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P551. Impaired Filtering and Hyperfocusing: Neural Evidence for Distinct Selective Attention Abnormalities in People With Schizophrenia

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Background: Although schizophrenia is classically thought to involve impaired attentional filtering, people with schizophrenia (PSZ) exhibit a more intense and more exclusive attentional focus than healthy control subjects (HCS), referred to as hyperfocusing, in many tasks. A potential explanation for this contradiction relates to the distinction between control and implementation of selective attention. Control processes steer the attentional focus to the relevant sources of information, while implementation of selective attention refers to the actual amplification of the selected source relative to others. Selective attention may be impaired because processing resources are directed toward the wrong input, reflecting control deficits. Previous findings indeed suggest attentional control deficits in PSZ. However, when control processes are not a limiting factor, stronger implementation of selective attention may result in a more intense and exclusive focus in PSZ. We hypothesized that PSZ would exhibit impaired selective attention when control demands were high, but hyperfocusing when control demands were low.

Methods: Functional Magnetic Resonance Imaging (fMRI) was employed to study attentional gain within higher-order visual processing regions referred to as fusiform face area (FFA) and parahippocampal place area (PPA), which are specialized for face and spatial scene processing, respectively. Forty-three PSZ and 43 HCS underwent fMRI while responding to face and house stimuli. Stimulus-induced activation in FFA and PPA was analyzed as a function of whether participants were looking for a pre-defined target face or target house. To test the impact of attentional control demands on the attentional modulation of FFA and PPA BOLD activity, stimuli were presented either individually (Sequential condition), or concomitantly, as semitransparent face-house overlays that challenged attentional control (Overlay condition). Activation was studied in FFA and PPA regions of interest defined by an independent functional localizer on an individual-subject basis.

Results: Coordinates of FFA ROIs did not differ between PSZ and HCS. PPA ROIs on average tended to be localized somewhat more posterior and inferior in PSZ than HCS. Importantly, the ROIs' sensory face-house discrimination did not differ between groups, for neither localizer nor task stimuli, which speaks against group differences in the basic functioning of these regions.

Task responses were slower for house than for face stimuli in the Sequential conditions, and slower when prioritizing houses over faces in the Overlay condition, suggesting a difference in salience between stimulus dimensions. Target detection in PSZ was most impaired in the Overlay condition, and least (not at all) impaired when responding to face stimuli in the Sequential condition, following attentional control demands.

BOLD activity reflected poorer attentional selectivity in PSZ than HCS when attentional control was challenged most, that is, when faces and houses were overlaid and the task required detecting the lower-salience house target. Specifically, HCS but not PSZ displayed larger PPA activation to overlay stimuli when the house dimension was attended than when the face dimension was attended [interaction of group x attention: $F(1,81) = 4.12$, $P = 0.046$]. By contrast, attentional selectivity was exaggerated in PSZ when control was challenged least, that is, when stimuli were presented sequentially and the task required detecting the higher-salience face target. Specifically, PSZ but not HCS displayed larger FFA activation to face stimuli when the face dimension was attended than when the house dimension was addended [interaction of group x attention: $F(1,81) = 4.54$, $P = 0.036$]. Time course analyses revealed that these effects were not due to group differences in the timing of the hemodynamic response. Thus, hyperactivation in PSZ did not reflect prolonged stimulus engagement.

Conclusions: The findings are consistent with two distinct attentional abnormalities in schizophrenia leading to impaired and exaggerated selection under different conditions: attentional control deficits, and stronger implementation of selective attention, or hyperfocusing, once attention has been directed toward a stimulus.

Keywords: Schizophrenia (SCZ), Selective Attention, Brain Imaging, fMRI, Fusiform Face Area (FFA), Parahippocampal Place Area (PPA)

Disclosure: Nothing to disclose.

P552. Abnormal Delta-Gamma Phase Amplitude Coupling in Patients With Schizophrenia at Rest

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Background: Human brain functions, including perception, attention, and other higher-order cognitive functions, are supported by neural oscillations necessary for the transmission

of information across neural networks. Previous studies have demonstrated that the rhythmic firing of neural populations is not random but is governed by interactions with other frequency bands. Specifically, the amplitude of gamma-band oscillations is associated with the phase of lower frequency oscillations in support of short and long-range communications among networks. This phase-amplitude coupling (PAC) is thought to reflect the temporal coordination of neural communication. While schizophrenia patients show abnormal oscillatory responses across multiple frequencies at rest including gamma-band oscillation, it is unclear whether the functional relationships among frequency bands are intact. We aimed to characterize the lower frequency (delta/theta, 1-8 Hz) phase and the amplitude of gamma oscillations in healthy subjects and patients with schizophrenia at rest.

Methods: Low frequency-phase (delta- and theta- band) angles and gamma-band amplitude relationships were assessed in 142 patients with schizophrenia and 128 healthy comparison subjects. We characterized resting-state PAC in patients with schizophrenia and healthy comparison subjects via a modified method that does not use the gamma amplitude as a scalar in calculating PAC, selecting the highest 5 % data of gamma power to test for PAC, thus avoiding the confound of group differences in baseline gamma.

Results: Significant delta-gamma PAC was detected across broadly distributed scalp regions in both healthy subjects and patients with schizophrenia. Delta-gamma PAC was significantly decreased at frontocentral, right middle temporal, and left temporoparietal electrodes but significantly increased at a left parietal electrode in patients with schizophrenia.

Conclusions: Delta-gamma PAC may reflect a core pathophysiologic abnormality in schizophrenia. Data-driven measures of functional relationships among frequency bands may prove useful in the development of novel therapeutics. Future studies are needed to determine whether these alterations are specific to schizophrenia vs. evident in other neuropsychiatric patient populations.

Keywords: Electroencephalography, Gamma Oscillations, Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P553. A Potent and Highly Selective VPAC2 Receptor Antagonist Peptide KS-133 Counteracts Cognitive Impairment in a Mouse Model Relevant to Schizophrenia

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Background: Clinical studies have shown that microduplications at 7q36.3, containing VIPR2, confer significant risk for schizophrenia and autism spectrum disorder (ASD). VIPR2 gene encodes the VPAC2 receptor, a seven transmembrane heterotrimeric G protein-coupled receptor (GPCR), for vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP). Lymphocytes from patients with these microduplications exhibited higher VIPR2 gene expression and VIP responsiveness (cAMP induction). These findings provide a genetic evidence for a specific receptor-mediated (and potentially drug targetable) signaling pathway linked to schizophrenia and ASD that is distinct from dopaminergic, glutamatergic, and serotonergic systems. There are other PACAP receptor subtypes than VPAC2, namely VPAC1 and PAC1, and all these receptors belong to class-B GPCRs. The PAC1, VPAC1, and VPAC2 receptors have moderate amino acid sequence similarities (about 50%) with each other and highly three-dimensional structural homology. Therefore, these molecular features have made it difficult to discover VPAC2 receptor-selective small molecule drugs. In 2018, Sakamoto et al. discovered an artificial 16-mer cyclic peptide VIpep-3: Ac-c(CPPYLPRRLC)TLLLRS-OH antagonizing human and rodent VPAC2 receptor signal pathways in vitro (Biochem. Biophys. Res. Commun. 503: 1973–1979, 2018). However, this peptide comprises all-natural amino acids and has high susceptibility to degradation by proteases. In vivo efficacy of this VPAC2 receptor antagonist also remains to be determined in animal models of psychiatric disorders such as schizophrenia. The aim of this study was to generate a VIpep-3 derivative for in vivo experiments.

Methods: In vitro antagonist activities of candidate peptides against VIP-VPAC1, VIP-VPAC2, and PACAP-PAC1 signaling pathways were analyzed by calcium influx assay. In vivo VPAC2 antagonistic activity was evaluated by western blot analysis. The cognitive function of mice was determined using the novel object recognition test. Golgi-Cox staining was used to visualize neurons for analysis of dendritic morphology. Experimental procedures that involved animals and their care were conducted in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), and all animal experiments were approved by the Animal Care and Use Committees at Hiroshima University (#A20-115) and Osaka University (#30-3-2). Pregnant lCR (CD1) mice at 16 days of gestation and male C57BL/6J mice at 7 weeks of age were purchased from Japan SLC Inc. (Shizuoka, Japan).

Results: Using existing structural information of the extracellular domain of VPAC2 (PDB ID: 2X57), the C-terminal structure of VIP (PDB ID: 2RRI), and docking model of VPAC1/VIP (Couvineau et al. Front. Endocrinol. 3:139, 2012), we constructed a molecular design concept. After amino acid substitution and structure optimization of VIpep-3, we successfully generated a novel peptide KS-133 with a VPAC2-selective and potent antagonistic activity and at least 24 h of stability in plasma. KS-133 had IC50 value of 24.8 nM against VPAC2, which was stronger antagonistic activity than parental VIpep-3 (40.6 nM), even though the molecular weight was reduced from VIpep-3 (1941.1 g/mol) to KS-133 (1558.8 g/mol). KS-133 did not antagonize VIP-VPAC1 and PACAP-PAC1 signaling pathways up to 5 μM. Subcutaneous and intranasal administration of KS-133 suppressed the VPAC2 activation-induced increase in CREB phosphorylation in the prefrontal cortex of neonatal and adult mice, respectively. We also found that repeated administration of Ro 25-1553, a selective VPAC2 agonist, during postnatal days 1-14 in mice caused cognitive impairment in adulthood and simultaneous treatment with KS-133 prevented this effect. The same postnatally restricted Ro 25-1553 treatment reduced the total branch number and length of apical and basal dendrites of the prefrontal cortex neurons in mice. These morphological abnormalities were counteracted by concomitant administration of KS-133 with Ro 25-1553.

Conclusions: We generated KS-133 that possessed drug-like properties as follows: (a) high selectivity and a potent antagonist activity against VPAC2; (b) remarkable resistant to protease degradation, a common problem with peptides; (c) prevention of cognitive decline in a pharmacological model of early postnatal VPAC2 overactivation, a relevant mouse model of schizophrenia. Our study is not only the first validation of the effects of a VPAC2 antagonist in disease animal models, but also a good example of drug discovery for class-B GPCRs.

Keywords: Schizophrenia Novel Treatment, GPCR, VPAC2 Receptor Antagonist, PACAP, Cyclic Peptide

Disclosure: Nothing to disclose.

P554. The Effect of Instability of Baseline Severity of Positive Symptoms on End of Treatment Change in the PANSS Negative Factor Score in Negative Symptom Schizophrenia Studies – An Exploratory Post-Hoc Analysis

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Background: High severity of positive symptoms is often an exclusion criterion in schizophrenia negative symptom clinical trials and commonly such studies do not allow subjects with the positive factor score above 22 to be randomized. There is a general understanding from the work of Dunayevich et al. 2014 and others that more severe positive symptoms decrease the level of improvement seen in the unadjusted PANSS negative subscale (Dunayevich, et al. 2014. In the current retrospective analysis, we assessed the impact of instability in the baseline positive symptom severity between screening and baseline on end of treatment change in negative factor score in blinded data collected across a number of negative symptom clinical trials.

Methods: Baseline data were pooled from six negative symptom schizophrenia clinical trials. The severity of baseline positive symptoms was derived from the PANSS positive subscale and the PANSS positive factor score. Change in the PANSS positive subscale and PANSS positive factor score between screening and baseline were calculated. Using regression analyses, correcting for baseline severity of negative symptoms and study, we assessed the impact of positive symptom severity – separate analyses for the PANSS positive subscale and PANSS positive factor, and the impact of positive symptom change between screening and baseline – separate analyses for the PANSS positive subscale or PANSS positive factor on end of treatment change in PANSS negative factor score change in the pooled blinded data. Given the exploratory nature of the analyses we did not correct for multiple testing.

Results: Our dataset consisted of data collected from a total of 2,474 subjects. The mean(sd) baseline severity of baseline PANSS positive scores was 13.2(3.4) points but varied significantly between the individual studies ($p < 0.001$). The mean(sd) PANSS positive factor score was 17.3(3.5) and varied significantly between the individual studies ($p < 0.001$). The mean change in the PANSS positive subscale between screening and baseline was -0.15(1.6) points and the mean change in the positive factor score was -0.08 (1.7), no differences were identified between the studies ($p > 0.05$). The change from baseline to last visit in the negative factor score was significantly reduced by increasing severity of the baseline PANSS positive subscale and PANSS positive factor scores, respectively. Change in the positive symptom subscale but not in the positive factor score significantly reduced the last visit change in the negative factor score (all $p < 0.05$).

Conclusions: Our data confirm the detrimental impact of baseline positive symptom severity on the last visit change from baseline in PANSS negative factor score in the pooled dataset of blinded data collected across a large number of subjects. A 1 point increase in the PANSS positive subscale translated into a reduction of improvement by approximately 0.08 points and a 1 point increase in the PANSS positive factor score into a reduction by approximately 0.15 points. A 1 point worsening in the PANSS positive subscale between screening and baseline significantly reduced the improvement in the negative factor score by 0.13 points. In contrast, a 1 point worsening in the positive factor reduced the negative factor last visit change by 0.08 points, which did not reach statistical significance. This difference is somewhat puzzling and we are planning additional analyses to identify whether it stems from any of the unique items that comprise the positive subscale and the positive factor score. Our findings need to be interpreted with caution. The absence of treatment allocation data did not allow for an unblinded analysis and therefore we cannot address the most critical question, whether or not the severity of positive symptoms and their change between screening and baseline impacts signal detection.

Keywords: CNS Clinical Trials, Negative Symptoms, Positive Symptoms, Schizophrenia

Disclosure: Signant Health: Employee (Self)

P555. Development of the California Collaborative Network to Promote Data Driven Care and Improve Outcomes in Early Psychosis (EPI-CAL): Preliminary Qualitative and Quantitative Findings

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Background: Team-based "coordinated specialty care" (CSC) for early psychosis (EP) is highly effective in promoting clinical and functional recovery. NIH's EPINET project (https://nationalepinet.org/) seeks to join EP programs across the US to facilitate large-scale data collection and analysis to promote rapid dissemination of best practices. As an initial EPINET hub, California's EPI-CAL project (https://epical.ucdavis.edu) joined 6 university- and 7 county-based EP programs to create a sustainable learning healthcare network and contribute de-identified data to the NIMH EPINET database. Initial goals focused on: 1) developing a core battery of evidence-based measures based on qualitative analysis of stakeholder input, 2) co-designing a data collection platform (Beehive) for use by EP programs, and 3) implementing Beehive in 3 pilot EP programs to collect baseline data on 70% of eligible EP clients.

Methods: Semi-structured qualitative focus groups with stakeholders (e.g. clients, family members, EP program staff/leadership, county/state representatives) were completed to: 1) explore stakeholder opinions on the domains of outcomes data and method of collection within the EPICAL network; 2) obtain feedback on the design, flow, and functionality of the Beehive application and its dashboard; and 3) determine how best to share core aspects of EPI-CAL and the Beehive platform (e.g., purpose of data collection, security and data sharing) with users to support informed participation and data-sharing decisions. Focus group data was analyzed utilizing a mixed-methods design, incorporating both qualitative and quantitative methods when possible. Beehive was then implemented in 3 pilot EP programs to determine feasibility and acceptability to staff, clients and families. Descriptive analyses summarize the data collected to date.

Results: 1) A review of core outcomes domains from the research literature and evidence-based measures from the PhenX toolkit (https://www.phenxtoolkit.org/) were presented for stakeholder consideration. Stakeholders reviewed all possible outcome domains and measures via 23 focus groups and four interviews ($N = 189$) between 10/08/2019 and 11/03/2020. Results supported inclusion of a broad range of client-centered outcomes and focused on client self-report as the primary approach to data collection to facilitate client engagement and reduce data burden for clinicians. Results were shared with EPINET to support development of its core assessment battery for all US EP programs. 2) Between 3/26/2020 and 5/18/2020, 14 stakeholder focus groups ($n = 82$) reviewed digital storyboards of the Beehive

application and dashboard. Participants provided feedback related to the look/feel of the application, visualizations of data, and how to best structure the application for various user roles. 3) 6 focus groups with stakeholders ($n = 24$) were conducted between 8/2020 and 1/2021, in two parts: to share experiences and preferences around data sharing in general and around their mental health and, based on these initial focus groups, provide feedback how Beehive presents information on data sharing. Participants watched an informational video created by the research team, which showed key points on Beehive's purpose, data security, the concept of a limited data set, who data will be shared with, and how users may opt-in or opt-out to data-sharing for research purposes. Participants appreciated the ability to opt out of data sharing at their discretion and agreed that the video increased their understanding of the project, application, and how their information will be stored for each data sharing permission level. This enhanced their willingness to share a limited data set for research. Feedback shaped the final video content, including a longer section on Beehive's potential benefits to the individual, state and country to increase buy in for data sharing. To date, 27 individuals have completed registration in Beehive. Of that group, 81.48% ($n = 22$) have agreed to share their data with the NIMH EPINET data base and 77.78% ($n = 21$) have agreed to share their data with EPI-CAL (52.38% female sex, 38.10% Latinx, 80.95% Minority, 61.90% first episode/4.76% clinical high risk).

Conclusions: Stakeholder input contributed to the development of a core assessment battery that addressed client-level outcomes relevant to all stakeholder groups, which increased motivation for the project among participating sites. Co-design of the Beehive platform ensured the technology meets provider needs across domains. Providing users with clear information on the purpose of the project, how data security is maintained, and options for data sharing outside of the clinic increased participants' knowledge, trust and willingness to share data outside of the clinic. Participants approved of the video format with clear and simple language to present this information. Changes to this video were made with feedback from participants to enhance buy-in and comprehension. Pilot data collection in 3 sites demonstrated preliminary feasibility of the data collection platform and allowed identification of challenges and solutions early in the implementation process, such as the need for clear workflow integration. Data collection is expanding to the remaining EPICAL sites and results from an increased sample will be reported.

