other disorders as evidenced by differences in experiences of constructs within these systems such as perceived stress, loneliness, sadness, positive affect, and emotional support. Abnormal inflammatory processes have also been implicated in mood disorders and can impact mood and cognitive symptoms. For example, inflammation has been shown to impact affective response inhibition and to impact on motivated behaviors such as reward valuation. It has also been shown that other PV symptoms (i.e. impaired motivation, impaired energy, and anhedonia) positively correlate with pro-inflammatory immunomarkers and that NV symptoms (i.e. anxiety and interpersonal sensitivity) negatively correlate with pro-inflammatory immunomarkers. Importantly, it has been suggested that women may be more vulnerable to inflammation-mediated depressive symptoms and behavior changes and display increased PV symptoms than men. However, sex comparisons in the relationship of peripheral inflammation to NVS and PVS factors including perceived stress, loneliness, sadness, positive affect, and emotional support are not well characterized. Therefore, we aimed to extend prior research in the link between valence systems and inflammation and examine the role of biological sex in the relationship between peripheral markers of inflammation [i.e. tumor necrosis factor-alpha (TNF-alpha) and Interleukin-6 (IL-6)] and function in NVS and PVS, measured by self-reports of positive affect, friendship, emotional support, loneliness, perceived stress, and sadness.

Methods: Twenty-six participants (19 women and 17 men) were recruited at Brigham and Women's Hospital, including both healthy participants and participants with bipolar disorder to enrich the sample for a range of function within NIMH Research Domain Criteria (RDoC) negative and positive valence systems. Participants were aged 35-63 ($M = 47.94$, $SD = 8.53$). Self-report positive affect, emotional support, friendship, loneliness, perceived stress, and sadness scores from NIH Toolbox were assessed to capture constructs in negative and positive valences systems. Peripheral markers of inflammation included pro-inflammatory

cytokines TNF-alpha and IL-6 and were assayed from serum from a fasted morning blood draw. We conducted Pearson correlation analyses of each inflammatory marker with self-report measures in men and women separately. As a validity analysis, we conducted separate partial correlations in men and women controlling for age in the relationship between inflammatory markers with negative and positive valence systems.

Results: We observed significant inverse correlations between TNF-alpha and loneliness ($r = -0.704$, $p = 0.011$), perceived stress ($r = -0.588$, $p = 0.035$), and sadness ($r = -0.605$, $p = 0.017$) scores in women. We also observed a significant positive correlation between TNF-alpha and emotional support scores ($r = 0.616$, $p = 0.033$) in women. Also in women, there was a trend in positive relationship between TNF-alpha with friendship ($r = 0.517$, $p = 0.085$) and positive affect ($r = 0.455$, $p = 0.088$) scores. No significant relationships emerged with IL-6 and we did not observe any significant associations in men or the combined sample of men and women. When controlling for age the relationship between TNF-alpha with loneliness ($r = -0.658$, $p = 0.028$) and sadness ($r = -0.539$, $p = 0.047$) scores remained significant in women and no other significant relationships emerged in men or the combined sample of men and women when controlling for age.

Conclusions: These results suggest that inflammation is a key pathway to consider in the function of negative and positive valence systems in women. Specifically, TNF-alpha is inversely correlated with constructs within NVS and positively related to constructs in PVS in women. This is in line with previous research in depression showing that PV symptoms positively correlate with pro-inflammatory factors while NV symptoms negatively correlated with pro-inflammatory factors. Moreover, our findings of a link between inflammation and NVS and PVS in women but not in men extends and supports previous research indicating that women are more vulnerable to inflammation-induced mood alterations. Accordingly, our findings suggest a role for TNF-alpha as a mechanistic factor underlying sex differences in NVS and PVS functioning. Despite the promising nature of these findings, they are preliminary in nature and should be interpreted with caution given the small sample size. If confirmed, these findings have the potential to inform clinical care regarding the sex-informed use of anti-inflammatory medications to improve outcomes in disorders involving aberrant functioning in NVS and PVS.

Keywords: Inflammation, Negative Valence System, Positive Valence, Cytokines, Mood Disorder

Disclosure: Nothing to disclose.

P798. CSF and Peripheral Inflammatory Biomarkers After COVID-19 Infection in a Pregnant Population

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Background: In light of growing evidence of long-term sequelae after COVID-19 infection (termed post-covid conditions, long COVID or Post-Acute Sequelae of COVID [PASC]), there is an urgent need to investigate the potential long-term neuropsychiatric effects of SARS-CoV-2 infection. It has been estimated that between 10 and 30% of individuals who are infected with the virus, including those with mild disease, could experience post-covid sequelae. The central nervous system (CNS) is potentially susceptible to long-term changes from systemic inflammation that may occur during infection. While some evidence suggests that pregnant women may be more vulnerable to the acute effects of SARS-CoV-2 infection than the general population, the risk for potential long-term sequelae is unknown. This research aims to examine cerebrospinal fluid (CSF) and peripheral cytokine levels in pregnant individuals at delivery in relation to SARS-CoV-2 infection and vaccination status, to assess any potential long-term differences in inflammation.

Methods: Participants were recruited from a prospective pregnancy cohort, Generation CSF, in the Mount Sinai Health System. CSF and plasma samples were drawn from 58 participants during labor and delivery (CSF $n = 45$, plasma $n = 56$, paired $n = 43$). A panel of fourteen cytokines was analyzed using a high sensitivity assay. In addition, SARS-CoV-2 infection and vaccination status were collected through self-report and extracted from medical records. Mann-Whitney U analysis was used to compare median cytokine levels between individuals who had tested positive for SARS-CoV-2 infection before or during pregnancy ($n = 8$) and individuals with no known prior SARS-CoV-2 infection ($n = 50$).

Results: There were no significant differences in median cytokine levels in CSF and plasma between SARS-CoV-2 infected (before or during pregnancy) and non-infected groups. Subgroup analyses by vaccination status were not performed due to low sample sizes in some subgroups.

Conclusions: This preliminary data analysis found no evidence of long-term central or peripheral inflammatory changes after SARS-CoV-2 infection in a pregnant population. This project is ongoing (Generation C-SF, target $N = 500$, R01MH127315) and further analyses in a larger sample including vaccination status are currently in progress.

Keywords: Cytokines, Inflammation, CSF, COVID-19, Pregnancy

Disclosure: Neurocrine Continental, Inc.: Consultant, Advisory Board (Self), Neurocrine Biosciences, Inc., Alkermes, Inc.: Consultant (Self), AICure, Millennium Pharmaceuticals, Inc. (Takeda Pharmaceutical Company Limited): Grant (Self).