Keywords: Early Psychosis, Learning Health System, Patient Outcomes, Technology, Relevance for Outcomes

Disclosure: Safari Health, Inc.: Founder (Self)

P556. Feasibility and Acceptability of Telehealth Community Reinforcement and Family Training (CRAFT) for Substance Use and Early Psychosis

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Background: Approximately 50% of people with early psychosis have a history of problems with substance use and many endorse recent cannabis, alcohol, and nicotine use. Co-occurring substance use significantly increases the likelihood of treatment disengagement and dropout. Community Reinforcement and Family Training (CRAFT) aims to increase clients' readiness to change their substance use, decrease families' distress, and improve client-family relationships. CRAFT topics include building motivation and self-care, communication, functional analysis of substance use, positive reinforcement of healthy/recovery-oriented behaviors, discussing treatment engagement, allowing for negative consequences, and problem solving. The CRAFT protocol has not targeted families of individuals with early psychosis, nor has it been adapted for administration via a telehealth platform (e.g., Zoom) prior to this study. The current project aims to develop and evaluate the feasibility and acceptability of a telehealth coaching intervention adapting CRAFT to improve treatment engagement and reduce distress among families of individuals diagnosed with early psychosis (EP) and co-occurring substance use.

Methods: Participants were family members ($N = 21$) of a relative with early psychosis (first episode in the past 6 years) and a history of substance use (cannabis, alcohol, nicotine in the past 90 days or no immediate interest in abstinence). The family members completed the intervention, which consisted of six to eight ~60-minute coaching sessions of CRAFT modified for psychosis and delivered via telemedicine (CRAFT-PT) by a study coach. Participants completed an assessment battery four times: pre-treatment, mid-treatment, post-treatment, and a three-month follow-up. At pre-treatment, participants completed the Structured Clinical Interview for DSM-5 (SCID) to characterize the sample. Families also completed a survey following each session to rate their satisfaction with treatment (average of how helpful and convenient the session was from 0=poor to 5=excellent), they also rated whether they experience technical difficulties (yes/no). After 10 families completed the program, participants completed focus groups, and we reviewed session feedback, participation, and retention data to guide further treatment development. Testing with the revised manual (now eight sessions) is nearing completion with 10 new family members. The percentage of session attendance and descriptive statistics of treatment satisfaction ratings will be used to evaluate the feasibility and acceptability of the intervention.

Results: As of August 5, 2021, of the family members who started coaching ($N = 20$: 18 mothers, 1 father, 1 sister), 75% had a DSM-5 diagnosis (e.g., major depressive disorder, mild alcohol use disorder, etc.). All had 100% session attendance, and they rated treatment satisfaction across sessions as being near excellent (mean(SD) = 4.85(0.32)) with 75% of sessions rated excellent despite experiencing technical difficulties in 20% of sessions. Of those who completed the program, participants reported 60-65% improvement in their current problems, and 100% of participants would recommend the program to others.

Conclusions: Preliminary data suggest that the CRAFT-PT protocol is highly feasible and acceptable to serve as the active treatment in a pilot randomized controlled trial comparing treatment as usual plus CRAFT-PT to treatment as usual alone. Given that 95% of participants who engaged in coaching were women and the majority endorsed having a DSM-5 diagnosis, our results also highlights the need to target support for female caregivers of people with co-occurring disorders and identify new ways to engage male caregivers.

Keywords: Early Psychosis, Substance Abuse, Telemedicine

Disclosure: Nothing to disclose.

P557. Kynurenic Acid Elevation Elicits Sex-Dependent Deficits in Sleep Spindle Dynamics

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Background: Sleep spindles are neural oscillations which occur during non-rapid eye movement (NREM) sleep within the 10-15 Hz frequency range for rodents and the 12-16 Hz frequency range for humans. Initiated by the thalamic reticular nucleus, sleep spindles are critical for synaptic plasticity associated with the integration of new information. NREM sleep spindle aberrations are commonly associated with psychotic disorders such as schizophrenia (SZ) and

bipolar disorder (BD) and are linked to deficits in procedural memory and cognition. Kynurenic acid (KYNA), a tryptophan metabolite synthesized from kynurenine via kynurenine aminotransferases (KATs) and glial-derived modulator of glutamatergic and cholinergic transmission, is elevated in the brain of patients with psychotic disorders. To further understand the role of KYNA in psychotic disorder etiology, we utilized two experimental systems. With embryonic kynurenine (EKyn) exposure, KYNA is elevated during the prenatal period, and brain KYNA levels are elevated in adult male EKyn but not female EKyn. With acute kynurenine challenge during adulthood, brain KYNA levels are transiently increased in both sexes. Taken together, we presently examined if sleep spindle dynamics are negatively influenced when KYNA levels are elevated.

Methods: Sleep spindle dynamics were evaluated during zeitgeber time (ZT) 0 to ZT 4, the first four hours of the light cycle, wherein rats readily accumulate NREM sleep. Male and female Wistar rats were implanted with telemetric transmitters to acquire polysomnographic recordings which combine electroencephalogram (EEG) and electromyogram (EMG) signals and activity levels. In Experiment #1, pregnant dams were fed chow laced with 100 mg kynurenine (EKyn) or control (ECon) wet chow from embryonic day (ED) 15 to ED 22. Sleep behavior was evaluated in young adult, postnatal day (PD) 56, offspring ($N = 7$-9 per group). In Experiment #2, adult rats were challenged acutely with kynurenine (100 mg/kg; i.p.) at ZT 0 ($N = 10$-14 per group). Manual analysis of sleep spindles during NREM was performed by applying a 10-15 Hz band pass filter to the EEG signal and identifying spindles by rectifying the band pass filtered signal with a cubed root mean square transform, within a 750 ms window.

Results: Male EKyn offspring exhibited a 32% decrease in NREM sleep spindle density compared to ECon (ECon: 8.4 ± 0.3 spindles/min; EKyn: 5.7 ± 0.5 spindles/min; $^{***}P < 0.001$). Average spindle duration (ECon: 1.2 ± 0.04 sec; EKyn: 1.1 ± 0.06 sec; $P > 0.05$) and peak spindle frequency remained between 11.5 to 12 Hz for both groups. NREM sleep spindle density remained unchanged in female EKyn rats (ECon: 10.1 ± 0.5 spindles/min; EKyn: 10.4 ± 0.5 spindles/min; $P > 0.05$) and the average spindle duration was also unchanged (ECon: 1.1 ± 0.02 sec; EKyn: 1.1 ± 0.04 sec; $P > 0.05$). In the females, peak spindle frequency was between 12 to 12.5 Hz for both groups. Preliminary analysis of sleep spindles ($N = 3$) in the acute kynurenine challenge study demonstrates a trend in reduced sleep spindle density (main effect of kynurenine treatment, $P = 0.16$).

Conclusions: The results from this study investigate the impacts of prenatal KYNA elevation and acute KYNA elevation, evaluating differences between long-lasting and transient changes in KYNA, on sleep spindle dynamics. We determined conspicuous sex differences, wherein sleep spindle dynamics are different between male and female subjects, and only male EKyn offspring have deficits in sleep spindles compared to counterpart controls. Future work will consider the mechanistic efficacy of KYNA synthesis inhibition using the compound PF-04859989, a KAT II inhibitor, to ameliorate NREM sleep spindle dysfunction provoked by KYNA elevation.

Keywords: Kynurenic Acid, Sleep Spindles, Psychotic Disorders, Polysomnography

Disclosure: Nothing to disclose.

P558. Microglia-Mediated Mechanisms Underlying the Synergistic Impact of Adolescent Cannabis Exposure and CNVs Conferring the Risks of Psychiatric Disorders on Brain Maturation and Cognitive Outcomes in Adulthood

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Background: Considering recent marijuana legalization in the US, the deleterious effects of cannabis use during adolescence, a critical period for prefrontal cortex (PFC) maturation, has gained further attention as an environmental factor increasing the risk for psychiatric disorders such as schizophrenia. Importantly, most cannabis users do not develop psychiatric symptoms, suggesting that cannabis exposure may be an environmental risk factor in individuals genetically predisposed to psychiatric disorders. Previous study showed the effect of delta-9-tetrahydrocannabinol (THC), a major psychoactive ingredient of cannabis on brain function, is mainly mediated by the cannabinoid receptor type 1 (CNR1) that is widely distributed in the presynaptic regions of various types of neurons and regulates retrograde synaptic suppression, thereby controlling cognitive and emotional behaviors. Intriguingly, recent studies highlighted the importance of CNR1 expressed in astrocytes, causally mediating THC-induced cognitive deficits. However, adolescent cannabis effect on microglia remains elusive. In this study, we investigate the impact of adolescent THC exposure on microglial function of which disturbance may be exacerbated by genetic insults conferring the risk of psychiatric disorders, leading to aberrant PFC maturation and producing adult pathophysiology.

Methods: C57BL/6 mice (both sex) were chronically exposed to THC (8.0 mg/kg) during adolescence by single daily subcutaneous injections, followed by immunohistochemical and biochemical assays to determine the effect of adolescent THC exposure on microglia. The THC doses were chosen based on our pilot tests and prior studies. We also examined whether adolescent THC treatment and 16p11.2 duplication (16p11dup), a copy number variation (CNV) associated with psychiatric disorders, synergistically impair microglial function, resulting in disturbance of PFC neuronal function and cognitive behaviors in adulthood.

Statistical significance for behavioral, biochemical, morphological and behavioral phenotyping data was assessed by two-tailed unpaired Student *t*-test with THC and vehicle treatment and two-way ANOVA with 16p11dup and control mice as well as THC and vehicle treatment as independent factors. Significant effects and interactions were followed up with multiple comparison testing with post hoc Bonferroni tests. The sample size was based on power analyses. A value of $p < 0.05$ was considered statistically significant.

Results: Adolescent THC treatment induced medial prefrontal cortex (mPFC)-specific microglial apoptosis via CNR1-mediated mechanisms ($p = 0.0001$, $t = 10.97$, $\mathrm{df} = 10$). These microglial phenotypes were specifically exacerbated by 16p11dup predisposition, leading to reduction of intrinsic excitability of a specific type of mPFC pyramidal neurons and abnormalities in social novelty recognition (16p11dup x THC interaction ($F1,49 = 6.392$, $p = 0.0147$) and memory ($F1,30 = 5.665$, $p = 0.0239$), causally mediated by microglial CNR1. Microglia-specific RNA-seq based gene expression profiling identified a key mediator for convergent effect of adolescent THC treatment and 16p11dup ($n = 4$-6 per groups, $p < 0.05$). We are currently investigating intracellular molecular communication between mPFC microglia and neurons that may mediate the synergistic effects of adolescent THC exposure and 16p11dup interaction.

Conclusions: We demonstrate that adolescent THC exposure induces mPFC-specific microglial apoptosis via CNR1-mediated mechanisms. Adolescent THC exposure and 16p11dup synergistically produce CNR1-mediated microglial apoptosis, leading to disturbance of mPFC neuronal maturation and social memory deficits. Our results indicate that modeling cell type-specific mechanisms of rare genetic risks for psychiatric disorders and adolescent environmental factors may contribute to better understanding of disease pathophysiology.

Keywords: Cannabis, Microglia, Apoptosis
Disclosure: Nothing to disclose.

P559. Olanzapine- and Haloperidol-Induced Changes in Hypothalamic Kinase Activity

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Background: Background: Second generation antipsychotics, such as olanzapine, are associated with significant metabolic adverse effects contributing to states of obesity and insulin resistance, which occur at exceedingly high rates in patients with psychosis spectrum illnesses. Independently of adiposity changes, antipsychotics dysregulate whole body glucose metabolism, via a mechanism directly connected to the central nervous system (CNS). Following a meal, maintenance of glucose homeostasis requires suppression of glucose production by the liver in the presence of increased glucose concentrations. It has been established that postprandially, the hypothalamus senses elevated glucose levels, leading to activation of hypothalamic ATP-sensitive potassium (KATP) channels, signaling the liver to suppress hepatic glucose production. Disruptions in these CNS-hypothalamic pathways result in dysglycemia and insulin resistance. Kinases upstream to KATP channels also modulate this central glucose sensing pathway. The objective of the current study was to identify olanzapine- and haloperidol-induced changes in hypothalamic protein kinase activity, particularly as it pertains to established modulators of KATP channels, in parallel with assessment of in vivo glucose kinetics.

Methods: Methods: Gold-standard, pancreatic euglycemic clamps were used to assess changes in glucose kinetics in response to a primed, continuous intracerebroventricular (ICV) infusion of glucose (2 mM, approximating postprandial CNS concentrations) or vehicle solution (5µl/hour, into the 3rd ventricle). Immediately before starting the clamps, male, Sprague Dawley rats were co-treated with acute subcutaneous injections of olanzapine (3 mg/kg), haloperidol (0.25 mg/kg), or vehicle with dosing based on clinical D2 occupancies. $N = 6$-9/group. Following euthanasia, the hypothalamus was harvested. Kinome array analyses of hypothalamus samples consisted of determining the degree to which samples phosphorylated the serine/threonine residues of reporter peptides on the PamChip (PamGene International B.V), followed by a random sampling analysis-based deconvolution of protein kinase peptide assignments using kinase-substrate databases such as GPS 3.0, Kinexus Phosphonet (Kinexus Bioinformatics), PhosphoELM, and PhosphoSite Plus.

Results: Results: As expected, ICV (central) glucose infusion suppressed hepatic glucose production ($p < 0.05$) versus vehicle, an effect abolished by treatment with olanzapine, but not haloperidol. In parallel, ICV glucose stimulated the activity of various kinases in the hypothalamus, including extracellular signal-regulated kinase (ERK), which is known to activate KATP channels. Haloperidol also increased the activity of kinases that can activate KATP channels, including ERK, cAMP-dependent protein kinase (PKA), and cGMP-dependent protein kinase (PKG) (Z score > 2, outside the 95% CI), while olanzapine decreased their activity (Z score > 2, outside the 95% CI). It appears that haloperidol- and olanzapine-induced changes in kinase activity may have primed the hypothalamus for or against suppression of hepatic glucose production, respectively, in the presence of ICV glucose.

Conclusions: Conclusion: Olanzapine and haloperidol have distinct effects on protein kinase networks that alter the activity of KATP channels in the hypothalamus, which may explain their differential effect on disruptions in hypothalamic glucose sensing and regulation of hepatic glucose production. It remains to be determined if these changes in kinase activity directly cause dysfunctional glucose metabolism associated with antipsychotics.

Keywords: Antipsychotic, Kinome Array, Hypothalamus
Disclosure: Nothing to disclose.

P560. Luvadaxistat, an Investigational D-Amino Acid Oxidase Inhibitor, was Associated With Signals of Efficacy in Cognitive Impairment Associated With Schizophrenia but Not Negative Symptoms: Results From the Interact Study

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Background: A lack of effective treatment options for negative symptoms and cognitive impairment associated with schizophrenia (CIAS) constitutes a major unmet medical need and adds to the related economic burden. Deficits in glutamatergic transmission are hypothesized to play an important role in the pathophysiology of these symptoms. Luvadaxistat – a potent D-amino acid oxidase inhibitor – is thought to increase NMDA-dependent glutamatergic signaling in the brain by elevating levels of D-Serine, an obligatory NMDA receptor co-agonist. Luvadaxistat was previously shown to improve social interaction and cognition in rodent behavioral models. Here we report efficacy and safety findings from INTERACT, a phase 2 study of adjunctive luvadaxistat in adults with schizophrenia with persistent negative symptoms.

Methods: INTERACT was a randomized, double-blind, parallel-group, placebo-controlled, dose-range finding study conducted in Europe and North America (NCT03382639). Eligible participants were aged 18–60 years with symptomatically stable schizophrenia, had a baseline Brief Negative Symptom Scale (BNSS) score of ≥ 28 (12-item, excluding item 4), and were receiving primary antipsychotic therapy. To overcome potential confounding effects, those with depressive and extrapyramidal symptoms were excluded. The study comprised a 28-day screening period, a 14-day single-blinded placebo run-in period to prospectively evaluate drug adherence and BNSS score stability, and a 12-week double-blind treatment period.

The primary endpoint was the change in negative symptoms, measured as the 12-week change from baseline in the Positive and Negative Syndrome Scale – Negative Symptom Factor Score (PANSS NSFS). To assess CIAS, secondary endpoints included the 12-week change from baseline in the Brief Assessment of Cognition in Schizophrenia (BACS) composite score, a measure of cognition across multiple domains, and the Schizophrenia Cognition Rating Scale (SCoRS) score, a measure of day-to-day cognitive functioning requiring input from an adult informant. Secondary endpoints were not corrected for multiplicity. Safety endpoints included assessment of treatment-emergent adverse events (TEAEs). Dose selection was based on target occupancy and elevation of D-Serine.

Results: Overall, 256 participants were randomized 3:2:2:2 to receive placebo, luvadaxistat 50 mg, 125 mg and 500 mg, respectively, of whom 228 (89.1%) completed the study. Participants' mean age was 40 years (range 18–60 years), 168 (65.6%) were male and 208 (81.3%) were white. Demographic and baseline characteristics were evenly distributed across treatment groups.

For negative symptoms, no significant improvements in PANSS NSFS versus placebo were observed with luvadaxistat 50 mg, 125

mg or 500 mg at Week 12 ($p = 0.426$, $p = 0.362$ and $p = 0.808$, respectively). From baseline to Week 12, least squares (LS) mean changes in PANSS NSFS were −3.3 (95% confidence interval [CI], −4.3 to −2.2), −3.4 (95% CI, −4.4 to −2.3) and −2.5 (95% CI, −3.6 to −1.5) with luvadaxistat 50 mg, 125 mg and 500 mg, respectively, and −3.1 (95% CI, −4.0 to −2.3) with placebo.

For CIAS, nominally significant improvements were observed with luvadaxistat 50 mg versus placebo in the BACS composite score ($p = 0.031$; effect size, 0.4) and the SCoRS interviewer total score ($p = 0.011$; effect size, 0.4), but not with luvadaxistat 125 mg or 500 mg. For the BACS composite score, a 12-week LS mean change from baseline of 4.6 (95% CI, 2.7 to 6.5) was observed with luvadaxistat 50 mg, versus 2.3 (95% CI, 0.7 to 3.9) with placebo. For the SCoRS interviewer total score, a 12 week LS mean change from baseline of −3.8 (95% CI, −5.3 to −2.3) was observed with luvadaxistat 50 mg versus −1.6 (95% CI, −2.9 to −0.3) with placebo.