P799. The Transdiagnostic Comparative Efficacy of Non-Invasive Neurostimulation Interventions for Suicidal Ideation: Initial Results From a Systematic Review and Meta-Analysis

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Background: Recent research has suggested that non-invasive neurostimulation may reduce suicide ideation (SI). However, the efficacy of non-invasive neurostimulation interventions for SI has not yet been comprehensively reviewed and compared across methods and psychiatric disorders. Here, we present initial findings from the largest meta-analysis to date on the comparative efficacy of non-invasive neurostimulation for SI across psychiatric disorders.

Methods: Embase, MEDLINE, PsycINFO, CINAHL, and Cochrane's CENTRAL were used to search for peer-reviewed studies published from inception to June 2021. Human clinical trials examining the efficacy of non-invasive neurostimulation on psychiatric symptoms and reporting on a continuous outcome using a suicide-specific measure (e.g., Beck Scale for Suicide Ideation) or a suicide item from a standardized measure (e.g., suicide item on the HAM-

D) were included. Random-effects meta-analyses of the standardized mean difference in SI scores from pre-to post treatment were used to estimate treatment effects. Where possible, effects of active v. sham treatment on SI were examined, as well as effects between psychiatric disorders and neurostimulation sites. Effect sizes are represented by Hedge's g.

Results: The search returned 10933 abstracts. 2591 studies underwent full text review. 39 studies were included in this preliminary analysis. Electroconvulsive Therapy (ECT): $N = 14$ ECT trials were identified, comprising 503 subjects with major depressive disorder (MDD). Only one trial was sham-controlled. There was a large and significant effect of ECT on SI (Hedge's $g = -1.56$, SE = 0.17 [95% CI -1.89 -1.22], $p < 0.001$). A funnel plot suggested the presence of publication bias. Three studies compared ECT to other neurostimulation types, including low amplitude seizure therapy (LAP-ST; $n = 1$) and repetitive transcranial magnetic stimulation (rTMS; $n = 2$). No significant difference between ECT and LAP-ST on SI was reported in the one study that compared these interventions ($p > 0.33$). In contrast, a large and significant effect on SI, favoring ECT over rTMS, was found (Hedge's $g = -1.29$, $p < 0.001$). Magnetic Seizure Therapy (MST): $N = 2$ trials examined the effects of MST on SI in 90 subjects with MDD. A large and significant effect of MST on SI was found (Hedge's $g = -0.82$, SE = 0.20, 95% CI: -1.05, -0.58, $p < 0.001$). Due to the small number of trials, publication bias could not be assessed. Transcranial Magnetic Stimulation (TMS): $N = 21$ TMS trials were identified, comprising 928 subjects. A large and significant effect of TMS on SI was observed (Hedge's $g = -0.91$, SE = 0.10, 95% CI: -1.10, -0.72, $p < 0.001$). Analysis of two adolescent trials suggested a medium but not significant effect of TMS on SI in this age group (Hedge's $g = -0.51$, $p = 0.06$). In contrast, a large and significant effect on SI was found for 17 adult studies (Hedge's $g = -0.84$, $p < 0.001$) and two geriatric studies (Hedge's $g = -2.01$, $p < 0.001$). Across all TMS trials, a funnel plot suggested publication bias. Of the 21 TMS trials identified, 11 were sham-controlled, comprising 342 subjects. Overall, results favored active over sham TMS with a small, significant effect on SI (Hedge's $g = -0.36$, SE = 0.08, [95% CI: -0.51, -0.21], $p < 0.001$). A funnel plot of sham-controlled TMS studies suggested a low chance of publication bias. Effect sizes across stimulation sites were similar and favored active over sham TMS with small and significant effects on SI: six trials stimulated the left dorsolateral prefrontal cortex (DLPFC)/prefrontal cortex (Hedge's $g = -0.41$, $p = 0.04$) whereas four studies applied bilateral stimulation (Hedge's $g = -0.32$, $p = 0.009$). One study stimulated the visual cortex and found no significant differences on SI in active v. sham TMS (Hedge's $g = -0.45$, $p = 0.12$). Diagnostically, nine sham-controlled trials examined subjects with MDD. Results favored active over sham TMS for reducing SI in MDD (Hedge's $g = =0.40$, $p = 0.001$). In contrast, two trials studied the effects of sham-controlled TMS in diagnoses other than depression ($n = 1$ borderline personality disorder and $n = 1$ posttraumatic stress disorder and/or traumatic brain injury). No significant differences between active v. sham TMS on SI in these diagnoses were found (Hedge's $g = -0.24$, $p = -.46$). Transcranial Direct Current Stimulation (TDCS): $N = 2$ open-label TDCS pilot trials reported on an SI outcome. One investigated anorexia nervosa and the other, treatment-resistant obsessive-compulsive disorder. There was no significant effect of TDCS on SI in these samples (Hedge's $g = -0.06$, $p = 0.81$).

Conclusions: Initial results suggest that ECT, MST, and TMS may be efficacious for reducing SI. Although the strongest effects on SI were associated with ECT in MDD, only one ECT study was sham-controlled and there was a risk of publication bias across ECT studies. MST appears promising but the findings are limited by a lack of published data. Overall, sham-controlled TMS trials provided the highest quality evidence; active over sham TMS was associated with small but significant effects on SI, and no evidence of publication bias. Crucially, there is a paucity of neurostimulation trials in psychiatric groups other than MDD and this limits the generalizability of these findings to other groups that frequently experience SI. To date, analyses of two TDCS pilot trials in anorexia nervosa and OCD showed that TDCS did not significantly reduce SI in these groups. Meta analyses of remission and relapse rates are forthcoming.

Keywords: Suicidality, Neurostimulation, Meta-Analysis

Disclosure: Nothing to disclose.

P800. CALM-IT: Feasibility, Reliability, Validity, and Clinical Relevance of a Novel Mobile Application Probing Inhibitory Control

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Background: The identification of brain-based mechanisms underlying psychopathology is critical to the development of efficacious interventions. Inhibitory control, or the ability to modulate prepotent behavioral responses, is mediated through established neural circuitry, including ventrolateral, ventromedial, and dorsolateral prefrontal cortices; the orbitofrontal cortex; the inferior frontal gyrus; and the dorsal, posterior, anterior cingulate cortices. Research has linked inhibitory control deficits to increased risk for psychological symptoms and disorders. However, existing tools for assessing inhibitory control are repetitive, time intensive, expensive, and laboratory based, posing barriers to broad dissemination. We examined the utility of "CALM-IT," a novel, gamified inhibitory control task based on the Go/No-Go paradigm delivered via mobile application platform to increase accessibility of an inhibitory control assessment in the clinical community. In four aims, we investigated the (1) feasibility, (2) reliability, (3) validity, and (4) potential clinical relevance of CALM-IT.