In total, 76 participants (29.7%) experienced a TEAE, of which 23 (9%) were considered drug related by the investigator. TEAEs occurring in > 5 participants (2%) were headache, insomnia and weight gain, which were observed at similar frequencies in the luvadaxistat and placebo groups. Mild, moderate and severe TEAEs occurred in 49 (19.1%), 24 (9.4%) and 3 (1.2%) participants, respectively. Severe events of rhabdomyolysis (placebo, $n = 1$) and chronic cholecystitis (luvadaxistat 50 mg, $n = 1$) were not considered drug related. Severe schizophrenia was reported in one participant taking luvadaxistat 125 mg and was considered a drug-related serious TEAE. Four serious TEAEs were reported in the placebo group; none were considered drug related. Five participants, including three taking placebo, experienced TEAEs resulting in discontinuation (blood creatine phosphokinase increased, liver function test increased, psychiatric disorders). No deaths were reported.

Conclusions: Luvadaxistat did not show significant efficacy in the treatment of negative symptoms of schizophrenia at the three doses evaluated. For CIAS, an inverted U-shaped dose response was observed with luvadaxistat 50 mg, 125 mg and 500mg. Only luvadaxistat 50 mg showed a signal of efficacy as measured by the BACS composite score and the SCoRS interviewer total score, with a clinically meaningful effect size of 0.4 for each. Additional clinical research is warranted to further evaluate this efficacy signal. Consistent with prior clinical experience, luvadaxistat was generally well tolerated in INTERACT.

Keywords: Cognitive Impairment Associated with Schizophrenia, Schizophrenia Negative Symptoms, D-Amino Acid Oxidase Inhibitor

Disclosure: Takeda Pharmaceutical Company Ltd, Cambridge: Employee (Self)

P561. Neuro-Oscillatory Measures During Reward Outcome Processing in Schizophrenia

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Background: Alpha band neural oscillation synchronization is a dominant EEG signal when people are awake, but at rest. In contrast, alpha desynchronization to salient events is thought to direct information processing resources away from the internal state to important external stimuli. In the current study, we hypothesized that alpha event-related desynchronization (ERD) would be deficient during reward processing in SZ (i.e., less alpha suppression to wins, relative to losses), and that reward-related ERD deficits would correlate with negative symptoms. Further, as ERD is thought to reflect a "desynchronization" of the resting state to ready the brain for external environmental engagement, we further hypothesized that trait rumination, reflective of excessive internal focus, would relate to reduced alpha ERD.

Methods: EEG was recorded while participants with schizophrenia (SZ = 54) and healthy controls (HC = 54) completed a slot machine gambling task. Event-related power measures from a principal components analysis were extracted in delta, theta, and alpha frequency ranges, and then compared between groups and equiprobable reward outcomes. Individual differences between alpha ERD and negative symptom (SZ) and trait rumination (HC, SZ) measures were also examined.

Results: A significant Group X Reward Outcome interaction ($p = .018$) was explained by HCs showing significant posterior-occipital alpha ERD to wins, relative to near miss losses ($p < .001$), compared to SZ who did not modulate alpha power to wins vs. losses ($p > .1$). Alpha power did note relate to negative symptoms ($p > .1$) among individuals with SZ. However, across all participants (HC + SZ), less alpha ERD to reward outcomes was related to more trait rumination, for both win ($p = .005$) and loss ($p = .002$) outcomes, with no group differences observed in the slopes of these relationships, and with rumination effects persisting after controlling for theta and delta event-related power.

Conclusions: These findings suggest that event-related modulation of alpha power is altered in schizophrenia during reward outcome processing, even when reward attainment places minimal demands on higher-order cognitive processes during a simple slot machine task. In contrast, theta and delta power bands did not show group differences in modulation by reward outcome. We did not observe negative symptom correlations with alpha ERD, however, high trait rumination was associated with less ERD to reward feedback, regardless of reward outcome valence and group membership.

Keywords: Alpha Oscillations, Reward Processing, Schizophrenia

Disclosure: Nothing to disclose.

P562. Developing and Validating a Combined Virtual Reality - Electroencephalogram Paradigm to Assess Biomarkers of Procognitive Treatment Response in Schizophrenia Patients

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Background: After decades of procognitive research in schizophrenia (SZ) with no FDA-approved procognitive drug in sight, therapeutic development has pivoted towards identifying biomarkers that predict procognitive treatment sensitivity. Mismatch negativity (MMN) and auditory steady-state gamma-band responses (ASSR), electroencephalogram (EEG)-based measures of early auditory information processing (EAIP) predict the clinical and cognitive benefits of targeted cognitive training (TCT) response in treatment-refractory SZ patients, albeit, with modest power. An important question is, can we enhance the ability of EAIP "biomarkers" to predict cognitive and functional gains from TCT in SZ patients? For instance, measures of MMN and ASSR utilize isolated sound fragments (tones, clicks), presented in the artificial context of a laboratory setting. It is possible that EEG measures generated using contextually relevant naturalistic sound stimuli might more accurately reflect the brain's capacity for EAIP, and thereby be more sensitive to both EAIP deficits and treatment sensitivity. However, EAIP measures require millisecond-level stimulus control within a structured test session and are thus not easily assessed in a naturalistic setting. Virtual reality (VR)

technology provides both the naturalistic context and tight experimental control needed to generate and assess potentially more ecologically relevant measures of EAIP. Here we developed a VR-based EEG paradigm to measure EAIP evoked by naturalistic sound stimuli (e.g., footsteps, jackhammer) presented in familiar VR-delivered contexts (e.g. walking down the street, walking past a construction site) and assessed the sensitivity of these VR-based measures to one-hour of TCT session.

Methods: Two VR-EEG paradigms were developed to measure MMN and ASSR. Carefully screened, medically stable, psychiatrically healthy adults (HS) and SZ patients ages 18-45 yo with an intelligence quotient (IQ) above 80 measured using wide range achievement test-3 (WRAT-3) completed a screening visit followed by a test day. Eligible subjects completed a comprehensive neurocognitive (MATRICS Consensus Cognitive Battery (MCCB)) and functional (Brief UCSD Performance-Based Skills Assessment (UPSA-B)) assessment during the screening visit and on test day completed both standard laboratory- and VR- based EEG measures before and after a one-hour "sound sweeps" TCT session in a randomized, order-balanced design. Pearson's correlation was used to determine the validity, reliability, and biomarker potential of VR-based MMN and gamma evoked power (γEP) in healthy subjects (HS) and SZ patients.

Results: 5 subjects have completed testing to date [$N = 4$(HS) and $N = 1$(SZ)]. Participants were in their late twenties (28 ± 10 yrs), high school educated (14.4 ± 2.3 yrs), women (60%) with a WRAT-3IQ of 111.7 ± 11.6, MCCB composite score of 14 ($n = 1$ (SZ)) and 52.2 ± 7.3 ($n = 4$ (HS)) and UPSA-B total score of 9 ($n = 1$ (SZ)) and 18 ± 1.4 ($n = 4$ (HS)). Participants registered a mild level of discomfort on the Simulator Sickness Questionnaire (SSQ) and high scores on the Presence Questionnaire (PQ) (80.5 (range: 58-120)) after the VR condition, the latter suggestive of a higher sense of presence in the VR environment. Collectively, these findings indicate that the VR-based EEG paradigms were immersive and well-tolerated. The average baseline auditory processing speed (APS) pre- TCT training = 65 milliseconds vs. Post- TCT training APS = 36 milliseconds ($n = 4$ (HS)) suggesting a 55% gain in APS. Overall participants completed an average of 15 (range 12-21) ($n = 4$ (HS)) and 6 ($n = 1$(SZ)) training blocks. Subject testing and analysis of VR-based neurophysiological (EEG) data are ongoing and results will be presented in full. However, a preliminary observation of the EEG data ($N = 1$ (HS) and $N = 1$ (SZ)) showed VR-based MMN and γEP to be comparable to standard laboratory-based MMN and γEP, with VR-based measures in this one SZ patient reduced compared to one HS and exhibiting malleability after one hour of TCT.

Conclusions: Our preliminary findings and observation of the VR-based EEG data suggest that VR-based EEG paradigms are well tolerated, immersive, and can generate robust MMN and γEP in both HS and SZ patients. Testing is ongoing, and complete comparison between VR-based EEG measures and TCT performance will be reported.

Keywords: Virtual Reality, Auditory Mismatch Negativity, Targeted Cognitive Training, Auditory Steady-State Response, Cognitive Impairment Associated with Schizophrenia

Disclosure: Nothing to disclose.

P563. Orphan Receptor GPR52 Profoundly Modulates Dopamine D2 Receptor Signaling: Discovery of GPR52 Druglike Agonists With Antipsychotic Activity

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Background: GPR52 is an orphan G protein-coupled receptor selectively expressed in the brain and has been identified as a promising drug target for psychiatric disorders including schizophrenia and substance use disorders. GPR52 is primarily expressed in the ventral striatum of the human brain in dopamine D2 medium spiny neurons where it may functionally regulate cAMP signaling and oppose the neural signaling of dopamine D2 receptors. Here we examine signaling crosstalk between GPR52 and the D2R and report our drug discovery effort to create novel GPR52 agonists and evaluate a lead agonist for antipsychotic-like activity.

Methods: To examine potential signaling crosstalk between GPR52 and D2R, the human GPR52 and D2 receptors were co-expressed in HEK293 cells and agonists or antagonists for both receptors were examined for cAMP signaling in living cells (Glosensor assay). Medicinal chemistry and pharmacology were subsequently used in a systematic structure-activity relationship study of an indoline-carboxamide based pharmacophore to illuminate ligand features crucial for GPR52 activation and to optimize a druglike GPR52 agonist. 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. Vehicle or increasing doses of select GPR52 agonists were tested in vivo for activity on amphetamine-induced locomotion using c57BL6J mice ($n = 12$ animals/treatment group). A one-way ANOVA for a between subjects design was employed to analyze horizontal activity.

Results: Expression of human GPR52 in HEK293 cells profoundly elevated basal cAMP levels by over 100-fold, indicating high constitutive receptor activity. We hypothesized this high GPR52 constitutive signaling may set the basal tone of cAMP in cells to oppose or modulate signaling by the Gi/o-coupled D2R. Expression of D2R alone in HEK293 cells produced low basal cAMP levels and cell treatments with D2R agonist quinpirole or antagonist haloperidol both yielded minimal changes to cAMP levels (~1-2 thousand light counts/sec). Notably, co-expression of GPR52 with D2R substantially increased basal cAMP levels and this elevated basal cAMP allowed for larger windows of agonism and inverse agonism by D2R ligands quinpirole (decrease of ~500,000 light counts/sec) and haloperidol (increase of ~150,000 light counts/sec). When the cells were treated concurrently with the GPR52 agonist PW0787 to further elevate cAMP, the D2R efficacies the inverse agonism by haloperidol grew even more significantly. Medicinal chemistry and pharmacology were subsequently used to illuminate ligand features crucial for GPR52 activation. We determined the two lower aromatic moieties of the agonist lead were amenable to further medicinal chemistry with substituents shown to modulate both agonist potency and efficacy. Surprisingly, when the nitrogen containing ring of the indoline system was broken into more flexible variants, this further increased agonist potency (EC50: ~40 nM) and efficacy, while retaining excellent target selectivity, plasma exposure and serum concentrations. 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: These studies indicate that striatal orphan receptor GPR52 may set the level of cellular cAMP and that GPR52 expression and activation profoundly modulates D2R signaling and pharmacology. The drug discovery efforts have also elucidated several agonists with optimized potency and efficacy, with PW0787 being a novel, orally bioavailable, brain penetrant GPR52 agonist with antipsychotic activity.

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Keywords: Drug Discovery - New Approaches, Schizophrenia, Antipsychotics, GPCR, Novel Targets, Preclinical Pharmacology

Disclosure: New Atlas Biotechnologies: Contracted Research (Self)

P564. Thalamocortical Relationships With Symptoms and Cognition in a Transdiagnostic Psychosis Sample

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Background: Thalamocortical dysconnectivity is consistently observed in psychosis and may characterize a clinical biomarker. However, its relevance to underlying symptomology across the psychosis spectrum remains unclear. Here we directly examine the relationship between thalamocortical connectivity and both clinical and cognitive variables in a transdiagnostic sample of patients with psychosis (including schizophrenia, schizoaffective disorder, and bipolar disorder with psychosis), their first-degree relatives, and healthy controls.

Methods: Patients with psychosis including those with a schizophrenia-spectrum diagnosis ($N = 99$) or a bipolar diagnosis ($N = 33$), their first-degree relatives ($N = 74$), and a group of healthy controls ($N = 43$) underwent resting fMRI in addition to a clinical and cognitive assessment as part of the Psychosis Human Connectome Project. Individual subject pre-processing and denoising used the ART toolbox and aCompCor in the 'CONN' toolbox. Next, seed-based connectivity analyses relied on an anatomically defined bilateral thalamus region of interest (ROI). Brain-wise effects across all subjects examined the relationship between thalamocortical connectivity and both clinical symptoms (measured using the Brief Psychiatric Rating Scale; BPRS) and global cognition (measured using the Brief Assessment of Cognition in Schizophrenia; BACS) controlling for subject age and gender. Two-way tests of significant clusters relied on a whole-brain false discovery rate (FDR) correction of $p < 0.01$.

Results: Individual differences in psychiatric symptoms across groups were negatively related to thalamocortical connectivity (higher BPRS scores correspond to lower connectivity) in prefrontal regions including the left and right middle frontal gyrus and anterior cingulate cortex (ACC). Individual differences in global cognition across groups were positively related (higher BACS scores correspond to higher connectivity) to thalamocortical connectivity in the ACC and right insula. Post-hoc analyses of significant ROIs determined that these effects were robust to individual subject motion (all p's $< .0001$). Last, we examined whether thalamocortical ROIs that significantly related to symptoms or cognition also differed between groups. Controlling for BPRS and BACS score respectively, each ROI showed a significant effect of group (all p's $< .012$), driven by reduced connectivity in the schizophrenia and bipolar subjects.

Conclusions: In a transdiagnostic sample, thalamocortical connectivity negatively correlated with psychiatric symptoms in prefrontal areas associated with cognitive control, and positively correlated with global cognitive scores in anterior cingulate and insular regions consistent with the salience network. Sensitivity to psychiatric symptoms and cognitive deficits across patients with psychosis and those with genetic liability may be driven by reduced thalamo-prefrontal and thalamo-salience connectivity respectively.

Keywords: Psychosis, Thalamo-Cortical Connectivity, Cognition, Symptomatology

Disclosure: Nothing to disclose.

P565. Frontostriatal Brain Wiring Organization in Early Psychosis Subjects Using a Novel Diffusion Imaging Fiber Cluster Analysis

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Background: Alterations in brain connectivity may underlie neuropsychiatric conditions such as schizophrenia. We here assess the geometric pattern of structural connectivity between the frontal cortex (FC) and caudate (Cd) in 56 healthy controls (HCs) and 155 Early Psychosis Patients (108 EP-Non-Affective and 47 EP-Affective subjects) from the Human Connectome Project (HCP); age: 18 to 33; Mean: 23.21 years; sex: 74 females and 137 males. We used our novel method of fiber cluster analysis of whole brain diffusion Magnetic Resonance Imaging (dMRI) tractography to assess the organization of frontostriatal brain wiring, which allows us to quantify the degree of deviation from a topographic, parallel, arrangement.

Methods: The data used in this study come from the shared data set from the Human Connectome Project for Early Psychosis (HCP-EP) study (MPI: Shenton, Breier). Diffusion MRI Data from 3 HCP sites (University of Indiana, Massachusetts General Hospital and McLean Hospital) were harmonized using our harmonization methodology (Cetin-Karayumak S et al. 2019). From this harmonized data set we generated whole brain tractography using our unscented Kallman filter (UKF) 2-tensor tractography methodology (Malcolm JG et al. 2010). To enable the identification of fiber tract parcels from the frontal cortex (FC) and the caudate (Cd), we used a data-driven fiber clustering atlas (Zhang et al. 2018) that allows for a whole brain tractography parcellation into 2000 fiber clusters according to the white matter (WM) anatomy (i.e., fiber geometric trajectory). Then, fiber clusters of interest (i.e., from FC to Cd) from the whole brain WM were identified for each subject in each subject group, according to their connected anatomical brain regions. We studied multiple Freesurfer FC regions including orbital, lateral and medial FC regions and the caudate. We identified 17 WM fiber clusters that connect FC and Cd in both left and right hemispheres in each subject group. To quantify the topographical relationship of these fiber clusters, we measured the inter-cluster mean distances between the endpoints of the fiber clusters within the frontal cortex (i.e., cortical distance) and the mean distances between the endpoints of the corresponding fiber clusters terminating in the caudate (i.e., caudate distance).

Results: We have performed the following preliminary analyses. For each group (HCs, EP-Non-affectives and EP-Affectives) in each hemisphere, we generated plots (not shown) based on the 17 fiber clusters (with 136 pairs of fiber clusters, yielding 136 data points), showing the relationship between the cortical distances and the corresponding caudate distances of the obtained fiber cluster pairs that connect the frontal cortex and the caudate. For the left hemisphere (LH) we found a non-linear relationship between inter-cluster cortical distances and caudate distances in HCs and in both patient groups (EP-Non-affectives and EP-Affectives) driven by the results from 10 cluster pairs. For the right hemisphere (RH), we found a similar non-linear relationship driven by the results from 10 cluster pairs in HCs, and a more flattened non-linear relationship in both EP-Non-affective and EP-Affective patient groups. Of note, a fiber cluster, originating in the inferior frontal gyrus, pars triangularis, was significantly over-represented in these 10 cluster pairs.

Conclusions: Using dMRI fiber cluster topography analysis in HCs, we show 1) the overall FC wiring projection pattern between the FC and the caudate in HCs deviates from a topographic, parallel, organization, due to a bilateral pattern of convergence in regionally specific fiber clusters; 2) this is similar to that which we found in our prior study of healthy subjects (Levitt, 2021); and, 3) patient groups (EP-Non-affective and EP-Affective subjects) appear to have a similar pattern of connectivity to controls in the LH but appear to have a more flattened trajectory versus controls in the RH. The poster will include a more refined regionally specific analysis of individual fiber clusters across all subject groups.

Keywords: Early Psychosis, Diffusion Weighted Imaging, Frontostriatal Circuitry, Brain Structural Connectivity

Disclosure: Nothing to disclose.

P566. Extreme Phenotype Sequencing Reveals High Impact Rare Genetic Variants in Severe Schizophrenia

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Background: While genome-wide association studies have implicated hundreds of common variant loci of modest effect in schizophrenia risk, identifying rare variants of large effect has proven challenging in comparison with other neurodevelopmental disorders. Across complex disorders, individuals with more severe or earlier-onset manifestations are more likely to harbor rare, deleterious variants of large effect. As a consequence, extreme phenotype sampling—exclusively studying individuals at the extreme ends of the distribution of a phenotypic trait—can substantially reduce the sample size needed to identify rare variants of large effect. We hypothesized that severely affected, chronically institutionalized individuals with schizophrenia would be more likely to harbor rare, damaging variants compared to individuals with typical forms of schizophrenia and controls.

Methods: We conducted whole genome sequencing of 114 individuals with severe, extremely treatment-resistant schizophrenia (SETRS), 198 individuals with typical schizophrenia, and 4,146 controls that passed quality control. We defined SETRS individuals as those whose illness severity has required continuous hospitalization for at least 5 years in a long-term New York State inpatient facility due to primary schizophrenia symptoms.

We compared the burden of rare, damaging missense and loss-of-function variants between SETRS, typical schizophrenia, and controls across "intolerant" genes depleted of functional variation in the general population. Prior studies of schizophrenia have shown that the case-control burden of rare loss-of-function (odds ratio, 1.26) and damaging missense variants (odds ratio, 1.06-1.25) is concentrated exclusively in these "intolerant" genes.

For the gene set burden analysis, we tallied the total number of rare, damaging variants in cases and controls for a given gene set and assessed the significance of this difference across genetically-determined ancestral clusters using a two-sided Cochran-Mantel-Haenszel test with effect sizes represented as odds ratios. We accounted for multiple comparisons using the Benjamini-Hochberg false discovery rate. Specifically, we accounted for each test of qualifying missense and loss-of-function variants across each of the gene sets analyzed.

Results: SETRS individuals had a mean age of 60.9 years, were predominantly male (69.3%), and had an average duration of lifetime hospitalization of 24.9 years. The average age of onset was 18.4 for males and 18.3 for females and only 14.9% of participants had children, consistent with a strong effect of negative selection.

SETRS individuals had a high burden of rare loss-of-function (odds ratio (OR), 1.97; 95% confidence interval [CI], 1.42-2.75; $P =$ 6.1 x 10-5) and damaging missense variants in intolerant genes (odds ratio, 2.59; 95% CI, 1.77-3.78; $P = 5.4$ x 10-7). 47.4% of SETRS individuals carried at least one rare, damaging missense or loss-of-function variant in intolerant genes compared to 30.3% of typical schizophrenia individuals (OR, 2.06; 95% CI, 1.25-3.43; $P = 3$ x 10-3) and 26% of controls (OR, 2.55; 95% CI, 1.72-3.78; $P = 1.5$ x 10-6).

Restricting to genes previously associated with schizophrenia risk further strengthened the enrichment with 8.8% of SETRS individuals carrying a damaging missense or loss-of-function variant compared to 2.5% of typical schizophrenia (OR, 4.62; 95% CI, 1.26-16.89; $P = 0.02$) and 1.5% of controls (OR, 7.57; 95% CI, 3.23-16.24; $P = 3$ x 10-9).

Conclusions: In this study, we found that selecting individuals with extremely severe forms of schizophrenia led to a significantly improved ability to detect disease-associated rare variants. The high prevalence of rare variant risk factors in SETRS individuals suggests future clinical opportunities for risk prediction, prognostic stratification, and genetic counseling. These findings have implications for the design of future genetic studies in schizophrenia and highlight a strategy to reduce phenotypic heterogeneity and improve gene discovery efforts in other neuropsychiatric disorders.

Keywords: Genomics, DNA, Whole-Genome, Sequencing, Schizophrenia, Treatment-Resistant Schizophrenia

Disclosure: Nothing to disclose.

P567. Metabolomic Measures of Oxidative Stress in the Dorsolateral Prefrontal Cortex, Dorsal Striatum and Ventral Striatum in Schizophrenia

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Background: Oxidative stress, and the resultant oxidative damage to fundamental cellular components, are proposed to contribute to the complex pathophysiology underlying the clinical syndrome of schizophrenia (SZ). The primary antioxidant system in the brain, glutathione (GSH), regulates the abundance of oxidant species, and consequently oxidative damage. An insufficient brain antioxidant defense, due to direct impairments to GSH availability and/or excess of oxidants that overwhelm the GSH system, has been proposed as a pathogenic mechanism in the SZ disease process.

Although some sources of oxidant species, such as mitochondrial production of reactive oxygen species, are ubiquitous throughout the brain, others are brain region-dependent. For example, the striatum has a particularly rich source of oxidant species, dopamine degradation, that is largely absent from other regions such as the dorsolateral prefrontal cortex (DLPFC), which receive sparse dopaminergic inputs. Further, in primates, measures reflecting presynaptic dopamine are normally greater within the dorsal striatum (DS) than in the ventral striatum (VS). SZ appears to be associated with an exaggeration of these normal differences in dopamine innervation in the striatum, such that an excess synthesis and release of dopamine is most pronounced in the DS and not in the VS. As dopamine degradation can be a substantial additional source of oxidant species, markers of oxidative stress may be most pronounced in the DS relative to the VS and DLPFC. To investigate this idea, we utilized quantitative mass spectrometry to quantify markers of 1) the GSH antioxidant system and 2) oxidative damage to lipids in the DLPFC, DS and VS in SZ subjects.

Methods: Brain specimens from 50 subjects were processed for metabolomic analyses. Each SZ subject ($n = 25$) was matched with an unaffected comparison (UC) subject for sex and as closely as possible for age and postmortem interval (PMI). Tissue samples from all subjects were processed together in an experiment. Subject groups did not differ in mean age, PMI, brain pH, RNA integrity number, or tissue storage time (all $p > 0.2$). DLPFC grey matter, DS and VS were utilized for targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) to quantify the abundance of free GSH and glutathione disulfide (GSSG). GSSG is produced from GSH antioxidant activities. LC-MS was utilized to quantify the abundance of malondialdehyde (MDA), a product of oxidative damage to lipids. Two-way ANCOVA models were

performed that included the relevant dependent measure, independent variables of diagnostic group and brain region, and any significant covariate. Cohen's *d* was calculated to determine the SZ disease effect size. Statistical significance was assessed at $p < 0.05$, and trend-level statistical significance assessed at $0.05 \leq p < 0.1$.

Results: For GSH abundance, no significant main effect of brain region was identified ($F2,96 = 1.9$, $p = 0.15$). However, a trend-level significant main effect of SZ diagnosis was present ($F1,47 = 3.6$, $p = 0.06$). Contrary to the observed effects of oxidative stress, the SZ disease effect was associated with greater GSH in the DS ($d = +0.43$) and VS ($d = +0.51$), and no effect in the DLPFC ($d = -0.04$) relative to UC. No significant interaction of diagnosis and brain region on GSH abundance was identified ($F2,96 = 2.4$, $p = 0.1$).

For GSSG, a significant main effect of brain region was identified ($F2,96 = 12.5$, $p = 0.00002$); post-hoc analyses revealed 44% greater abundance of GSSG in DLPFC relative to DS and VS (all $t = 2.9$, $p < 0.005$). No significant main effect of SZ was identified for GSSG abundance ($F1,48 = 0.76$, $p = 0.4$). No significant interaction of diagnosis and brain region on GSSG abundance was identified ($F2,96 = 0.61$, $p = 0.5$).

For MDA, a significant main effect of brain region was identified ($F2,96 = 13.6$, $p = 0.000006$); post-hoc analyses revealed 22% greater abundance of MDA in DS relative to DLPFC ($t = -3.3$, $p = 0.002$). No significant main effect of SZ on MDA abundance was identified ($F1,48 = 0.6$, $p = 0.5$). However, a trend-level significant interaction of SZ diagnosis and brain region on MDA abundance was present ($F2,96 = 3.1$, $p = 0.05$). Post-hoc analysis revealed no significant effect of SZ on MDA abundance in DLPFC, DS or VS (all $t \leq 1.6$, $p > 0.3$), and SZ disease effect sizes in DS ($d = -0.12$), DLPFC ($d = +0.24$) and VS ($d = +0.42$).

Conclusions: Contrary to the effects of oxidative stress on GSH abundance, we identified a greater concentration of free GSH in the DS and VS in SZ and no effect of SZ on GSH abundance in the DLPFC. These findings suggest that the GSH system can increase antioxidant capacity in regions that may have a greater oxidant load in SZ. The lack of a significant SZ disease effect on MDA, a product of lipid oxidation, further suggests that the GSH antioxidant system is appropriately defending against oxidative damage. Finally, the significantly greater abundance of MDA in DS across subjects is consistent with the greater oxidant load potential in this striatal region, given its greater dopamine innervation relative to the VS and DLPFC. Together, these data suggest that an impaired GSH antioxidant system and increased cellular oxidative damage are not present in the DLPFC, DS or VS in SZ subjects, and therefore may not represent common pathogenic mechanisms in SZ.

Keywords: Glutathione, Lipid Oxidation, Postmortem, Schizophrenia, DLPFC

Disclosure: Nothing to disclose.

P568. Plasma Clozapine and N-Desmethylclozapine: Relationship With Cognition and Changes in Basal Forebrain Functional Connectivity in Treatment-Resistant Schizophrenia

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Background: Clozapine (CLZ) demonstrates a unique clinical efficacy relative to other antipsychotic drugs. Previous work has linked CLZ's major metabolite, N-desmethylclozapine (NDMC) with pro-cognitive effects, by putative action on the cholinergic system. Here, we further examine and extend this relationship with functional neuroimaging in a homogeneous cohort of nineteen acutely ill participants with treatment-resistant schizophrenia (TRS) undergoing CLZ treatment.

Methods: We followed the cohort across 12 weeks of CLZ treatment. Measures of neurocognition and plasma levels of NDMC and CLZ were obtained in addition to resting-state functional neuroimaging scans, which were captured at baseline and after 12 weeks of CLZ treatment. Cognition and changes in basal forebrain functional connectivity with the dorsolateral prefrontal cortex were examined in relation to the CLZ/NDMC ratio.

Results: Consistent with previous findings, we demonstrate a positive relationship between NDMC levels and measures of overall cognition ($p = 0.006$), working memory ($p = 0.00025$) and attention ($p = 0.0055$). In addition, we observed a significant correlation between basal forebrain-DLPFC connectivity and CLZ/NDMC ratios across CLZ treatment ($p = 0.019$) and a negative correlation with working memory scores ($p = 0.04$).

Conclusions: These findings may reflect the impact of CLZ and NDMC on the cholinergic system through action on the muscarinic receptors. Results of this work further support the potential pro-cognitive effects of NDMC and demonstrate a longitudinal neuroimaging correlate of plasma drug ratios.

Keywords: Schizophrenia, Clozapine, N-desmethylclozapine, Functional Connectivity, Working Memory

Disclosure: Nothing to disclose.

P569. The Relationship of Inflammation With Antibodies to Gliadin (AGA IgG) in Persons With Schizophrenia

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Background: It has been established that people with schizophrenia experience higher than normal levels of inflammation (1). One important example of this is that prenatal maternal infection is associated with an increased risk of offspring developing schizophrenia (2). Additionally, the presence of an autoimmune disease is associated with a 45% increased risk of developing schizophrenia (3). Research has also shown associations between immune reactions to gluten (gliadin) and the presence of neurologic/psychiatric symptoms (4). Recently, it has been found that about 30% of persons with schizophrenia have high levels of antibodies to gliadin (AGA IgG) (5). In preliminary studies, a gluten-free diet has shown to decrease negative symptoms among people with schizophrenia who have high AGA IgG (6). Results also demonstrated associations between AGA IgG and several biomarkers, including TNFα and IL-1β, both of which decreased among participants on the gluten-free diet. This suggests that inflammation is linked to these antibody levels. These findings prompted an interest to understand which pro-inflammatory cytokines are related to high AGA IgG in order to better understand the underlying mechanisms of the nearly 30% of people with schizophrenia with high antibodies to gliadin.

Methods: Serum biomarkers were analyzed from an existing dataset of participants with a DSM-5 diagnosis of schizophrenia or schizoaffective disorder between the ages of 18 and 64 ($N = 417$). Serum samples were collected to determine the levels of AGA IgG as well as a battery of pro-inflammatory markers (GM-CSF, IFNγ, IL-17A, IL-1β, IL-6, and TNFα). AGA IgG was measured and analyzed using semi quantitative ELISA assays from Inova Diagnostics. Pro-inflammatory cytokines (GM-CSF, IFNγ, IL-17A, IL-1β, IL-6, and TNFα) were measured using EMD Millipore's MAP Human Cytokine Magnetic Bead panel (Luminex bead-based immunoassays

(Millipore, Billerica NY)). The readout was completed using a Bioplex 200 platform (Biorad, Hercules CA) to determine the concentration of multiple target proteins in the specimens. Participants were grouped based on the level of AGA IgG in their sera (≥20 = positive; <20 = negative). Mean cytokine values were compared between the two groups (negative vs. positive AGA IgG) and analyzed for significant differences using a Wilcoxon statistical test. Correlations between AGA IgG (positive vs. negative) and all six cytokines were also analyzed using a Wilcoxon statistical test.

Results: The mean age for the sample was 41.96 (SD = 12.46), with 134 females and 282 males. The racial demographics of the sample were as follows: 56% were Black or African American (N = 236), 34% were white (N = 145), 2% were Asian (N = 10), and 6% were other reported racial categories or unknown. The overall rate of AGA IgG positivity among the sample was 38.1% (N = 159). Mean cytokine values were all significantly higher for those in the positive AGA IgG group compared to those in the negative AGA IgG group ($p < 0.001$). Positive AGA IgG (≥20U) was moderately associated with all pro-inflammatory cytokines: GM-CSF ($R = 0.36$, $p < 0.001$), IFNγ ($R = 0.28$, $p < 0.001$), IL-17A ($R = 0.23$, $p < 0.05$), IL-1β ($R = 0.33$, $p < 0.001$), IL-6 ($R = 0.37$, $p < 0.001$), and TNFα ($R = 0.20$, $p < 0.05$). However, in the group found to be negative for AGA IgG (<20U), R values were all less than 0.16, with only two correlations meeting the threshold for significance: IL-1β ($R = 0.16$, $p < 0.05$), and IL-6 ($R = 0.13$, $p < 0.05$). The sample of over 400 participants provides a high statistical power of 1.00 to detect the relationship between anti-gliadin antibodies and pro-inflammatory cytokines, with accompanying moderate to high effect sizes between $r = 0.24$ and $r = 0.38$ for all 6 cytokines.

Conclusions: All pro-inflammatory cytokines were moderately associated with positive AGA IgG, but no relationship was seen in the larger group considered negative for AGA IgG. This suggests that the relationship between anti-gliadin antibodies and the inflammatory biomarkers exists only for those with high anti-gliadin antibodies (AGA IgG ≥ 20). These results replicate previous data that demonstrated relationships between positive AGA IgG and both TNFα and IL-1β (7). These results further support a subgroup of schizophrenia patients with high inflammation and immune activation that may be different from those without this component. The concept of a potential subgroup of schizophrenia patients whose symptomatology may be associated with underlying inflammation and immune activation could help identify new treatment targets and better personalize treatment.

Keywords: Schizophrenia (SCZ), Gluten, Gliadin, Inflammation, Anti-Gliadin Antibodies (AGA)

Disclosure: Nothing to disclose.

P570. Tracking the Developmental Trajectory of 22q11.2 Deletion Syndrome in a Mouse Model

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Background: 22q11.2 deletion syndrome (22q11DS) is a genetic syndrome with high penetrance for developmental neuropsychiatric disorders (Drew et al. 2011; Gur et al. 2017). Human imaging studies have shown alterations in structural and functional connectivity across brain regions in individuals with 22q11DS (Schreiner et al. 2017; Jalbrzikowski et al. 2014). These observations have promoted a conceptualization of 22q11DS as a 'developmental dysconnectivity' syndrome, which may involve atypical circuit formation due to abnormal neurodevelopment. However, multidimensional, longitudinal investigations of the macroscale developmental trajectory in 22q11DS are lacking.

To probe the developmental trajectory in 22q11DS, we longitudinally mapped brain morphoanatomy, resting-state fMRI (rsfMRI) connectivity and socio-cognitive behavior in LgDel/+ mice, a well characterized mouse model of 22q11DS (Merscher et al. 2011; Meechan et al. 2015). To relate changes in neural synchronization to functionally dysconnectivity as measured with rsfMRI, we also probed developmental rescue produced by pharmacological inhibition of the GSK3β pathway hyperactivity that characterizes these mice (Mukai et al. 2008; 2015; Moutin et al. 2017; Tamura et al. 2016), as previous research showed that this procedure normalizes fronto-hippocampal synchrony in LgDel/+ mice.

Methods: We longitudinally mapped rsfMRI-based functional connectivity, diffusion tensor imaging and brain morphometry in pre-pubertal (p33-p37) and young adult (p105-p120) LgDel/+ mice (LgDel/+ $n = 22$; WT $n = 22$, mixed sexes). We also assessed socio-communicative functions using tests for social preference and recognition, social reward and temporal order memory tasks. rsfMRI time series were analyzed as previously described (Cole et al. 2010) using voxel-wise mapping. High resolution morpho-anatomical T2-weighted images were acquired ex vivo on PFA-fixed brains and analyzed as in Pagani et al. 2019 using voxel-based morphometry. Rescue experiment was performed by injecting mice every second day from p7 to p27 with SB216763 or vehicle as in Tamura et al. 2016.