Methods: Eighty-six youth (56.2% male) between 8 and 18 years old (M = 13.29, SD = 2.75) were recruited from the community. Youth with primary diagnoses of attention deficit hyperactivity disorder (ADHD; $n = 25$), anxiety disorder ($n = 17$), and disruptive mood dysregulation disorder (DMDD; $n = 19$); youth with clinically significant irritability not meeting the threshold for DMDD ($n = 8$); and youth without clinical diagnoses ($n = 17$) were included in the sample using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (KSADS-PL). At home, youth completed CALM-IT on a mobile device across two sessions approximately one week apart (Mdays = 10.94, SDdays = 9.94). In the laboratory, youth completed canonical inhibitory control paradigms, including the Antisaccade, AX Continuous Performance, Flanker, and Stop Signal tasks. On average, there were 429.51 days (SDdays = 359.64) between laboratory paradigm and CALM-IT completion. In addition, parents and youth completed a series of dimensional measures of psychopathology including assessments of irritability, anxiety, ADHD, and depression symptoms, respectively: Affective Reactivity Index (ARI), Screen for Anxiety Related Emotional Disorders (SCARED), Conners Comprehensive Behavior Ratings Scale (Conners), and Mood and Feelings Questionnaire (MFQ). Detailed information on study methods are available in the CALM-IT Registered Report. To determine feasibility, we calculated the percent of youth who completed CALM-IT with usable data, along with the percent of targets hit (indicating correct performance) as a measure of youth engagement. The intraclass correlation

coefficient (ICC) between the two CALM-IT sessions provided an estimate of test-retest reliability. We operationalized inhibitory control performance during CALM-IT using d', which assesses an individual's ability to distinguish signal from noise. Specifically, we used d' to determine the standardized difference between hits (successfully swiping at a target) versus false alarms (failing to inhibit response to hit a target). We then calculated a latent factor of inhibitory control from all four of the canonical laboratory-based assessments. Finally, we calculated the correlation between CALM-IT d' and the latent factor of inhibitory control (validity) and the dimensional measures of psychopathology (clinical relevance).

Results: Aim 1. Approximately 95.56% of CALM-IT levels produced complete and usable participant data. On average, youth hit 76.89% of targets (SD = 9.35%), demonstrating engagement with the task. Aim 2. Consistency was good for target hit rate (ICC = 0.85, $p < 0.001$) and moderate for d' (ICC = 0.66, $p < 0.001$). Aim 3. On average, CALM-IT d' = 0.00 (SD = 1.02). Greater inhibitory control as measured by CALM-IT d' was associated with greater latent inhibitory control (r = 0.26, p = 0.05). Aim 4. Decreased inhibitory control as measured by CALM-IT d' was associated with higher levels of youth-reported irritability on the ARI (r = −.27, p = 0.01) and parent-reported hyperactivity/impulsivity on the Conners (r = −.24, p = 0.04). Associations between inhibitory control as measured by CALM-IT d' and parent-reported irritability on the ARI (r = −.13, p = 0.27), parent-reported inattentiveness on the Conners (r = −.12, p = 0.30), youth-reported (r = 0.04, p = 0.71) and parent-reported (r = 0.08, p = 0.46) anxiety on the SCARED, and youth-reported (r = 0.21, p = 0.06) and parent-reported (r = 0.16, p = 0.16) depression on the MFQ were nonsignificant.

Conclusions: Taken together, findings provide robust support for the feasibility, reliability, validity, and clinical utility of CALM-IT. First, CALM-IT produced largely complete and usable data, and youth showed moderate levels of task engagement. Second, CALM-IT exhibited moderate-to-good test-retest reliability. Third, CALM-IT d' was positively associated with a latent factor of inhibitory control with a medium effect size. Finally, preliminary support emerged for the clinical relevance of CALM-IT; lower levels of CALM-IT inhibitory control were related to greater youth-reported irritability and parent-reported hyperactivity/impulsivity. Future work will examine associations between youth CALM-IT performance and neural activation during inhibitory control tasks.

Keywords: Inhibitory Control, Mobile Application, Developmental Psychopathology

Disclosure: Nothing to disclose.

P801. Differences in Personality, Cognitive Abilities, Illicit Drug Use, and White Matter Structural Integrity Between Hallucinogen Users and Matched Controls

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Background: Classic psychedelic drugs have potential positive enduring effects on personality (MacLean et al., 2011; Erritzoe et al., 2018) and cognition (Bouso et al., 2012; Doss et al., 2021; Rucker et al., 2022) as well as mood (Davis et al., 2021; Carhart-Harris et al., 2016, 2022) and substance use disorders (Johnson et al., 2014, 2017; Bogenschutz et al., 2015). However, enduring effects of psychedelic on human brain structure are unknown, and neural mechanisms supporting the enduring psychedelic effects are not well-understood. Preclinical findings suggest micro-scale structural neuroplastic changes after psychedelic administration (Ly et al., 2018; Shao et al., 2021). The current study investigated the association between psychedelic use, macroscale brain structure, personality, cognitive ability, and illicit drug use in a naturalistic sample.

Methods: Data from 53 subjects (age M = 43.1, SD = 15,1; 32 F) reporting ever having used hallucinogens and 53 approximately matched controls (age M = 43.2, SD = 15.8; 31 F) who reported never having used a hallucinogen were drawn from the Nathan Kline Institute-Rockland Sample database (Nooner et al., 2012). All participants completed diffusion tensor imaging (137 directions, 2mm3 voxels, b = 0, 1500 s/mm2), psychological assessments (NEO Five Factor Inventory, Wechsler Abbreviated Scale of Intelligence II or WASI-II, Wechsler Individual Achievement Test second edition abbreviated or WIAT-IIA) and psychiatric assessments (CASI-A and NIDA drug use questionnaires, Structured Clinical Interview for DSM-IV-TR). Controls were matched to hallucinogen users by age, sex, and presence vs absence of a psychiatric diagnosis. Groups were compared on measures of personality, cognitive ability, history of illicit drug use, and the density of white matter tracts determined from probabilistic tractography using linear statistics.

Diffusion image preprocessing and analysis was conducted using the FSL toolbox (Jenkinson et al., 2021). Preprocessing included skull stripping, eddy current and motion correction, and local fitting of diffusion tensors using DTIFIT. Data were normalized to MNI template space using FLIRT. Tractography was then performed in native space using BEDPOSTX and the cross-species tractography toolbox (XTRACT; Warrington et al., 2020). XTRACT performs probabilistic, anatomically constrained tractography using a library of validated tractography protocols for 42 white matter tracts. 2 tracts (Left and Right corticospinal tract) were removed due to insufficient number of subjects with full brain data coverage for these areas. White matter tract density for each of the remaining 40 tracts was compared between groups using multi-covariate regression, controlling for sex, age, and the presence or absence of lifetime use of stimulants, cocaine, narcotics, and inhalants. Additional linear models were estimated for each white matter tract that showed a significant difference in density between groups, to evaluate the association between differences in white matter density and differences in questionnaire assessment data. For statistical inference, including correction for multiple comparisons across space, maximal statistic permutation testing with threshold-free cluster enhancement was conducted (Smith et al., 2006).