Results: rsfMRI mapping revealed significant alterations in connectivity both at the prepubertal and adult stage. However, the direction and anatomical distribution of these changes was dramatically different across development. Specifically, we found evidence of generalized hyperconnectivity in pre-pubertal LgDel/+ mice, an effect that however reverted to focal fronto-hippocampal hypoconnectivity in young adulthood ($T > 2.1$, FWER cluster-corrected, with cluster defining threshold of $t > 2$, $p < 0.05$). Brain morphometry revealed small and focal gray matter reduction in olfactory and cerebellar areas in the pre-pubertal phase, with post-pubertal emergence of increased gray matter volume in cortico-limbic and basal ganglia, as well as focal reduction in gray matter in ventral hippocampal areas ($T > 2.1$, FWER cluster-corrected, with cluster defining threshold of $t > 2$, $p < 0.05$). Connectivity and neuroanatomic changes were not predictive of sociability (two-way ANOVA, genotype, $p = 0.005$), reward processing (t test, $p = 0.04$) and cognitive impairments (t test, $p = 0.0004$) assayed behaviorally in the same mice across development. Interestingly, developmental Gsk3β kinase inhibition completely rescued juvenile hyperconnectivity as well as cognitive functions (temporal order memory test) in adult mice, but did not rescue adult fronto-hippocampal rsfMRI decoupling, neuroanatomic changes or any other of the socio-behavioral alterations assayed at both developmental timepoints (sociability and social memory).

Conclusions: Our data document robust developmental alterations in brain connectivity and morphometry in LgDel mice. Of interest, connectivity changes appeared to present divergent expression across development, with generalized hyperconnectivity during the juvenile phase, but not fronto-hippocampal decoupling in adulthood, being critically dependent on Gsk3 pathway overactivity. The lack of a robust association between our imaging findings and corresponding behavioral deficits suggests that the observed imaging phenotypes, while potentially useful biomarkers for disease progression, might possibly be mechanistically distinct from the socio-cognitive dysfunction that characterizes 22q11DS.

Keywords: 22q11.2 Deletion Syndrome, fMRI, Neuroanatomy, Behavior, LgDel

Disclosure: Nothing to disclose.

P571. Intrinsic Motivation

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

Methods: Data collection for Time1 recently completed and participants are now returning for 2-year longitudinal follow-up. Preliminary results here utilized the fMRI-analyzable Time1 sample of 94 individuals with PS and 30 typically developing controls (TD) aged 16-26 (50 females, group-balanced). During fMRI, participants performed a visual fractal memory task, under three counterbalanced feedback conditions: 1) none 2) accuracy information 3) monetary. On each trial, participants identified which of two presented fractals was the one viewed previously during pre-scan encoding. Trials included three phases: choice, confidence rating, and feedback, separated by jittered delays. fMRI analysis focused on VS, together with secondary regional and whole-brain analyses. Additional study measures include clinical negative symptoms (CAINS), prodromal symptoms (SIPS, PRIME), self-report of trait and task IM, a behavioral free-choice measure of task IM, and a behavioral effort-discounting task. MRI data also included structural MPRAGE and resting state BOLD (ABCD sequences).

Results: The PS group showed greater clinical amotivation (CAINS) ($t = 6.2$, $p < 0.001$) and impaired trait IM ($t = 2.6$, $p = 0.01$). However, trait EM was intact in PS ($t = 0.14$, $p = .89$), and PS exhibited a relative reduction in IM compared to EM (IM-EM difference scores, $t = 3.1$, $p = 0.003$). Across all participants, CAINS amotivation correlated more strongly with self-reported trait IM ($r = -0.36$, $p = 0.01$) than EM ($r = -0.08$, $p = 0.9$). Mean memory task accuracy was 63%, indicating that the task was challenging but achievable, conditions designed to elicit IM. Across-trial confidence ratings correlated with correct/incorrect outcome on average $r = 0.21$ across the sample, indicating that confidence ratings are an (imperfect) indicator of actual memory accuracy. There were no group differences in task accuracy, confidence, or confidence-accuracy correlation (p's > 0.5). fMRI revealed robust VS activation to the fractal stimulus (memory choice) phase and to correct vs. incorrect feedback ($p < 0.01$) but not the outcome phase overall. There was a significant relationship between VS activation and across-trial confidence ratings during the choice phase, consistent with a valuation signal, and with across-trial prediction errors (outcome – confidence) at the outcome phase ($p < 0.01$). All these contrasts showed stronger activation as feedback salience increased (money>information>none). Group differences in VS activation were not significant, but trait intrinsic motivation was dimensionally related across the full sample with VS activation, especially during the choice phase ($p < 0.01$).

Conclusions: Our findings support the hypothesis that intrinsic motivation is selectively impaired in PS and selectively related to clinical amotivation, relative to EM. The fMRI results demonstrate that VS responses during a challenging cognitive task indeed reflect internally generated reinforcement signals related to accuracy and confidence, as well as to self-reported trait IM. Ongoing work will directly compare differential fMRI response to IM vs. EM task feedback conditions in relation to self-report IM and EM measures. Longitudinal data will permit evaluation of how these neurobehavioral phenotypes develop over time. Our ultimate goal is to characterize neural mechanisms of amotivation and develop biomarkers for neurobehaviorally-defined amotivation dimensions or subtypes. These biomarkers will be tested for prognostic utility and as moderators or mediators for early interventions in at-risk youth.

Keywords: Psychosis Risk, Intrinsic Motivation, fMRI, Ventral Striatum

Disclosure: Nothing to disclose.

P572. Using a BSNIP Biomarker Fingerprint to Stratify Participants for Clinical Trials: Testing the Kynurenic Acid Hypothesis of Cognitive Impairment

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Background: The brain concentration of kynurenic acid (KYNA), a metabolite of the kynurenine pathway of tryptophan degradation and antagonist of both N-methyl-D-aspartic acid and α7 nicotinic acetylcholine receptors, is elevated in the prefrontal cortex of individuals with schizophrenia (SZ). This increase may be clinically relevant because hypofunction of both of these receptors is implicated in the pathophysiology, and especially in the cognitive deficits, of the condition.

Two enzymes, kynurenine 3-monooxygenase (KMO) and kynurenine aminotransferase II (KAT II) play critical roles in regulating brain KYNA levels. Thus, a reduction in KMO activity, as seen in the brain of people with SZ, increases brain KYNA, and inhibition of KAT II, which produces most KYNA in the brain, is being considered a fundamentally new approach to prevent cognitive impairments (Schwarcz et al. 2012).

Examined postmortem in the frontal eyefield (BA6) of SZ cases, the KMO SNP rs2275163 CC (but not KMO SNP rs2275163 CT/TT) is associated with reduced KMO mRNA expression enzyme activity as well as elevated KYNA levels. Moreover, and unrelated to a SZ diagnosis, this genotype reduces predictive pursuit function and worsens visuospatial working memory (Wonodi et al. 2011).

Methods: Based on these results, we developed a "biomarker fingerprint" of KMO SNPs rs2275163 CC (the "high KYNA" phenotype) and rs2275163 CT/TT in a large cohort ($N = 250$) of SZ individuals belonging to Biotypes 1 and 2, as defined by the Bipolar and Schizophrenia Network (Clementz el al., 2015), using genotype and specific biomarkers together to distinguish "high KYNA" individuals. To this end, we conducted four separate canonical discriminant analyses with the biotypes (each bootstrapped 1000x with 95% sampling), evaluating i) cognition (BACS and Stop Signal Task), (ii) EEG

(evoked and intrinsic activity), (iii) oculomotor function (pro-/anti-saccades and smooth pursuit), (iv) cortical thickness, (v) cortical surface area, and (vi) cortical and subcortical gray and white matter volume.

Results: The results revealed a 0.7 SD separation between rs2275163 CC and rs2275163 CT/TT probands. Notably, probands with z-scores >0.5 had a > 95% likelihood of carrying the rs2275163 CC genotype. This generated not only a genotype, but also a phenotype of interest for identifying "high KYNA" individuals.

Conclusions: These findings support the application of biomarkers as co-primary outcome measures and, specifically, the use of the KMO SNP rs2275163 CC genotype along with the CC genotype biomarker fingerprint to select the study population in clinical trials that are designed to examine the possible pro-cognitive effects of KAT II inhibitors in humans. This approach of prospective enrichment of the patient population through biomarker phenotyping is expected to reduce the size of trials as well as increase the likelihood of success by targeting patients with a "high KYNA" fingerprint. Compounds are currently being prepared for hypothesis testing in humans.

Keywords: CNS Clinical Trials, Kynurenic Acid, Biomarker Fingerprint, Psychosis, Cognition

Disclosures: Kynexis, Karuna, Astellas, Sunovion: Advisory Board (Self) Karuna: Stock / Equity (Self)

P573. Medications Matter: Impact of Anticholinergic Medication Burden on Biomarkers of Early Auditory Information Processing in Schizophrenia

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Background: Mismatch negativity (MMN) and P3a are crucial biomarkers of early auditory information processing in schizophrenia (SZ) and are currently lead candidates for deployment in biomarker-guided SZ clinical trials. However, the impact of medication related factors--specifically anticholinergic medication burden--on these biomarkers has not been sufficiently explored. Previous studies have demonstrated that cumulative anticholinergic medication burden stemming from routinely prescribed psychotropic and non-psychotropic medications negatively impacts cognitive performance across multiple measures in SZ. As MMN and P3a have been mechanistically linked to cognitive impairment in SZ, we aimed to investigate: 1) how anticholinergic medication burden affects MMN/P3a response, and 2) whether anticholinergic medication burden alters previously examined mediation pathways linking MMN/P3a, cognitive functioning, symptoms and functional outcomes.

Methods: Anticholinergic medication burden was calculated using a modified Anticholinergic Cognitive Burden (ACB) scale for participants with SZ ($n = 774$) enrolled in the multi-site Consortium on the Genetics of Schizophrenia (COGS-2) case-control study who had complete medication records available and who had undergone measurement of MMN/P3a. ACB scores ranging from 0 to 3 were assigned to individual medications based on anticholinergic properties and were summed to generate a cumulative anticholinergic medication burden score for each participant. SZ patients were separated into groups based on ACB as previously described (ACB = 0, $n = 52$; ACB = 1 or 2, $n = 194$; ACB = 3 or 4, $n = 185$; ACB = 5 or 6, $n = 93$; ACB > 6, $n = 87$). Structural equation modeling was used to test mediation effects.

Results: Degree of ACB was significantly associated with MMN ($F = 5.15$, $p < 0.001$) and P3a ($F = 4.34$, $p < 0.01$). Compared to SZ individuals with an ACB of 0, those with ACB > 6 had more attenuated MMN and P3a responses (average [st dev]; ACB = 0, MMN = -1.52μV [0.87], P3a = 1.83μV [1.48]; ACB > 6, MMN = -0.82μV [0.96], P3a = 1.01μV [1.01]. ACB's effect on functional outcomes was mediated by direct effects on MMN/P3a, cognitive functioning and negative symptoms (i.e., ACB score was associated with attenuated MMN/P3a amplitudes, poorer cognitive functioning, and increased negative symptoms, which ultimately predicted poorer functional outcomes). Results suggested that an increase of an ACB ~3 in an SZ patient's medication regimen (equivalent to one strong anticholinergic medication) corresponded to a ~$d = -0.3$ on functional outcomes.

Conclusions: Accounting for anticholinergic medication burden may clarify relationships between electrophysiological biomarkers, cognitive functioning and outcomes for future studies. Such effects could be salient in large-scale genetic studies of SZ and cognition, biomarker-guided SZ clinical trials, and the development of novel pro-cognitive therapeutics in SZ.

Keywords: Cognition, Psychosis, Anticholinergic, Anticholinergic Medication Burden

Disclosures: Astellas Pharma, Heptares Therapeutics, NeuroSig, Takeda Pharmaceutical Company, Ltd: Consultant (Self)

P574. Mismatch Negativity as an Index of Target Engagement for Excitation/Inhibition-Based Treatment Development: A Double-Blind, Placebo-Controlled, R Single-Dose Cross-Over Study of the Serotonin Type-3 Receptor Antagonist CVN058

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Background: Serotonin type-3 receptor (5-HT3R) antagonists show potential as a treatment for cognitive deficits in schizophrenia. CVN058, a brain-penetrant, potent and selective 5-HT3R antagonist, shows efficacy in rodent models of cognition and was well-tolerated in Phase-1 studies. We evaluated the target engagement of CVN058 using mismatch negativity (MMN) in a randomized, double-blind, placebo-controlled, cross-over study.

Methods: Subjects were stable outpatients with schizophrenia or schizoaffective disorder treated with antipsychotics. Subjects were not permitted to use other 5-HT3R modulators or serotonin reuptake inhibitors. Each subject received a high (150mg) and low (15mg or 75mg) oral dose of CVN058 and placebo in a randomized order across 3 single-day treatment visits separated by at least 1 week. The primary pre-registered outcome was amplitude of duration MMN. Amplitude of other MMN deviants (frequency, intensity, frequency modulation and location), P50, P300 and auditory steady state response (ASSR) were exploratory endpoints.

Results: 19 of 22 randomized subjects (86.4%) completed the study. Baseline PANSS scores indicated moderate impairment. CVN058 150mg led to significant improvement vs. placebo on the primary outcome of duration MMN ($p = 0.02$, Cohen's $d = 0.48$). A significant treatment effect was also seen in a combined analysis across all MMN deviants ($p < 0.001$, $d = 0.57$). Effects on location MMN were independently significant ($p < 0.007$, $d = 0.46$). No other significant effects were seen for other deviants, doses or EEG measures. There were no clinically significant treatment related adverse effects.

Conclusions: These results show MMN to be a sensitive target engagement biomarker for 5-HT3R, and support the potential utility of CVN058 in correcting the excitatory/inhibitory imbalance in schizophrenia.

Keywords: Auditory Mismatch Negativity, Auditory Steady-State Response, NMDA Receptor, 5-HT3 Receptors, Cognitive Impairment Associated With Schizophrenia

Disclosures: Glytech, NeuroRx, Promentis,: Stock / Equity (Self) Biogen: Advisory Board (Self)

SK Life Sci, Boehringer Ingelheim, Celerion: Consultant (Self)

P575. Penetrance and Pleiotropy of Polygenic Risk Scores for Schizophrenia, Bipolar Disorder, and Major Depression in 660,000 US Veterans: A Strategy for Validation of EHR Diagnoses

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Background: Schizophrenia and bipolar disorder are heritable, multifactorial disorders and major causes of disability. Polygenic risk scores (PRS) aggregate variants identified from genome-wide association studies into individual-level estimates of liability to disease, and are a promising tool for risk stratification in clinical settings. With the advent or large-scale genomic data collection, understanding the validity or EHR diagnoses as phenotypic entities is critical.

Methods: Leveraging the VA's extensive electronic health record (EHR) and a cohort of 9,378 individuals with confirmed schizophrenia or bipolar disorder diagnoses, we validated case-control assignments based on ICD codes, and benchmarked the performance of PRS in 660,000 Million Veteran Program (MVP) participants. We explored relationships between PRS and 1,700 disease categories and 70 laboratory values via phenome-wide association studies (PheWAS). Given the substantial genetic overlap between neuropsychiatric disorders, we applied genomic structural equation modeling (gSEM) to derive novel PRS indexing common and disorder-specific latent genetic factors.

Results: Among 3,953 and 5,425 individuals with confirmed diagnoses of schizophrenia or bipolar disorder, 95% were correctly identified using ICD codes (>1), with 25% also meeting criteria for the incorrect diagnosis. Encouragingly, PRS performed best in comparisons of confirmed cases, followed hierarchically by individuals who received inpatient treatment, individuals who received any treatment, and spectrum diagnoses. Our PheWAS confirmed that higher neuropsychiatric PRS increases risk for many psychiatric and physical health problems, with many findings generalizable to African Americans. A protective effect of schizophrenia PRS on tinnitus was driven by schizophrenia-specific effects. Our LabWAS yielded positive associations between schizophrenia PRS and cholesterol levels and white blood cell count and negative associations with glucose and electrolyte levels.

Conclusions: Using current neuropsychiatric PRS, we have demonstrated the validity of EHR-based phenotyping approaches across diverse US populations. Our PheWAS uncovered novel associations and replicated previously reported relationships between schizophrenia, bipolar disorder, and major depression PRS and a range of clinical traits and outcomes, highlighting the potential of PRS for disentangling biological and mediated pleiotropy.

Keywords: Polygenic Risk Scores, DNA, Whole-Genome, Sequencing, Schizophrenia, Schizophrenia, Bipolar Disorder, Major Depression, Diagnosis

Disclosure: Nothing to disclose.

P576. Testing Amphetamine Effects on the Therapeutic Impact of Targeted Cognitive Training in Antipsychotic-Medicated Schizophrenia Patients: Study Design and Feasibility

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Background: Computerized targeted cognitive training (TCT) improves neurocognition and function in patients with schizophrenia (SZ). This TCT consists of a suite of exercises that target auditory and verbal working memory and verbal learning performance, with an iterative delivery that continuously adjusts task difficulty. While many patients exhibit lasting gains after 20-30 hours of TCT training, some exhibit only modest gains, and "non-response" rates of 40-50% are reported even after 40 hours of TCT. This heterogeneous TCT response presumably reflects heterogeneity in the amount of learning from the TCT exercises. We hypothesize that TCT-associated gains will be enhanced and/or accelerated by interventions that enhance TCT engagement and learning. Because we know that attentional deficits impede engagement and learning, we hypothesized that interventions that enhance attention in patients with attentional deficits will also enhance clinical gains from TCT. Consistent with this hypothesis, we reported that the pro-attentional drug, d-amphetamine (AMPH; 5 or 10 mg po), enhances learning during the TCT "Sound Sweeps" module in antipsychotic (AP)-medicated SZ patients, and specifically among patients with attentional deficits. We are now studying the impact of AMPH (5 mg) on the therapeutic impact of 30 hours of training from a full TCT suite in AP-medicated SZ patients. Here we describe our study design and initial "feasibility" data.