Results: Hallucinogen users reported greater lifetime use of stimulants ($(\chi)2 = 5.194$, p = 0.023), cocaine ($(\chi)2 = 12.791$, p = 0.000), narcotics ($(\chi)2 = 8.477$, p = 0.004), and inhalants ($(\chi)2 = 6.360$, p = 0.012), but not tobacco ($(\chi)2 = 1.178$, p = 0.278), cannabis ($(\chi)2 = 2.038$, p = 0.153), alcohol ($(\chi)2 = 3.087$, p = 0.079), or tranquilizers ($(\chi)2 = 1.504$, p = 0.22). Higher scores were observed in hallucinogen users on the personality trait of openness (t = 2.321, p = 0.014), WASI-II total score (Mann-Whitney U = 1033, p = 0.012), and WIAT-IIA composite standard score (Mann-Whitney U = 8897, p = 0.006). Greater density of white-matter tracts was observed in hallucinogen users in the left superior thalamic radiation (p = 0.031), left arcuate fasciculus (p = 0.026), left perigenual cingulum (p = 0.009), left ($p < 0.001$) and right (p = 0.035) frontal aslant, right superior longitudinal fasciculus (p = 0.004), and left inferior longitudinal fasciculus (p = 0.017). Less white matter tract density was observed in hallucinogen users in the right inferior longitudinal fasciculus ($p < 0.001$). Positive associations between white matter tract density and cognitive abilities were observed for WIAT-IIA composite score and the left inferior longitudinal fasciculus (p = 0.0009), and for WASI-II full scale score and the right superior longitudinal fasciculus (p = 0.038).

Conclusions: Hallucinogen exposure may result in structural consequence in canonical white matter tracts associated with perception, cognition, and affect. If greater white matter density confers a processing benefit, and lower density confers a deficit,

the current findings are consistent with previous findings suggesting improved cognitive function and reduced negative affect among hallucinogen users (Bouso et al., 2012; Barrett et al., 2020; Doss et al., 2021). Given proposed psychoplastogenic effects of hallucinogens (Ly et al., 2018; Shao et al., 2021), it may be that circuits or pathways that are acutely altered during hallucinogen drug action undergo some lasting structural change that can be observed with diffusion imaging methods. These novel findings provide clues to potential neural mechanisms underlying therapeutic effects of hallucinogens.

Keywords: Psychedelics, Potential Mechanism, Structural Connectivity, Big Five Personality Factors, Cognitive Performance

Disclosure: Wavepaths, Ltd, MindState Design Labs, Inc.: Advisory Board (Self).

P802. The Placebo Response in Classic Psychedelics: A Systematic Review of Clinical Trials and Qualitative Analysis

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Background: The use of classic psychedelics (e.g., psilocybin, ayahuasca, lysergic acid diethylamide [LSD]) as potent mental health treatments is gaining traction, yet significant challenges remain in conducting trials with these substances. Both the role of placebo controls in psychedelic therapy trials and the importance of placebo mechanisms in explaining the efficacy of psychedelic therapy remain understudied. Several factors pose significant challenges to blinding in psychedelic therapy trials. Successfully blinding participants to respective treatment arms is challenging, in part due to strong subjective effects of psychedelic compounds. This issue is important because it may overestimate outcomes in psychedelic therapy trials by increasing expectancy effects in active arm conditions and by diminishing effects in placebo conditions via participant disappointment in not receiving a psychedelic. This is supported by the fact that one study reported a death by suicide in one of their participants 11 days after receiving a very low dose of psilocybin intended as a placebo (Griffiths et al., 2016). The authors note that the subject reported feeling bored, possibly reflecting a nocebo effect. While blinding is clearly a difficult area in this field, surprisingly, it has not been well studied. One previous review suggests that only half of modern psychedelic clinical studies reported on subject blinding and nearly all subjects were able to ascertain whether they were given the psychedelic or placebo. The role of expectancy in placebo response also remains understudied and is a vital component of response in these studies. We review and synthesize the entire existing literature on the placebo response in the context of clinical trials that involve the administration of classic psychedelics to humans in order to guide future clinical trial design and enhance our understanding of this complex area.

Methods: We conducted an extensive PROSPERO registered (CRD42020185100) systematic review in accordance with PRISMA guidelines to identify any studies with the use of placebo in the context of a classic psychedelic clinical trial in a psychiatric population. EMBASE, PsycINFO, and PubMed were electronically searched with a combination of medical subject headings and free text keywords from their inception dates to present. The following data was extracted: study authors, publication year, study design, study duration, inclusion/exclusion criteria, participant demographics and baseline characteristics, sample sizes and descriptions of groups, details on interventions/exposures, outcome measures used, outcomes/effects reported, and adverse events. The characteristics and findings of included studies are presented as a systematic narrative synthesis and summarized in tabular format. The narrative synthesis explores findings within and between included studies and includes qualitative outcomes. We assessed the methodological quality of included studies using the Oxford Centre for Evidence Based Medicine (OCEBM) Levels of Evidence.

Results: We identified a total of 55 out of 1053 studies in our search that were eligible for inclusion, with publication dates ranging from 1963-2020. Studies were either RCTs or within subject cross-over design. There was also one single-blind non-randomized placebo-controlled within subject trial. In the RCTs, there were 10 on LSD, 17 on psilocybin, 5 on ayahuasca, and 1 on N,N-Dimethyltryptamine (DMT). Among RCTs, 29 studies were on healthy subjects, 3 on life-threatening cancer, 2 on schizophrenia, and 2 on major depressive disorder. For cross-over design studies, 7 were on LSD, 2 on psilocybin, 1 on ayahuasca, and 1 on DMT/6-hydroxy-N-dimethyltryptamine. There were 8 studies on healthy subjects, 2 on schizophrenia, and 1 in alcohol use disorder in these studies. The most common forms of placebo used were empty capsules, niacin, and IV saline. Expectancy was not measured in the vast majority of studies. Clinical outcomes in our review include subjective mental states, physiological measures, creative imagination and mental imagery tests, BDNF and cortisol levels, eyeblink responses, as well as formal measures of clinical depression and anxiety.

Conclusions: Our review suggests that the vast majority of placebo-controlled psychedelic therapy studies involve studies in healthy participants with the use of LSD or psilocybin. There is a very limited number of placebo-controlled studies in psychiatric populations, and the quality of placebo controls have been questionable. The use of an adequate placebo control, and assessment and balancing of expectancy, is severely lacking in existing clinical trials. Future psychedelic clinical trials should include adequate assessment of blinding, use of more appropriate controls, and should randomize both treatment arms and treatment expectancy. Active psychopharmacological controls, such as other rapid acting agents, and head-to-head comparison with active treatments should be considered as alternatives.

Keywords: Psychedelic Therapy, Placebo, Clinical Trials, Blinding, Expectancy

Disclosure: Consultant for GoodCap Pharmaceuticals Inc.: Consultant (Self).

P803. Effects of Psychoactive Drugs MDMA, Methamphetamine, and Buprenorphine on Affective Touch Processing in Healthy Humans

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Background: MDMA has shown great promise in the treatment of several psychiatric disorders when used in combination with psychotherapy. However, the components of the psychotherapy that accompanies MDMA treatment have not been fully investigated. One novel, yet understudied, component of psychedelic-assisted psychotherapy is therapeutic touch, raising the possibility that drugs like MDMA affect treatment response in part by affecting the somatosensory experience. The effects of psychoactive drugs on the experience of touch are not fully understood. In controlled studies with healthy volunteers, we tested the effects of three different psychoactive drugs on responses to affective touch; 1) the prosocial drug MDMA, 2) the prototypical stimulant methamphetamine, which shares some properties with MDMA without producing reported experiences of social closeness, and 3) the opioid buprenorphine, which has been shown to enhance some dimensions of social reward processing.

Methods: Healthy men and women participated in two double-blind placebo-controlled studies using a within-subjects crossover design. In Study 1 ($N = 36$; two sessions), they received buprenorphine (0.2 mg) or placebo in randomized counterbalanced order. In Study 2 ($N = 36$; four sessions), they received MDMA (0.75 mg/kg and 1.5 mg/kg), methamphetamine (20 mg), and placebo. In both studies, during expected peak drug effect, they completed an affective touch task to assess pleasantness of either affective touch (slow forearm brushing; 3 cm/s) or nonaffective touch (fast forearm brushing; 30 cm/s). Pleasantness of the touch was assessed with facial electromyography (EMG) and subjective ratings. These measures were examined in relation to ratings of subjective drug effects measured by the Drug Effects Questionnaire (DEQ, e.g. liking the drug and wanting more).

Results: As expected, slow touch was rated as more pleasant than fast touch in both studies. Both MDMA (both doses) and buprenorphine (0.2 mg) increased pleasantness ratings of affective touch [MDMA: $F(2,70)=7.27$, $p < 0.05$; buprenorphine $t(34)=-1.70$, $p < 0.05$], but methamphetamine did not. For MDMA, increases in pleasantness ratings were significantly correlated with "like drug" ($r = 0.39$, $p < 0.05$) and "want more" ($r = 0.50$, $p < 0.01$).

Conclusions: The finding that MDMA, and not methamphetamine, enhanced pleasantness ratings of affective touch is consistent with reports that the drug enhances the experience of touch in recreational settings. This finding suggests that changes in somatosensory processing may also contribute to the drug's effectiveness in MDMA-assisted psychotherapy. Our finding the opioid buprenorphine also enhances pleasantness of affective touch suggests that this effect is not specific to serotonergic stimulant drugs, but may also occur with other drug classes, and may be dissociable from euphorigenic effects. Future studies with symptomatic participants are needed to extend these findings to a clinical population.

Keywords: MDMA, Buprenorphine, Methamphetamine, Affective Touch, Psychophysiology

Disclosure: Nothing to disclose.

P804. Monosynaptic Circuitry Based CNS Drug Development: A Proven Approach to De-Risk Such Work

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Background: Most major pharmaceutical companies have abandoned psychiatric drug development. A principal reason is the riskiness of such development. However, there is a proven way to de-risk such development which will be presented in this poster. The reason to why ACNP as an organization and its membership should be interested in this topic is two-fold: (a) psychiatric medication is the major treatment modality for patient suffering from psychiatric disorders and abandonment of such development stagnates the development of new treatment for such illnesses and (b) the research that ACNP members do provides the necessary knowledge to take this circuitry approach to drug development.

Methods: The successful development of 7 different single mechanism of action CNS drugs will be present and the features that they have in common. The seven drugs include: aprepitant, lorcaserin, ondansetron, ramelteon, suvorexant, varenicline, and most recently most recent is sublingual dexmedetomidine (SLD). The poster will explain why these 7 drugs were selected as well as why drugs such as ketamine/esketamine and acamprosate.

Briefly, the criteria were:

1. A well-defined, monosynaptic circuit mediating the behavior broadly defined such as appetite, agitation, nausea, sleep, and smoking.
2. How a single mechanism of action drug could impact the circuitry to modify the behavioral in a predictable and desirable way,
3. The drug has a single mechanism thus reducing off target effects
4. The behavior is observable rather than subjective and is either dichotomous (e.g., vomiting or not) or highly correlated (e.g., the factors on the Excitatory Component of the Positive and Negative Symptom Scale, PANSS) in contrast to the less correlated symptoms domains in scales used to measure antidepressant (i.e. HDRS or MADRS) or antipsychotic (total PANSS).
5. An objective, surrogate marker may be able such as polysomnography encephalography (PSE) or blood pressure to assess target engagement.

Results: In contrast to drugs which do not meet the above criteria such as antidepressants and antipsychotics, the results of the studies with these 7 drug include the following:

1. good agreement between preclinical models and human studies
2. good agreement between phase 2 and phase 3 human studies.
3. few, if any, negative studies in phase 3.

Table and graphs will be presented in this poster to illustrate and support the above points.

The development of such drugs is not without some development hurdles. One example is that there may be a shifted dose-response curve individuals with the target illness versus normal volunteers. That is generally accepted for dopamine-2 receptors blockers in normal volunteers versus patients with schizophrenia. Another example is blood pressure effects of SLD in normal volunteers versus agitated individuals with bipolar disorder or schizophrenia. However, these differences have been readily addressed in early phase 2 studies to properly determine the dose range for the phase 3 studies.

Conclusions: Encouraging the development of mechanistically new drugs to treat psychiatric illness should be a prime concern of ACNP. This poster reviews the development of 7 new psychiatric medication based on well-defined monosynaptic circuitry mediating specific behavioral abnormalities over the last two decades. This approach is also being used for drug development and discovery relative to the model of the Nucleus Accumbens as the central common pathway to drug addiction developed by George Koop. This topic can be discussed during the poster presentation.