Methods: Carefully characterized male and female AP-medicated SZ or schizoaffective disorder (depressed) patients are assessed for baseline neurocognitive function (MATRICS Comprehensive Cognitive Battery; MCCB). Next, on two test days separated by one week, comprehensive measures of clinical symptoms and function are obtained, together with potential predictive behavioral (TCT "Sound Sweeps" performance), electroencephalographic (EEG: event-related potentials, auditory steady-state response) and auditory fidelity measures (Words-in-Noise; Quick Speech-in-Noise), after either placebo (Test 1) or AMPH (5 mg po; T2). For the next 10-12 weeks, participants complete 30 training sessions of a complete TCT suite (1-1.5h/session; 2-3 sessions/week), 60 min after ingesting placebo or AMPH (5 mg) in a double-blind, randomized design. Outcome measures (MCCB, clinical symptom and function scales) as well as EEG and auditory fidelity measures are acquired after 10, 20 and 30 sessions of TCT training, and 12 weeks post-training (final target $n = 54$; blinding to be broken at $n = 10$ for preliminary analyses).

Results: To date, 5 AP-medicated subjects (M:$F = 3{:}2$; age (mean (range)) = 39 (33-47)) have completed screening, test days 1-2 and at least 10 sessions of TCT training with post-TCT testing (divided across the 2 drug conditions); 3 have completed 30 hours of training, 1 has also completed 12-week follow-up testing. Data are reported up to and including post-TCT session 10. There have been no adverse events, and there has been no participant attrition despite heavy training and testing schedules. Clinical ratings for general and psychosis-specific psychiatric symptoms (PHQ-9, BPRS, PANSS, PSYRATS, YMRS) and suicidality (CSSR-S), as well as neurocognitive performance (MCCB) were unchanged or modestly improved between baseline and post-TCT session 10. Blinded drug group sizes are too small to permit meaningful analyses of AMPH effects but updated larger group comparisons will be reported.

Conclusions: Testing the effects of AMPH on the therapeutic impact of TCT in AP-medicated SZ patients is feasible. Participants to-date exhibit no evidence of "test fatigue" or other adverse effects of testing, training or AMPH exposure. In this small sample, changes from baseline symptoms, neurocognition and function are not pronounced after 10 sessions of TCT training, though blinding prevents any assessment of drug effects at this point. Unblinded preliminary analyses will be presented after completing $n = 10$ subjects.

Keywords: Computerized Cognitive Training, Schizophrenia (SCZ), Amphetamine

Disclosure: Nothing to disclose.

P577. Anthranilic Acid and 3-Hydroxykynurenine Plasma Levels: Effect of Antipsychotic Drugs

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Background: Converging evidence suggests dysregulation of the kynurenine (Kyn) pathway of tryptophan (Trp) metabolism in schizophrenia. Kyn is a substrate for formation of 3-hydroxykynurenine (3HK), and kynurenic (KYNA) and anthranilic (AA) acids. A postmortem study revealed reduction of gene expression and deficient activity of kynurenine-3-monooxygenase (KMO), enzyme that catalyzes Kyn conversion into 3HK, in brains of schizophrenia patients[1]. KMO deficiency increases Kyn availability as a substrate for formation of KYNA and AA. Elevated (at the expense of 3HK formation from Kyn) concentrations of KYNA, an NMDA receptor antagonist, were found in brains and CSF (but not plasma/serum levels) of schizophrenia patients [2,3], and suggested to be causally associated with major psychopathology of schizophrenia (KYNA hypothesis of schizophrenia)[4]. Elevated AA levels were previously reported in serum[5] and plasma[6] of schizophrenia patients. However, we are not aware of studies that evaluated the effect of antipsychotic treatment on plasma AA levels in schizophrenia.

Methods: Severity of schizophrenia was assessed by the Positive and Negative Syndrome Scale (PANSS). Plasma levels of Trp and kynurenines were evaluated (HPLC/MC)[7] in fasting plasma samples of schizophrenia patients before and after treatment with risperidole ($n = 21$), olanzapine ($n = 10$), or seroquel ($n = 11$). The study was approved by University of Magdeburg Review Board, and written informed consent was obtained. Paired test was used to assess statistical significance of the obtained data.

Results: PANSS scores decreased from 88.3 to 56.7 ($p < 0.0001$). Plasma levels of 3HK were increased by 47% from 14.64 to 21.94 µmol ($p < 0.001$), while AA levels were decreased by 64% from 16.36 to 10.56 µmol ($p < 0.006$). There was smaller elevation of Kyn from 1.32 to 1.67 nmol ($p < 0.0001$) and Trp levels from 49.86 to 55.34 nmol ($p = 0.055$). Plasma KYNA and XA levels were unchanged. There were no gender differences of plasma levels of studied metabolites after antipsychotic treatment.

Conclusions: This is a preliminary report of the first observation of drastic decrease of plasma AA levels after 6 weeks of treatment with antipsychotics. Concurrent elevation of 3HK levels suggests that antipsychotic treatment attenuates KMO deficiency, and restores the pattern of downstream Kyn metabolism to preferential (under physiological conditions) conversion of Kyn into 3HK and KYNA, rather than AA [8]. Plasma levels of measured metabolites most likely reflect their brain concentrations, except KYNA that does not penetrate the blood-brain-barrier [9]. Evaluation of AA plasma levels might be developed into a marker of KMO activity in schizophrenia patients. Future studies of large patient populations might allow to analyze gender differences and correlation of plasma AA levels with severity of schizophrenia symptoms after antipsychotic treatment.

References:

1. Wonodi I, Stine CO, Sathyasaikumar KV, Robert RC, Mitchell BDL, Elliot Hong L, et al. Downregulated Kynurenine 3-Monooxygenase Gene Expression and Enzyme Activity in Schizophrenia and Genetic Association With Schizophrenia Endophenotypes. Arch Gen Psychiatry. 2011;68:665–674. https://doi.org/10.1001/archgenpsychiatry.2011.71

2. Schwarcz, R., Rassoulpour, A., Wu, H.Q., Medoff, D., Tamminga, C.A., Roberts, R., 2001. Increased cortical kynurenate content in schizophrenia. Biol. Psychiatry 50, 521–530

3. Erhardt S, Blennow K, Nordin C, Skogh E, et al. (2001). Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. Neuroscience Letters 313, 96–98

4. Erhardt, S., Schwieler, L., Nilsson, L., Linderholm, K., Engberg, G., 2007. The kynurenic acid hypothesis of schizophrenia. Physiol. Behav. 92, 203–209.

5. Fazio, F., Lionetto, L., Curto, M., Iacovelli, L., et al. 2015. Xanthurenic acid activates mGlu2/ 3 metabotropic glutamate receptors and is a potential trait marker for schizophrenia. Sci. Rep. 5, 17799–17813. https://doi.org/10.1038/srep17799 (PMID: 26643205).

6. Oxenkrug, G., van der Hart, M., Roeser, J., Summergrad, P., 2016. Anthranilic Acid: A Potential Biomarker and Treatment Target for Schizophrenia. Ann Psychiatry Ment Health 4, 1059-1062. pii: 1059.PMID: 27042691

7. Oxenkrug G, H-G Bernstein, P. Guest, P. Summergrad, J. Steiner. (2019) Plasma Anthranilic Acid Correlates With Obesity but Not Insulin Resistance Markers in Schizophrenia. Neuropsychopharmacology, 44, 166 https://doi.org/10.1038/s41386-019-0545-y

8. Moroni F. Tryptophan metabolism and brain function: focus on kynurenine and other indole metabolites. European Journal of Pharmacology 375 1999 87–100

9. Fukui S, Schwarcz R, Rapoport SI, et al. Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. J Neurochem 1991; 56(6):2007-2017.

Keywords: Schizophrenia, Antipsychotics, Anthranilic Acid, 3-hydroxykynurenine

Disclosure: Nothing to disclose.

P578. A Cortico-Cortical Circuit Underlying Adolescent Stress-Induced Social Cognitive Impairment in the Postpartum Period

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Background: Early life stress, such as psychosocial stress during adolescence, is known as the predominant risk factor for the development of postpartum mental disorders. Nonetheless, the biological mechanisms by which adolescent psychosocial stress influences postpartum behaviors later in life have not been well characterized. Previously, we have established a novel mouse model in which mild adolescent isolation stress results in social behavioral deficit at one week postpartum. Furthermore, our in vivo microdialysis study revealed that our novel mouse model showed an aberrant reduction of extracellular glutamate levels in the prelimbic cortex (PrL). Human imaging studies have linked functional changes in the anterior insula (AI) and dorsal anterior cingulate cortex [dACC, homologous to the PrL in rodents] to postpartum behavioral changes. Based on our in vivo microdialysis data and on human imaging studies conducted by other groups, we examined whether adolescent social isolation, in conjunction with the stressful events of pregnancy/delivery, leads to deficits in postpartum behaviors related to social cognition via functional alternations of the glutamatergic pathway from the AI to the PrL.

Methods: To perform optogenetics for neuronal manipulation, adeno-associated viruses (AAV) 5-Syn-FLEX-ChrimsonR or AAV5-EF1a-DIO-eNpHR3.0 viruses were injected into the AI of virgin vesicular glutamate transporter 1 (Vglut1)-Cre female mice at 5 weeks of age. To conduct in vivo calcium imaging with a mini-

epifluorescence microscope for activity readouts, we injected AAV expressing the calcium indicator GCaMP6f in a conditional manner (AAV1-Syn.Flex-GCaMP6f) and implanted a gradient refractive index lens above the PrL at the same time. This strategy enabled us to manipulate the glutamatergic projections from the AI to the PrL and monitor the calcium dynamic of glutamatergic neurons in the PrL at the same time. After surgery, animals were exposed to mild social isolation during late adolescence (from 5 to 8 weeks of age), which alone caused no endocrine or behavioral changes. Each mouse was then mated with a C57BL/6J male and gave birth to pups. Three-chamber social interaction tests (SIT) were performed at one week postpartum. During SIT, calcium image recording with or without optical manipulation of the AI-PrL pathway was conducted. Single-cell neural signals were extracted with regions of interest (ROI) detection using principal and independent component analysis, and analyzed with receiver operating characteristic (ROC) analysis to quantify each neuron's response during social interaction events.

Results: Calcium imaging of glutamatergic neurons in the PrL during SIT was used to identify the subsets of neurons that are excited or suppressed during interactions with a familiar or novel mouse. We found that the inhibition of sniffing behaviors to a novel mouse in stressed dams was accompanied by the decreased fraction of excited glutamatergic neurons and the increased fraction of suppressed glutamatergic neurons during interactions with a novel mouse. Optical activation of the AI-PrL pathway in stressed dams increased the time they spent sniffing a novel mouse by normalizing the disturbance in the excited and suppressed glutamatergic neurons during interactions with a novel mouse. In contrast, optical inhibition of the pathway in unstressed dams led to behavioral deficits in social novelty recognition. Under the condition of neural silencing, the fraction of excited glutamatergic neurons was decreased while the fraction of suppressed glutamatergic neurons was increased in the PrL during interactions with novel mice. The fractions of excited or suppressed glutamatergic neurons in the PrL during interactions with a familiar mouse were not affected by social isolation nor optical manipulation of the pathway.

Conclusions: Our present study demonstrated that adolescent social isolation, in conjunction with the stressful events of pregnancy/delivery, induces hypofunction of the AI-PrL pathway and leads to subsequent social behavioral deficits in the postpartum period. These data provide the opportunity to understand the causal role of the AI-PrL pathway, a novel cortico-cortical circuit, in postpartum social novelty recognition. Our novel mouse model may be a promising model to study the pathological mechanism underlying postpartum behavioral deficits and to explore novel therapeutic strategies for it.

Keywords: Social Cognition, Adolescent Stress, Postpartum, Prelimbic Cortex, Anterior Insula

Disclosure: Nothing to disclose.

P579. Gaba Alteration in Patients With Ultra-Treatment-Resistant Schizophrenia: A 1H-MRS Study

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Background: Gamma-Aminobutyric Acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system. GABAergic dysfunction has been implicated in the pathophysiology of schizophrenia. Clozapine, the only approved drug for treatment-resistant schizophrenia (TRS), involves the GABAergic system as one of its targets. However, no studies have investigated the relationship between brain GABA levels, measured by proton magnetic resonance spectroscopy (1H-MRS), and clozapine response in patients with TRS.

Methods: This study enrolled patients with TRS who did not respond to clozapine (ultra-resistant schizophrenia: URS) and who responded to clozapine (non-URS), patients with schizophrenia who responded to first-line antipsychotics (first-line responders: FLR), and healthy controls (HCs). We measured GABA levels in the midcingulate cortex (MCC) using 3T 1H-MRS MEGA-PRESS and compared these levels between the groups. The associations between GABA levels and symptom severity were also explored within the patient groups.

Results: A total of 91 participants (URS: $n = 22$; non-URS: $n = 21$; FLR: $n = 15$; HCs: $n = 33$) completed the study. We found overall group differences in GABA levels after adjusting for smoking status. Specifically, patients with URS showed higher GABA levels compared to HCs. GABA levels in the MCC showed no associations with any of the symptom severity scores within each group or the patient group as a whole.

Conclusions: Our study is the first to report elevated GABA levels in the MCC in patients with schizophrenia resistant to clozapine treatment. Longitudinal studies are required to evaluate if GABA levels are a suitable biomarker to predict clozapine resistance.

Keywords: Clozapine, Schizophrenia Novel Treatment, GABA, 1H-MRS

Disclosure: Nothing to disclose.

P581. Effect of Daridorexant on Sleep Macro-Architecture by Quarter of the Night in Patients With Insomnia: Exploratory Analysis of Data From an International, Randomized, Double-Blind Placebo-Controlled Phase 3 Trial

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Background: In two pivotal phase 3 trials (Trial 1: NCT03545191; Trial 2; NCT03575104), we evaluated the effect of daridorexant on sleep macro-architecture over the whole night in adults and elderly patients with insomnia disorder. Daridorexant improved sleep variables without altering the proportion of sleep stages at Month (M) 1 and M3 of treatment. The current analysis further examines sleep macro-architecture by quarter of the night (QoN) in patients with insomnia from Trial 1.

Methods: Patients eligible for the trial were aged ≥18 years, had insomnia disorder (per DSM-5) with an insomnia severity index [ISI] score ≥15, and a self-reported history of disturbed sleep on >3 nights per week. Patients ($N = 930$) were randomized (1:1:1) to double-blind treatment with oral daridorexant 25mg, 50mg, or placebo nightly for 3 months. Total sleep time (TST) and sleep stages (non-rapid eye movement [NREM, N]1, N2, N3, REM) were measured by polysomnography (PSG) in a sleep laboratory on two consecutive nights during single-blind placebo run-in period (baseline, BL) and at Months 1 and 3 (M1, M3) of treatment. Change from baseline in TST and sleep stages by the quarter of the night were exploratory endpoints. Data are presented as change from baseline in absolute time (min).

Results: Among 930 randomized patients, the mean age was 55 years and 67% were female. At BL, mean wake time after sleep onset ranged from 95–103 minutes across the three groups, mean latency to persistent sleep ranged from 64– 67 minutes, mean total sleep time ranged from 319-328 minutes, and mean ISI

scores were 19 in each group. Data from 923 patients (363 aged ≥65 years) were included in this analysis.

TST spent in N1, N2 and REM over the whole night increased from BL in each treatment group by 2-7 min, 16-38 min, and 9-16 min respectively, with no to little change in time spent in N3. The proportion of TST spent in each sleep stage over the whole night was similar in each treatment group (daridorexant 25mg, 50mg, and placebo): the proportion of time spent in N2 and REM increased, the proportion spent in N3 decreased, and the proportion spent in N1 was unchanged.

The absolute increase from BL in N1 was similar in each group and in the order of 1–2 minutes in all quarters of the night; however, the proportion of N1 decreased in the first quarter of the night (Q1) but was unchanged in subsequent quarters (Q2–4) in all treatment groups.

The absolute increase from BL in N2 was consistently higher in recipients of daridorexant than placebo in Q1–4, with the largest increase (14-15 min in daridorexant recipients) observed in Q1. Despite the largest increase in duration of N2 in Q1, the proportion of TST spent in N2 in Q1 was similar to BL (52-57%) in all groups. In contrast, the proportion of TST spent in N2 increased in Q2–3 in all groups, and in Q4 in recipients of daridorexant only.

The absolute time spent in N3 increased in Q1 by approximately 5–7 min in daridorexant recipients, then decreased throughout Q2–4 by approximately 2 min across all groups. The proportion of TST in N3 remained unchanged from BL in Q1 (21 -23%) and decreased to a similar extent in all groups in Q2–4.

The absolute time spent in REM increased in all groups across Q1–4. The change in the proportion of time spent in REM during Q2–4 was similar across all groups.

Conclusions: Daridorexant increased the time in most sleep stages over the whole night while the proportion of each sleep stage was comparable to placebo, suggesting preservation of sleep macro-architecture. The largest changes were seen in Q1, but effects were still detected until Q4 suggesting overall benefit of daridorexant throughout the night and preservation of sleep stage cycle progression. Following daridorexant treatment, the increase in time spent in N2 together with the increase in REM sleep in the first quarter of the night suggests that these stages are involved in generating better sleep quality in patients with insomnia. However, it remains to be determined how these subtle changes in sleep macro-architecture are reflected in the symptoms of insomnia, such as daytime functioning.

Keywords: Sleep Architecture, Insomnia, Dual Orexin Receptor Antagonist

Disclosure: Employee of Idorsia (trial sponsor): Employee (Self)

P582. Effects of Daridorexant and Zolpidem on the Distribution of Wakefulness Throughout the Night in Adults With Insomnia: Exploratory Analysis of Data From an International, Randomized, Double-Blind, Placebo-Controlled Phase 2 Trial

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Background: A key symptom of insomnia disorder is the increased duration of wake bouts during the night. Daridorexant, a new dual orexin receptor antagonist, reduced wake time after sleep onset (WASO) in patients with insomnia disorder in a phase 2 dose-finding study. The objective of this post-hoc analysis was to evaluate the duration and progression of wake bouts during treatment with daridorexant, in comparison to zolpidem or placebo over an 8-hour polysomnography (PSG) night after 1 day and 29 days of treatment.

Methods: Eligible patients were aged 18–64 years with insomnia disorder as per DSM-5 criteria, a self-reported history of time to fall asleep ≥30 minutes, WASO ≥ 30 minutes, and total sleep time (TST) ≤ 6.5 hours on ≥3 nights per week. After a single-blind, placebo run-in period, patients were randomized (1:1:1:1:1:1) to double-blind treatment with oral daridorexant (5, 10, 25, or 50 mg), zolpidem 10 mg or placebo for 30 days.

The primary efficacy outcome was the change in wake time after sleep onset (WASO) from baseline to Days 1 and 2 as measured by PSG. In the current analysis, wake bouts at baseline, Day 1, and Day 29 were examined in patients who received daridorexant 25 or 50 mg, zolpidem or placebo. Day 1 and 29 were chosen to assess the immediate and 1-month effects of daridorexant, respectively. Daridorexant 25 mg and 50 mg doses were selected for this analysis as they have demonstrated efficacy on sleep variables in pivotal phase 3 trials (NCT03545191).

Analyses were conducted using wake bout data for every patient, computing the cumulative time in wake bouts over the whole 8-hour PSG night, in half minute intervals (i.e., 1 epoch). Data were aggregated and averaged across patients within each treatment group.

Results: Among 359 randomized patients, the mean age was 45 years and 64% were female. This post-hoc analysis included data from patients treated with daridorexant 25 mg ($n = 60$), 50 mg ($n = 61$), zolpidem 10 mg ($n = 60$) and placebo ($n = 60$).

At baseline, there was no difference in cumulative average time in wake bouts between treatment groups. The cumulative average time in wake bouts progressively increased to a greater extent with zolpidem and placebo as compared with daridorexant 25 mg and 50 mg at Day 1 and Day 29. At Day 1 and Day 29, most of the time awake after sleep onset occurred during the last two quarters of the night in patients in each of the four groups.

During the last two quarters of the night, the cumulative average time in wake bouts was similar in recipients of placebo and zolpidem on Day 1 and greater in recipients of zolpidem than placebo on Day 29, indicating an increase in time spent awake when compared to the first two quarters of the night. Daridorexant 50 mg resulted in consistently lower cumulative average time in wake bouts than either zolpidem or placebo during the last two quarters of the night while the cumulative average time in wake bouts was similar in recipients of daridorexant 50 mg and zolpidem during the first two quarters of the night. This reduction of time spent in wake bouts was statistically significant in the last part of the night with daridorexant 50 mg when compared to zolpidem.

All treatments increased the number of short wake bouts and reduced longer wake bouts on Day 1 and Day 29 compared to baseline, with the biggest effect observed on daridorexant 50 mg thus reducing the number of awakenings that contributed most to the total time awake. Differences in cumulative wake time between daridorexant, zolpidem and placebo were driven largely by reductions in the amount of time spent in longer wake bouts in recipients of daridorexant.

Conclusions: In this analysis, daridorexant (25 and 50 mg) consistently reduced wakefulness throughout the entire night when compared with placebo; this maintenance of sleep throughout the entire night on daridorexant might be explained by the optimized pharmacokinetic profile of daridorexant. Patients who received daridorexant had less wakefulness during the second half of the night when compared with zolpidem 10 mg and placebo. Daridorexant reduced wakefulness mainly by decreasing the duration of longer wake periods, thereby targeting a key symptom of insomnia.

Keywords: Daridorexant, Dual Orexin Receptor Antagonist, Insomnia Disorder, Sleep Architecture

Disclosure: Nothing to disclose.

P583. Sex-Specific Sleep Characteristics Among Adults With Opioid, Cannabis and Cocaine Use Disorder

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Background: Sex differences exist in substance use disorder (SUD) risk and treatment. Thus, achieving a deeper understanding of the role that sex, as a key biological variable, plays in SUD is warranted. One area receiving increased attention as a target for SUD is sleep. Sleep and SUD demonstrate a bi-directional relationship, bringing complexity to SUD trajectories. Substance use itself can negatively impact sleep quality. Simultaneously, sleep health may heighten or buffer risk for SUD development and treatment response. In the general population, sex differences exist across sleep parameters, with sleep disturbance generally being more common among females than males. Prior efforts attempting to assess sex-specific associations between sleep and SUD have been limited and inconclusive. The study objective was to compare, within sex, sleep parameters between individuals with SUD and nonsubstance misusing controls.

Methods: This preliminary, hypothesis generating study included secondary analyses of a parent cross-sectional study examining the feasibility and acceptability of a novel neurofunctional phenotyping assessment battery (NIDA PhAB). The PhAB is designed for eventual use in clinical trials to allow for classification of individuals with SUD along neurofunctional domains (e.g., behavioral phenotype), and to eliminate heavy reliance on DSM-5 criteria and primary drug of use to determine treatment strategies. In brief, subjects with SUD were 18 to 70 years old and met DSM-5 criteria for a current SUD with opioids, cannabis, and/or cocaine as the primary drug diagnosis. Severe comorbid alcohol use disorder was exclusionary. Non-substance misusing controls met the same criteria, with the exception that they could not meet DSM-5 SUD criteria. SUD and control subjects were recruited through local advertising and an established research registry. Subjects with SUD were also recruited through a university-based outpatient SUD treatment clinic. Self-reported sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI). This 19-item self-report tool assesses overall sleep quality (range 0-21; higher scores indicate worse global sleep) along with seven component scores (range 0-3, higher scores indicate worse sleep). Sex-stratified *t*-tests compared sleep between SUD and control subjects while Crosstab analyses explored group differences in the proportion of individuals reporting poor sleep (defined as PSQI > 5).

Results: Data from 162 males (44 controls, 118 SUD) and 146 females (64 controls, 82 SUD) were included in the present study. More SUD subjects identified as Black race (males 79%, females 73%) compared to controls (males 39%, females 39%). Among male SUD subjects, about a third had a primary drug diagnosis for opioid use disorder (OUD; $n = 53$), followed by cocaine ($n = 37$) and cannabis ($n = 28$) use disorder. For female SUD subjects, OUD ($n = 46$) was the most common primary drug diagnosis followed by cannabis ($n = 22$) and cocaine ($n = 14$) use disorder.

In males, PSQI global scores were better among controls (M = 5.11, SD = 3.08) than in SUD subjects (M = 6.92, SD = 3.72), $t(159) = -2.88$, $p = .005$. Male controls also had significantly lower PSQI global scores than males with OUD (M = 7.62, SD = 3.80) and cannabis use disorder (M = 7.36, SD = 3.43), $p = .001$, and .005, respectively. Generally, male controls reported statistically shorter sleep latency, longer sleep duration, and less sleep disturbances than SUD males with any primary drug diagnosis. In females, PSQI global scores were better among controls (M = 5.66, SD = 2.53) than in SUD subjects (M = 7.27, SD = 2.99), $t(143) = -3.45$, $p = .001$. Female controls also had significantly lower PSQI global scores than females with OUD (M = 7.63, SD = 2.83) and cannabis use disorder (M = 7.19, SD = 3.12), $p < .001$ and .05, respectively. Unlike their male counterparts, female controls did not report any PSQI component that was statistically better across all primary drug diagnoses for SUD subjects. Lastly, the proportion of males who reported PSQI-defined poor sleep did not differ between controls and SUD subjects, $\chi2(1, N = 161) = 2.05$, $p > .05$, nor primary drug diagnosis SUD subgroups. However, for females, a significantly lower proportion of controls reported PSQI-defined poor sleep than SUD subjects, $\chi2(1, N = 145) = 5.64$, $p < .05$, or subjects with a primary drug diagnosis of OUD, $\chi2(3, N = 145) = 8.63$, $p < .05$.

Conclusions: Sleep problems and SUD substantially overlap neurobiologically as well as in their socio-ecological complexity. Sleep dysfunction and SUD differ by sex, as sex is one of the critical variables that shape an individual's overall health and daily functioning. In our sample of individuals with SUD, we found worse sleep quality compared to a control group across sex, but clinically significant poor sleep was more prevalent only among SUD females compared to their control counterparts. These sleep differences were most notable for individuals with opioid and cannabis use disorder. Overall, our findings found that sleep holds promise as an avenue to address SUD within a biopsychosocial model. Future work at the intersection of SUD and sleep should prioritize investigations of their interplay with sex to identify targets for tailored SUD interventions. These findings have important implications for providers and addiction researchers especially given the increased awareness of SUD amongst the overdose crisis.

Keywords: Sleep Disturbances, Sex Differences, Opioid Use Disorder, Cocaine Use Disorder, Cannabis Use Disorder

Disclosure: Nothing to disclose.

P584. Assessing the Abuse Potential of Daridorexant, an Investigational, New Dual Orexin Receptor Antagonist: Nonclinical Examinations in Rats

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Background: Orexin neuropeptides stabilize wakefulness and promote vigilance. This is particularly important at the end of the day when orexin neuropeptide levels rise to compete with increasing sleep pressure. Orexins also increase arousal and attention levels in response to internal and external environmental stimuli, are secreted during pleasant experiences, and enhance motivation to gain rewards.

Dual orexin receptor antagonists (DORAs), including the FDA-approved drugs suvorexant and Lemborexant, promote sleep and are indicated for the treatment of insomnia. Daridorexant is a new DORA under investigation for the treatment of insomnia in adults. It was developed with an optimized pharmacokinetic and pharmacodynamic profile for timed action covering the night. It is currently under FDA review.

Regulatory safety requirements for sleep medications include nonclinical testing for abuse potential. Such testing is required for DORAs despite the known biological function of the orexin system in the brain reward pathway, based on which antagonism of orexin receptors is expected to have "anti-rewarding" properties and not to induce reinforcement.

We used a well-established set of animal experiments for assessing the abuse potential of daridorexant, fulfilling regulatory requirements. Neither suvorexant nor lemborexant have so far revealed signals of abuse potential in nonclinical studies at clinically relevant concentrations. Real-world evidence (based on reports from FEARS, NSDUH, and other US federal surveys) for suvorexant, the first DORA approved in

2014, has so far only revealed very rare cases of non-medical use or abuse, which supports the absence of such findings in animal studies.

Methods: Potential reinforcing properties of daridorexant were tested in an intravenous self-administration model using female rats that had been originally trained to self-administer cocaine. Following successful training, vehicle or daridorexant (0.1, 0.3, and 1 mg/kg/infusion) were substituted for cocaine during a total of 6 daily substitution sessions ($n = 9$/group).

Interoceptive similarity of vehicle or daridorexant (15, 30 and 60 mg/kg; p.o.) to the standard sleep medication and positive GABA-A receptor modulator zolpidem (3 mg/kg; p.o.) was tested in an operant drug-discrimination study in female rats ($n = 11$/cross-over design).

Physical dependence to daridorexant was tested by monitoring possible withdrawal symptoms in female rats upon treatment discontinuation following chronic (4 weeks) oral gavage with 20 or 200 mg/kg/d of daridorexant ($n = 10$/group). The prototypical benzodiazepine and sleep-inducing agent chlordiazepoxide (up-titrated to 200mg/kg/d) was used as a positive control.

Results: Daridorexant did not support self-administration in rats with a previous history of cocaine self-administration. Rats infused with daridorexant or vehicle showed reduced responding starting upon the first substitution session as compared to the baseline with cocaine ($p < 0.01$; ANOVA). Furthermore, the rate of extinction of cocaine-seeking behavior during the substitution phase was similar between all daridorexant doses and vehicle (ANOVA; $p > 0.4$).

The potential interoceptive effects of daridorexant did not generalize to those of zolpidem. Rats treated with different doses of daridorexant almost exclusively selected the operant lever that had previously been associated with vehicle. This was true for the first food reward obtained and the entire test session. The response rate with daridorexant was not different from vehicle (ANOVA; $p > 0.7$).

Discontinuation of chronic daridorexant treatment did not induce any symptoms indicative of withdrawal. During the chronic treatment period, daridorexant caused a slight reduction of body temperature and locomotion, consistent with its pharmacological, sleep-promoting action. Upon discontinuation of treatment, body temperature and locomotion normalized. No relevant changes in physiological, neurobehavioral, or locomotor activity indicative of withdrawal were noted. In contrast, upon discontinuation of chronic chlordiazepoxide treatment, rats showed a marked decline in body weight (ANOVA; $p < 0.01$), paralleled by a reduction in food consumption (ANOVA; $p < 0.01$), and occurrence of neurobehavioral signs such as piloerection and chewing.

Conclusions: In rats, daridorexant did not evoke any signs indicative of abuse or dependence potential in the three well-established models employed at exposures comparable to or exceeding the human exposure; similar to what was observed previously with suvorexant and lemborexant. These nonclinical data do not indicate any potential for abuse of daridorexant in humans.

Keywords: Abuse Liability, Dual Orexin Receptor Antagonist, Rat Models, Drug Discrimination, Self-Administration

Disclosure: Nothing to disclose.

P585. Respiratory Safety of Lemborexant in Adult and Elderly Subjects With Moderate to Severe Obstructive Sleep Apnea

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Background: Central respiratory depression is a safety risk of commonly prescribed sleep-promoting drugs (benzodiazepine and nonbenzodiazepine receptor agonists), especially in vulnerable elderly individuals and/or those with concurrent pulmonary disorders (i.e., obstructive sleep apnea [OSA]). It is important to determine if sleep-promoting drugs with different mechanisms of action would present similar risks. Lemborexant (LEM) is a dual orexin receptor antagonist (DORA) approved for the treatment of insomnia in several countries, including the United States, Japan, Canada and Australia. In a previous study with the higher approved dose of LEM (10 mg, LEM10), there were no differences between LEM10 and placebo (PBO) on peripheral oxygen saturation (SpO2) and the apnea-hypopnea index (AHI) in adult and elderly subjects with mild OSA following single and multiple doses (NCT03471871). The current study, a post-marketing requirement, will be the first to establish the respiratory safety of LEM and DORAs in patients with moderate to severe OSA.

Methods: This study (NCT04647383) is a multicenter, multiple-dose, randomized, double-blind, PBO-controlled, 2-period crossover study in adult (age ≥45 to <65y) and elderly (age ≥65 to ≤90y) subjects with moderate ($15 \leq AHI < 30$) to severe ($AHI \geq 30$) OSA. All subjects have been recruited and were randomized to two 8-night treatment periods (separated by a washout ≥14d) with either LEM10 or PBO. In-lab polysomnography was performed at screening and on the first and last nights of both treatment periods.

Results: Forty-eight subjects were screened; of these, 33 (68.8%) were randomized. Only one randomized subject did not complete treatment, which was due to an adverse event classified as not being related to the study drug (COVID). The majority of subjects were White (24/33 [72.7%]) and male (23/33 [69.7% each]). Mean age was 60.6y; 22/33 subjects (66.7%) were ≥45 to <65y and 11/33 (33.3%) were ≥65 to ≤90y. Median BMI was 31.5; 8/33 subjects (24.2%) had a BMI ≥ 25 but <30, and 22/33 subjects (66.7%) had a BMI ≥ 30. During total sleep time, mean baseline SpO2 was 93.5% and mean AHI was 44.2.

Conclusions: This study will provide important new data on LEM, the first DORA to be evaluated in patients with severe OSA. These data, which will be available at the ACNP Annual Meeting, will supplement the known respiratory safety of LEM with multiple and single dosing in adult and elderly subjects with mild OSA, with those with moderate to severe OSA as objectively measured by SpO2 and AHI.

Keywords: Lemborexant, Respiratory Safety, Obstructive Sleep Apnea

Disclosure: Eisai Inc.: Employee (Self)

P586. Frontal White Matter Associations With Sleep Quality and the Role of Stress

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Background: Sleep is essential for both physical and psychological health and is a crucial physiological function of the human nervous system. An important measure of brain health is the integrity of brain connectivity structures such as white matter tracts that connect brain regions. Studies have found an association between sleep characteristics and white matter integrity. However, many factors impact both sleep and white matter measures; from relatively straightforward demographic variables to more complex factors that are harder to measure for instance environmental factors such as stress.

Our study used a sample from a rural Amish population whose members are exposed to a more homogeneous environment, compared to the general US population, for example education commonly ending in 8th grade and limited access to technology that may interfere with sleep. Our objective was to test whether the

association between sleep quality and white matter was present after specifically accounting for environmental factors such as stress.

Methods: The study included 240 members of Old Order Amish/Mennonite families [137 female and 103 males, age (37.9 ± 17.9, mean ± s.d.)] from Pennsylvania and Maryland with no major lifetime psychiatric or medical disorders. White matter integrity of 42 tracts was measured by diffusion tensor imaging to obtain fractional anisotropy values using a 3 Tesla Prisma scanner. Current stress was measured with the perceived stress scale and lifetime stress was measured with the lifetime stressor inventory. Sleep quality was determined by the self- reported Pittsburgh Sleep Quality Index (PSQI).

Results: Integrity of several white matter tracts were significantly associated with sleep quality all of which were located at the frontal lobe areas (all $p < 0.05$ after correction for multiple comparisons using the false discovery rate).

In multiple regression analyses to account for stress factors, models showed PSQI remained a significant predictor of white matter integrity in these frontal tracts after accounting for age, sex, current stress and lifetime stress (all $p < 0.01$) while current and lifetime stress were not significant predictors in any of the four models. Meanwhile, current stress was a significant predictor on sleep quality (all $p \leq 0.01$) in a model where both white matter tracts and current stress were predictors, further suggesting that current stress is linked to poorer sleep quality as expected, which was independent of and additional to the effects of these white matter tracts on sleep quality. Life-time stress was not a significant predictor of sleep quality.

Conclusions: Sleep quality was found to be significantly associated with several frontal white matter tracts that connect brain structures important in the regulation of sleep. For example, the anterior internal capsule contains fibers connecting subcortical nuclei and the prefrontal cortex. Similarly, the corona radiata contains ascending fibers that connect the thalamus to the cerebral cortex and descending fibers that connect the frontal cortex to subcortical nuclei where the pons, hypothalamus, thalamus and prefrontal cortex are important structures for the regulation of sleep and wakefulness. Stress may impact sleep and white matter integrity, but despite stress having a strong relationship with sleep, the data showed that stress level is not a significant confounder in this study of white matter integrity and sleep quality.