This knowledge is important for each member of ACNP which will in turn aid the ACNP in advocating for and promoting such drug development in the future. An issue will be how to translate this approach to more complex disease states such as major depression and schizophrenia. That may well be done by defining the circuits underlying the overall syndromic diagnosis. An adage here is that behaviors are the output of brain functions whereas syndromic diagnoses are human made conventions.

Keywords: Drug Development, Circuitry-Based Approach, Mechanism of Action

Disclosure: Nothing to disclose.

P805. Endocannabinoid Contributions to Affective Touch Processing in Humans With or Without Trauma Exposure Histories

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Background: Touch is a vital component of social interactions, contributing to the development and maintenance of social bonds throughout life. The pleasant, affective quality of touch is mediated by C-tactile (CT) afferent nerve fibers that respond most robustly to slow, gentle touch. CT-mediated affective touch is believed to play a role in buffering the effects of psychosocial stress and promoting prosocial behaviors. Thus, our first aim was to explore reactions to affective touch in adults who were exposed to trauma during childhood or adolescence. We then explored how individual variation in the endocannabinoid system, a neuromodulatory system implicated in stress and social behavior, may influence reactions to affective touch in this population. Finally, in a separate study, we assessed how pharmacological manipulation of the endocannabinoid system influenced affective touch processing in healthy adults.

Methods: In the first study, adult participants ($N = 101$) with documented childhood trauma ($N = 51$) or no trauma history ($N = 50$) completed tasks assessing perception of affective (e.g., CT-optimal) touch or non-affective (non-CT-optimal) touch. Blood samples were collected to analyze levels of endocannabinoids and cortisol. In the subsequent study, healthy adults ($N = 46$) were randomized to placebo or a drug that potentiates endocannabinoid signaling and responses to touch were measured.

Results: As expected, CT-optimal touch was rated as more pleasant overall (main effect of velocity: $F(1,81) = 44.5$, $p < 0.001$, partial $\eta 2 = 0.36$), though this did not differ between trauma-exposed and non-exposed individuals (touch type x group interaction: $p = 0.30$). However, this effect was mediated by levels of the endocannabinoid ligand anandamide (AEA; AEA x touch type interaction: $F(1,63)=7.13$, $p = 0.01$, partial $\eta 2 = 0.10$) such that greater AEA levels were associated with reduced preference for CT-touch ($r = -0.32$; $p = 0.01$). This was specific to ratings of the affective quality ("pleasantness") of touch, and not evident in ratings of touch intensity (touch type x group interaction: $p = 0.18$).

In study 2, we further demonstrated the role for AEA in affective touch processing by using PF-04457845 (now known as JZP150) to enhance AEA levels via reduced enzymatic degradation in healthy adults. Here, we again found that individuals with elevated AEA had no preference for affective, CT-optimal touch (group x touch type interaction: $F(1,42 = 50.9$, $p = 0.002$, partial $\eta 2 = 0.20)$). This was again specific to ratings of pleasantness and not intensity (group x touch type interaction: $p = 0.68$)

Conclusions: Overall, we find that increases in the stress-buffering endocannabinoid ligand AEA may play a critical role in mediating the perceived pleasantness of affective touch, as evidence both by individual variation in AEA levels and pharmacological intervention. This data suggests that when AEA is high, the impact of the stress buffering qualities of affective, CT-optimal touch may be minimal. As such, it is possible that individuals with lower AEA, or states that induce decreases in AEA (i.e., following stress exposure), may be particularly responsive to the stress buffering qualities of socially relevant, affective touch, providing insight into populations who may most benefit from social support via affective touch. In addition, it suggests that the endocannabinoid system may be a viable treatment target in individuals whom experience insufficient social contact.

Keywords: Affective Touch, Endocannabinoids, Childhood Trauma

Disclosure: Nothing to disclose.

P806. Lifestyle Factor Influence on TMS-Induced Excitability

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Background: Neurophysiology measures, such as motor-evoked potentials (MEPs), have been used with receptor-influencing pharmacology to test purported mechanisms of repetitive transcranial magnetic stimulation (rTMS) in humans. As such studies have been small in number and sample sizes, we sought to retest our previous findings (only ten subjects) with NMDA receptor partial agonist d-cycloserine which showed enhancement of rTMS effects with NMDA agonism. We hypothesized that d-cycloserine would again enhance rTMS excitatory effects. Additionally, MEPs are also known for their high variability, which limits our ability at times to interpret their outcomes. We accordingly gathered demographic and lifestyle information to include in our post-hoc exploratory analyses.

Methods: We conducted a double-blind crossover replication study with 10 healthy subjects whereby we gave NMDA receptor partial agonist d-cycloserine (100 mg) or placebo on separate visits. In each visit, drug was given first, followed by baseline excitability probes assessed by motor-evoked potentials (MEPs), then a 300 pulse 10 Hz rTMS "plasticity protocol" at 80% of resting motor threshold, finally followed by repeat MEPs over 1-hour. Post-hoc analysis was performed of covariate data including self-identified musicians and athletes ($n = 4$), and of self-identified caffeine users ($n = 7$). Time course data was analyzed with repeated measures ANOVA with and without drug for each covariate condition. Paired-pulse measures were analyzed with t-tests. Significance was set at $p < 0.05$.

Results: The primary outcome measure of the replication study was the 1-hour time course, and was not significant ($p = 0.12$), but was underpowered to determine a non-effect (this was a replication study which was previously significant with this sample size). However, post-hoc analysis of covariates revealed a significant enhancement of rTMS plasticity effects in musicians and athletes while taking d-cycloserine ($p = 0.03$). A separate but overlapping analysis of caffeine drinkers revealed blunting of plasticity effects in caffeine drinkers, and a marked enhancement from rTMS only in non-caffeine drinkers in the d-cycloserine condition. ($p = 0.01$). Paired-pulse measures, which can reveal underlying changes in glutamatergic or GABAergic tone, revealed enhanced intracortical facilitation, reflecting glutamatergic changes ($p = 0.02$).

Conclusions: Our underpowered primary outcome emphasizes the need for better powered motor neurophysiology experiments which are considered foundational for our mechanistic understanding of rTMS. Interestingly, our exploratory post-hoc covariate findings may reflect Hebbian and homeostatic plasticity mechanisms, induced by long-term practice and chronic caffeine intake, respectively. In addition, these extemporaneous factors may account for cortical excitability outcomes, and by extension, clinical outcomes, as they could conceivable affect rTMS effects on target neurons.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Neurophysiology, Pharmacology, NMDA Receptors

Disclosure: Nothing to disclose.

P807. Mesolimbic Dopamine Release Conveys Causal Associations

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Background: Learning to predict rewards based on environmental cues is essential for survival. It is widely believed that animals learn to predict rewards by updating predictions whenever the outcome deviates from expectations. Such violations of predictions are called reward prediction errors (RPEs) and are thought to facilitate learning. RPEs are the critical teaching signal in the most widely accepted model for cue-reward associative learning—temporal difference reinforcement learning (TDRL). Despite a few exceptions, TDRL RPE has been largely successful at explaining the activity dynamics of dopaminergic cell bodies and release in the nucleus accumbens. Hence, TDRL RPE has become the dominant theory of dopamine's role as the critical regulator of behavioral learning.

An alternative approach to learn cue-reward associations is to infer the cause of meaningful outcomes such as rewards. Since causes must precede outcomes, a viable approach to infer whether a cue causes reward is to learn whether the cue consistently precedes reward. This approach is advantageous as predicting the future is highly demanding in a cue-rich environment but inferring the cause of a rarer meaningful outcome simply requires a memory of previous experience. In other words, if an animal knows that a stimulus it just received is meaningful (e.g., a reward), it can look back in memory to infer its cause. Using this intuition, here we propose a causal inference algorithm that infers whether a cue is a cause of reward. Based on this algorithm, we denote stimuli (cues or rewards) whose cause should be learned by the animal as "meaningful causal targets" and propose that mesolimbic dopamine signals whether a current event is a meaningful causal target using an adjusted net contingency for causal relations (ANCCR) of that event in relation to other meaningful causal targets. We found that ANCCR makes similar predictions as RPE under commonly studied experimental settings. Hence, to distinguish between the two hypotheses (RPE or ANCCR signaling by mesolimbic dopamine release), we performed eight experimental tests by measuring dopamine release in nucleus accumbens core.

Methods: Eight adult wild type (C57BL/6; Jackson Laboratory) mice were used for the main experimental tests (six males). Additionally, six adult wild type mice were used to show that mice learn average reward rates in an environment with random delivery of rewards (four males). The animals were individually housed after surgery under a 12 h light/dark cycle. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the UCSF Institutional Animal Care and Use Committee.

Behavioral experiments were conducted under water deprivation, in which animals were maintained at ~85–90% of their pre-deprivation weights. Animals underwent surgery for injection of a virus causing expression of a dopamine sensor (AAVDJ-CAG-dLight1.3b) in the nucleus accumbens core. Dopamine measurements were made using photometry of dLight signals using standard frequency modulation approaches.

Trace conditioning was done with an auditory tone (3 kHz pulsing tone or 12 kHz constant tone, 75–80 dB) lasting 2 s or 8 s paired with a sucrose reward (15%, ~2.5 µL) 1 s after tone offset. This reward paired tone (CS+) was presented randomly interleaved with another tone (12 kHz constant tone or 3 kHz pulsing tone, 75–80 dB) that did not predict reward (CS−). In another experiment, random unpredictable sucrose drops were delivered with an exponential inter-reward interval at an average of once every 12 s.

We first simulated standard temporal difference reinforcement learning algorithms used to model dopamine dynamics. These included the use of a complete serial compound, microstimulus and semi-Markov state space. We compared the expected dynamics of dopamine from these models against those predicted by our ANCCR model. Using this comparison, we developed eight discriminative experimental tests that allowed us to distinguish TDRL RPE from a signaling of ANCCR. The collected data were analyzed across these conditions using standard statistical approaches.

Results: We found that mesolimbic dopamine conveys causal associations but not RPE in every case ($n = 8$ tests in $n = 7$ animals, $p < 0.05$ in each test). These included two tests using the delivery of random Poisson trains of rewards, five tests using standard variations assaying a cue-reward association, and one test in a new "trial-less" cue-reward association task. In all cases, we ruled out the standard TDRL RPE signals commonly used to model dopamine dynamics but observed that the results remained consistent with ANCCR, thereby challenging the dominant theory of reward learning in the brain.

Conclusions: In summary, we observed that the dynamics of mesolimbic dopamine release in nucleus accumbens core are inconsistent with TDRL RPE across a multitude of experiments but remain consistent with a causal learning algorithm. Our results provide a new conceptual and biological framework for associative learning.

Keywords: Dopamine, Associative Learning, Reinforcement Learning

Disclosure: Nothing to disclose.

P808. Interdigitated Visual and Personal Space-Sensitive Cortical Columns in Human Parietal Cortex: An Ultra-High Resolution fMRI Study

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Background: Personal space (PS) is the "safety zone" around the body that people prefer to maintain between themselves and unfamiliar others. Intrusion into one's PS evokes discomfort and an urge to move away. Numerous studies have described PS abnormalities in neuropsychiatric disorders such as schizophrenia and autism, suggesting that understanding the mechanisms underlying PS regulation may provide insights regarding the pathophysiology of these conditions. Physiological studies in non-human primates have shown that sensory and motor responses to stimuli that intrude into PS rely on the parietal cortex among other brain regions. However, the neural processes involved in calculating and regulating interpersonal distances remain unclear. Thus here we used ultra-high resolution (7Tesla, 1.1 mm isotropic) functional magnetic resonance imaging (fMRI) to investigate the hypothesis that the spatial encoding of interpersonal distance is transformed from purely sensory (retinotopic) to PS-related (body-centric) coordinates within human parietal cortex.

Methods: Seven healthy individuals (4 females) participated in multiple MRI scan sessions. In each session, subjects viewed one of three types of stimuli: 1) three-dimensional (3D) face stimuli (8 male and 8 female, with neutral facial expressions) that appeared to move either toward or away from (Approach vs. Withdrawal) the participant (2 sessions); 2) stationary 3D face stimuli that appeared at 9 different virtual distances from the participant, based on the participant's preferred personal space boundary (one session); 3) geometric random-dot stimuli forming a stereoscopic percept of a regular array of cuboids varying in-depth, either 'near' or 'far', relative to the fixation plane (one session). The fMRI data were analyzed using FreeSurfer (on the individual cortical surface, 14 hemispheres) without spatial smoothing. A region-of-interest (ROI) in the inferior parietal cortex was defined in each hemisphere using anatomical criteria. To test for a columnar organization of the fMRI responses, correlations were calculated between the selective activity in vertices

distributed along each of the three axes in the flattened cortical maps: X and Y within the surface-parallel cortical map, and Z along the perpendicular (radial) axis, at two different cortical depths (superficial and deep). For each of the three axes, correlation values were calculated independently for each subject and compared (following Fisher's r-to-z transformation) using paired t-tests. To measure the topographic relationships among the four main activity maps (approach-biased, withdrawal-biased, disparity-near biased and disparity-far biased), the degree of overlap of the columnar activity maps across them was calculated (as a percentage of the surface area) when the maps were correctly aligned compared to when the maps were randomly misaligned (i.e., spatially shuffled) 1000 times.

Results: In all fourteen hemispheres, activity within the parietal ROI showed a significantly higher correlation in the Approach-vs.-Withdrawal functional contrast when sampled along the radial axis, compared to functional variation along either of the surface-parallel axes (superficial layers: $t = 11.38$, $p < 0.001$; deep layers: $t = 16.82$, $p < 0.001$). Similarly, comparisons across maps sampled at different cortical depths revealed that disparity-selective Near and Far- biased clusters of activity in parietal cortex were also radially elongated, exhibiting a columnar organization. Lastly, the overlap analyses showed that maps of the PS-sensitive columns were systematically interdigitated (highly non-overlapping across multiple thresholds) with the disparity-sensitive columns in the parietal cortex ROI.

Conclusions: Across these experiments, two types of distance encoding were identified in two corresponding columns of activity within human parietal cortex. One category of columns responded selectively to moving and stationary face images presented at virtual distances that were nearer (but not further) than each subject's behaviorally-defined PS boundary. In the majority of these columns, BOLD response amplitudes increased monotonically and nonlinearly with increasing virtual face proximity (an approach bias). In a complementary subset of these columns, BOLD responses decreased with increasing proximity (a withdrawal bias). A second set of columns responded selectively to disparity-based distances (near or far) in random-dot stimuli, similar to disparity-selective columns described previously in occipital cortex. These results suggest that the transformation of spatial information, from visual to body-centric, may be computed within the parietal cortex via communication across short-range projections between columns. In future studies, similar approaches could be applied to determine whether the PS system, and the laminar organization of association cortices more broadly, are altered in neurodevelopmental disorders.

Keywords: Parietal Cortex, High Resolution fMRI, Cortical Columns, Personal Space, Depth Perception

Disclosure: Nothing to disclose.

P809. Behavioral Economics of Striatal Dopamine

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Background: Although striatal DA release is crucial for reward learning and decision-making, prior studies have disagreed over its role encoding costs, benefits, and motivation. Manipulations of DA function with lesions or pharmacology imply that striatal DA primarily mediates cost calculations; that is, how much effort an animal exerts to obtain rewards. However, these studies often confounded cost and benefit by varying both simultaneously, and used tools that could not examine how DA activity dynamics underlie these behaviors. In contrast, electrophysiological studies of DA activity dynamics suggest that striatal DA more reliably encodes benefit, not cost. These studies, however, were correlational and did not exploit causal tools to explore how DA release influences behavior. Furthermore, DA neuron recording studies have rarely explored changes in motivational state between individuals or across time except in studies of addiction, where there is debate over whether striatal DA release is sensitized or depleted once individuals transition to a highly-motivated, addicted state.

Methods: We established a simple operant task that independently varies costs and benefits to generate behavioral economic demand curves in response to sucrose rewards. During a single session, the fixed ratio (FR, or "cost" of reward) varied in 10-minute bins over 50 minutes, while the reward "benefit" remained identical. Between sessions, we varied the concentration and quantity of sucrose reward, such that mice experienced four different "benefits". By plotting reward consumption across cost for each session, we extracted a quantitative metric of motivation, defined by the willingness to overcome cost, independently from reward dose. At the same time, we measured DA release in both the nucleus accumbens (NAc) and dorsolateral striatum (DLS) using the genetically-encoded sensor GRAB-DA, allowing us to monitor how striatal DA release reflects costs, benefits, and motivational state. In a second cohort of mice, we used the excitatory opsin ChRMINE to stimulate DA inputs to the NAc or DLS while simultaneously recording DA release. These mice performed the identical task, except instead of working for sucrose reward, they worked for optogenetic stimulation of DA release. Finally, in a third cohort of mice, we used the inhibitory opsin NpHR3.0 to inhibit DA inputs to the NAc or DLS while mice worked for sucrose reward. In total we recorded from 84 mice (39 female).

Results: Mice successfully learned the task, increasing the number of rewards they earned in each session (Friedman test, $P = 0.0002$) and decreasing their latency to consume each reward (Friedman test, $P = 0.0026$). As expected, mouse behavior was sensitive to both reward benefit (Mixed-effects model, fixed effect of quantity, $F1,11 = 17.9$, $P = 0.0014$; fixed effect of concentration, $F1,11 = 176$, $P < 0.0001$) and cost (Friedman test, $P < 0.0001$). Examining DA release aligned to the reward-predicting cue, we found that increased sucrose concentration (Wilcoxon signed rank test, $P = 0.024$) and quantity (Wilcoxon signed rank test, $P = 0.003$) enhanced DA release in both regions. Surprisingly, increased cost also enhanced DA responses in both regions, despite the cue and reward being held constant (Friedman test, NAc: $P = 0.0043$; DLS: $P < 0.0001$). We then generated demand curves for each session, and took advantage of daily variation in subjects' performance to determine how striatal DA release relates to motivation. Strikingly, we found an inverse relationship between DA release and motivation level. In sessions when mice were more motivated for a given reward, less DA was released in response to fixed rewards in both striatal regions (Mann-Whitney test; NAc: $P = 0.001$; DLS: $P = 0.0049$). Furthermore, mice with higher average motivation levels exhibited lower average DA responses in both ventral and dorsal striatum (Spearman correlation; NAc: $P = 0.048$; DLS: $P = 0.028$). When we repeated all the above experiments with optogenetic stimulation of DA inputs, we found the same results. As cost increased, optogenetically-evoked DA release in both the NAc (Friedman test, $P = 0.0005$) and the DLS (Friedman test, $P < 0.0001$) increased, despite using identical light stimulation parameters. Furthermore, optogenetically-evoked DA release was greater during low motivation sessions in both the NAc (Mann-Whitney test, $P = 0.0061$) and DLS (Mann-Whitney test, $P = 0.0038$), and this inverse relationship between motivation and optogenetic striatal DA release held both within and between subjects. Finally, we tested the causal relationship between motivation

and striatal DA release in two ways. First, we reduced subjects' motivation for sucrose by prefeeding them with sucrose before each session. We found that this manipulation increased striatal DA release to the same reward (Wilcoxon signed-rank test; NAc: $P = 0.016$; DLS: $P = 0.016$). Second, we used optogenetics to inhibit DA release for sucrose reward, and found that this inhibition increased motivation (Wilcoxon signed-rank test; NAc: $P = 0.031$; DLS: $P = 0.031$). In all experiments, we did not observe any behavioral or neural differences between sexes.

Conclusions: We reveal that striatal DA release integrates benefit and sunk cost to encode value, and surprisingly, that high motivation dampens these signals. These findings reconcile discrepancies between prior studies on DA and help clarify the role of striatal DA signals in motivated behavior.

Keywords: Striatal Dopamine Signaling, Behavioral Economics, Circuit Optogenetics, Intrinsic Motivation, Effort-Cost Benefit Task

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