Furthermore, our findings are from a population with far less environmental heterogeneity than the general population which may indicate that environmental factors known to effect sleep and/or white matter such as technology and education are not major confounding factors when studying the relationship between sleep quality and white matter integrity.

In conclusion better sleep quality is associated with higher white matter integrity in frontal areas of the brain, specifically tracts that connect structures implicated in the physiology of sleep.

Keywords: Sleep, Perceived Stress, Diffusion Tensor Imaging (DTI), White Matter Integrity

Disclosure: Nothing to disclose.

P587. A Longitudinal Assessment of Decision-Making on Problematic Cannabis Use and Polydrug Use Trajectories Among Adolescent Cannabis Users

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Background: Based on recent national estimates, one out of four adult cannabis users will develop a Cannabis Use Disorder (CUD) over their lifetime. Multiple factors have been associated with CUD onset among adolescents. However, most studies have employed cross-sectional designs and few have examined the role of Decision-Making (DM) as a potential risk factor. The current longitudinal study examined the role of DM in predicting the development of problematic cannabis use and escalation in polydrug use among adolescents who use cannabis.

Methods: The study included five bi-annual assessments over two years among 315 adolescents ages 14-17, who reported having used cannabis at baseline. Decision-making (DM) was assessed at odd-numbered timepoints using the Iowa Gambling Task (IGT). Problematic cannabis use was assessed at all timepoints using the reported number of symptoms of abuse and dependence using the Structured Clinical Interview for DSM-IV Cannabis Use Disorder (CUDn) and the Marijuana Problem Scale (MPS) score. Polydrug use was assessed at all timepoints using the Drug Use History Questionnaire, based on the number of Drugs Other Than Cannabis (DOTC). Latent growth curve models were applied to examine bidirectional influences between DM and the outcomes of interest.

Results: Baseline DM ($b = .06$, $p = .635$) and the rate of change of DM ($b = .26$, $p = .08$) did not predict escalation in CUDn. Similarly, baseline DM ($b = .16$, $p = .189$) and rate of change of DM ($b = .14$, $p = .501$) failed to predict escalation in MPS scores. Neither baseline DM, nor its rate of growth were associated with escalation in the number of DOTC used. Results from post-hoc exploratory analyses revealed that a one-unit increase in the number of DOTC overtime was associated with increases in problematic cannabis use based on the DSM-IV Cannabis Use Disorder and the MPS score.

Conclusions: Results do not support a role for DM (as assessed by the IGT) as a risk factor for problematic cannabis use or polydrug use among adolescents who use cannabis. Future studies examining fine-grained aspects of DM or assessing the association under study among particular population subgroups may help to clarify our findings.

Keywords: Cannabis Use, Cannabis Use Disorder, Decision-Making, Polydrug Use, Adolescent

Disclosure: Nothing to disclose.

P588. Emotional Facial Expression During Impulsive Choice in Opioid Users Experiencing Craving

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Background: Drug craving often leads to reuse and relapse events. Craving can amplify the value of specific goods resulting in the decision to seek and consume them. Similarly, emotional states influence decision-making, and craving is often preceded or presents in conjunction with negative affective states. While much has been investigated about the neurobiology of craving and emotion, we still lack understanding about how craving relates to emotion and how these may influence decision-making so as to become a barrier to recovery, even for highly committed treatment seekers. In this study, we evaluated how different levels of craving affect emotional expression during a delay discounting task (a measure of impulsivity). Our approach involved electromyographic (EMG) analysis of muscles specifically involved in positive valence emotional expression (zygomaticus muscle) and in negative valence emotional expression (corrugator muscle). We chose facial EMG because it allows for continuous measurement of emotional expression rather than discrete self-reported measures of emotional state.

Methods: 31 Individuals with Opioid Use Disorder (OUD) who endorsed recent craving for heroin or other opioids were recruited from a methadone treatment program to participate in 2 sessions: one prior to the participant receiving methadone, and the other after, i.e., at "trough" and at "peak" methadone levels. The order of the two sessions was randomized across subjects. We employed validated instruments to assess craving, subjective withdrawal symptom severity, and current levels of anxiety. Participants then completed a 12-minute delay discounting task. We estimated a discount rate parameter for each session. Corrugator (necessary for frowning) and zygomatic (necessary for smiling) surface electromyography (EMG) were measured during the task. The resulting EMG signal was filtered (30-400 Hz) and rectified. Muscle activity was calculated as the Root Mean Square (RMS) across the session or across specific epochs within each trial.

Results: Participants reported higher craving for opioids in the session before methadone administration, than in the session after ($t = 3.66$, $P = 0.001$). In the post-medication session, the discount rate across participants was negatively correlated with zygomaticus activity (positive valence) and positively correlated with corrugator activity (negative valence). Interestingly, these relationships were inverted in the pre-medication session, such that negative valence now was inversely related to the discount rate.

Conclusions: Taken together, these preliminary results indicate that opioid users' facial emotional expression during impulsive choice is not invariant to context. In high craving states, more impulsive individuals show less negative valence expression, suggesting that craving may dampen the emotional response to decisions about immediate versus delayed rewards. Further elucidation of the physiological relationship between craving, emotion and decision-making could help understand, predict, and prevent relapse in OUD.

Keywords: Facial Emotion Processing, Opioid Addiction, Impulsivity, Craving

Disclosure: Nothing to disclose.

P589. Neurobiological and Behavioral Consequences of Repeated THC or Nicotine E-Cigarette Vapor Inhalation

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Background: The use of electronic nicotine delivery systems (ENDS) or e-cigarettes continues to be a popular method of drug delivery. Recent preclinical models of vapor inhalation have shown that exposure to vaporized Δ9-tetrahydrocannabinol (THC) may produce lasting behavioral and neurobiological changes. Further, investigations have confirmed that e-cigarette aerosols, with and without nicotine, pose a considerable risk to the developing nervous system. Understanding the lasting effects of repeated e-cigarette exposure will be critical for assessing harms and for identifying mechanisms for potential therapeutic targets.

Methods: Male Wistar rats were repeatedly exposed to vaporized Δ9-tetrahydrocannabinol (THC; 200 mg/mL), nicotine (30 mg/mL), or propylene glycol (PG) vehicle twice daily for up to 2 weeks. Rat brain tissues (hippocampus and frontal cortex) were collected for immunohistochemical and Western blot analyses. Tissues were immmunostained for cannabinoid receptor 1 (CB1), glial fibrillary acidic protein (GFAP) and ionized calcium binding adapter molecule 1 (IBA1). Western blot analyses of CB1, GFAP, IBA1, peroxisome proliferator-activated receptor-alpha (PPAR-α), and C3 compliment protein were performed using tissue from the frontal cortex. In a separate study, pregnant Wistar rats were repeatedly exposed to THC (100 mg/mL) or PG vapor for up to 2 weeks. The offspring were tested for anxiety-like behavior using the elevated plus maze procedure.

Results: Repeated THC vapor inhalation significantly reduced CB1 receptor expression in the hippocampus ($P < 0.05$). In addition, repeated exposure to PG vehicle vapor increased GFAP + cells ($P < 0.05$) and IBA1 + cells ($P = 0.06$), whereas repeated exposure to THC or nicotine resulted in differential effects. Western blot analyses confirmed partial changes in GFAP and IBA1, but only a modest change in PPAR-α or C3. Lastly, rats born to mothers exposed to repeated THC vapor exhibited decreased time spent in the open-arms compared to rats born to mothers exposed to PG vehicle, confirming the effects of prenatal e-cigarette exposure.

Conclusions: Overall, this study confirms that repeated THC vapor inhalation via e-cigarettes downregulates CB1 receptor density in rats, consistent with tolerance effects, and may produce lasting age-dependent effects on behavior. These data also suggest that repeated exposure to e-cigarette vapor inhalation may selectively modulate microgliosis and astrogliosis in rat brains.

Keywords: Electronic Cigarette (e-cigarette), delta9-tetrahydrocannabinol, THC, Nicotine

Disclosure: Nothing to disclose.

P590. Pharmacokinetic Assessment of High Affinity D4R-Selective Ligands to Attenuate Cocaine Self-Administration

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Background: Dopamine receptors (D1-like (D1R, D5R) and D2-like (D2R, D3R, D4R)) are G-protein coupled receptor proteins that regulate physiological functions such as movement, emotion, and cognition. The D4R is enriched in the prefrontal cortex where it plays important roles in cognition, attention, decision making and executive function. Studies have indicated D4R-selective ligands as a promising medication development to treat neuropsychiatric conditions, including Alzheimer's disease, ADHD and cocaine use disorders (CUD). D4R ligands have been shown to alter cognition and behavior in animal models of drug addiction and variations in the DRD4 gene are associated with novelty-seeking and risk behavior, as well as ADHD. A better understanding of D4R-mediated signaling is essential to understanding and treating D4R-associated disorders, including substance abuse disorders. Despite its clinical importance, there are currently no FDA approved medications that target the D4R and CUD treatment. The present study focuses on the design of D4R ligands based on the parental phenylpiperazine scaffold with pharmacokinetic analysis in rat and human liver microsomes, followed by preliminary in vivo behavioral analysis.

Methods: Based on the 4-phenylpiperazine scaffold, a series of high affinity and selective D4R ligands were designed by using computational modelling. Final compounds were purified and analytically characterized followed by CHN combustion elemental analysis. Their in vitro receptor affinities were determined using HEK293 cells expressing dopamine D2-like receptors (D2R, D3R, D4R). These binding studies were coupled with functional studies using β-arrestin recruitment and cAMP inhibition assays. For several D4R-selective ligands, we calculated in silico brain penetration using central nervous system multiparameter optimization of chemical features (CNS MPO) and performed Caco-2 membrane permeability tests. For selected compounds, we

performed in vitro and in vivo pharmacokinetic analysis and preliminary in vivo behavioral analysis in rats.

Results: The D4R-selective ligands predicted based on the parental scaffold were synthesized characterized. Compounds were profiled using radioligand binding displacement assays, β-arrestin recruitment assays, cAMP inhibition assays, and computational modeling. We identified several compounds with high binding affinity and D4R selectivity (Ki ≤ 100 nM and >100-fold vs. other D2-like receptors) with diverse partial agonist and antagonist profiles. Based on binding profiles, a subset of analogues was evaluated using functional assays in human D2-, D3- and D4-receptor transfected CHO cells. Several analogues displayed potent partial agonist and antagonist profiles in functional assay studies, and a few were selected for in vitro metabolic stability in rats and human liver microsomes of which some displayed acceptable stability profile. Of these CAB-01-019 (with good stability profile) was selected for in vivo pharmacokinetics in rats where it displayed excellent brain penetration with AUCbrain/plasma>3. The full antagonist CAB-01-019 (5, 15 and 30 mg/kg (IP)) was tested in preliminary cocaine self-administration studies in rats, using a within-session multidosing self-administration procedure. CAB-01-019 was found to dose-dependently decrease the number of infusions obtained for three unit doses of cocaine under an FR3 schedule of reinforcement, suggesting that the selected D4R antagonist reduced the rewarding effects of cocaine.

Conclusions: One of our most selective and high affinity D4R antagonists, CAB-01-019, was discovered as a lead drug candidate. Both in vitro rat and human metabolism data and in vivo rat pharmacokinetic data support its development. Some of the partial agonist ligands, even though metabolically stable in human liver microsomes assays, were unstable in rat species. The structural modifications on the parental phenylpiperazine scaffold backbone led to discovery of novel compounds with high D4R binding affinity and subtype selectivity. In preliminary efficacy studies, we found that the selective D4R antagonist CAB-01-019 attenuated cocaine self-administration in rats.

Keywords: Dopamine D4 Receptor, Antagonist Ligands, Partial Agonist Ligands, Cocaine Use Disorder

Disclosure: Nothing to disclose.

P591. Protracted Alcohol Abstinence Induces Proteomic Disruptions in the Dorsomedial Prefrontal Cortex That are Associated With Alcohol-Induced Cognitive Inflexibility

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Background: Alcohol (ethanol; EtOH) intoxication is known to engage homeostatic mechanisms that attempt to normalize central nervous system function, but over the course of repeated use, give rise to neuroadaptations that facilitate the transition to dependence. Under these conditions, the discontinuation of EtOH exposure induces a transient increase in excitatory (glutamatergic) signaling that is associated with varying degrees of neurotoxicity. Generally, these changes are remediated quickly during withdrawal, although the emergence of behavioral inflexibility during extended periods suggests a more lasting influence within cognitive regions of the brain. Here, we utilized proteomic and neurobehavioral analyses to explore whether withdrawal-induced hyperexcitable states mobilize an undercurrent of glutamatergic signaling processes in the medial prefrontal cortex (mPFC) that may underlie vulnerability to alcohol-induced cognitive dysfunction.

Methods: The animal studies were conducted with the approval of the Institutional Animal Care and Use Committee at the University of Texas at Austin and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Briefly, Long-Evans male rats received passive administration of vaporized EtOH or uncontaminated air ($n = 12$ per group) into their home cages (14h on/10h off) for 5-7 weeks (average blood EtOH concentration = 169.1 ± 13.3 mg/dL). Separate cohorts of rats ($n = 9$ per group) underwent similar procedures under a modified schedule consisting of 5 days of EtOH exposure and 2 days of abstinence in their home cages (average BEC = 158.1 ± 12.6 mg/dL). During abstinence, rats were trained in an operant setting to press either of 2 levers for a palatable food reward. After dependence induction, rats underwent a 10-14 day period of EtOH abstinence before testing in an operant model of strategy set-shifting. Briefly, performance was evaluated in a "set" task where the active lever was paired simultaneously with a cue light directly above it. After achieving 10 consecutive correct responses, the program sequence switched to the "shift" task where the active lever was held to one side of the chamber (left or right) independently of the cue lights. Rats proceeded with testing until criterion was reached, from which we analyzed the total trials and error types committed during testing. All rats were then humanely euthanized and the brains were extracted and dissected for the dorsal and ventral mPFC regions. This dual-region approach allowed us to compare molecular processes of cognitive behaviors thought to be functionally distinct in these brain regions. The mPFC samples were digested into fragment peptides and labeled with isobaric Tandem Mass Tags for quantitation.

Results: The analyses led to the identification of approximately 5000 proteins per brain region, of which 200-400 proteins were significantly changed by chronic intermittent EtOH exposure ($n =$ 3 biological replicates per group). Among these changes, we identified peptides belonging to the Grm2 gene encoding for metabotropic glutamate receptors (mGluRs) known to modulate glutamatergic signaling via a presynaptic mechanism. In this regard, EtOH rats experiencing withdrawal displayed reduced Grm2 levels in the ventral mPFC, as well as reductions in overlapping peptides of the Grm3 gene in the dorsal mPFC ($p <$ 0.05). We examined the potential of a mGluR2 positive allosteric modulator biphenylindadone A (BINA; 20 mg/kg, 5 mL/kg, IP) to modulate cognitive performance in the strategy set-shifting task. In this regard, EtOH rats experiencing withdrawal displayed an increase in perseverative-like errors committed during the set-shift task ($p < 0.05$). Alternatively, BINA pretreatment in EtOH rats significantly reduced the number of trials needed to achieve criterion, in part by reducing this propensity for error-prone behavior ($p < 0.05$).

Conclusions: Overall, our investigations of the mPFC proteome revealed common protein signaling elements that normally temper glutamatergic tone, but are likely downregulated as a result of CIE-induced hyperglutamatergic states. Follow-up work is utilizing phosphoproteomic and antibody capture tools to determine whether modulation of mGluR2 activity interacts with other signaling elements (NMDA or calcium-calmodulin kinases) that may stabilize glutamatergic signaling during this period of increased susceptibility to alcohol-induced cognitive dysfunction.

Keywords: Glutamatergic Transmission, Alcohol Withdrawal, Proteomics, Cognitive Function, Behavioral Flexibility

Disclosure: Nothing to disclose.

P592. A Translational Investigation of Morphine-Induced Neuroimmune Signaling: Implications for Opioid Use Disorder

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Background: Preclinical studies indicate that opioid administration evokes pro-inflammatory responses in the periphery and brain. These pro-inflammatory responses influence both appetitive and dysphoric addiction processes and thus, may contribute to the development of opioid use disorder (OUD) and/or perpetuate continued opioid use. Here, across two studies, we investigated the neuroimmune effects of morphine using Positron Emission Tomography (PET) imaging with [11C]PBR28. [11C]PBR28 is a radiotracer that binds to the 18kDa translocator protein (TSPO), a marker that is sensitive to pro-inflammatory neuroimmune stimulation.

Methods: Study #1: The first study included 10 healthy individuals with prior medical opioid exposure (3F; 34.7yrs [range = 26-49yrs]; BMI = 26.4 [range = 20-33]). In one day, subjects completed two 120-minute [11C]PBR28 PET scans: one before and one 2-hours after morphine administration (0.04mg/kg IM or 0.07mg/kg IM ['low' vs. 'high' dose]). Two subjects did not complete the post-morphine PET scan due to nausea: both received the 'high' morphine dose. Arterial blood was acquired during each scan to measure the metabolite-corrected arterial input function. Total volume of distribution (VT), i.e., TSPO availability, was estimated in 12 brain regions of interest (ROIs) using multilinear analysis-1 (MA-1; t^*=30). The effect of morphine on regional [11C]PBR28 VT was evaluated using linear mixed effects models with rs6971 Genotype ('high' vs. 'moderate' affinity binders), morphine Dose ('low' vs. 'high'), and Time ('pre-' vs. 'post'-morphine) as fixed factors and regional VT as the within-subject repeated factor. Subjects also completed the visual analogue scales (0-100mm) to measure morphine's subjective effects. Peripheral biomarker findings were not available at abstract submission but will be presented in the poster. Study #2: In non-human primates, we investigated the putative neurobiological mechanism underlying morphine's neuroimmune effects: Toll-like receptor-4 (TLR4). Two adult male rhesus macaques (9yr and 10kg; 11yr and 15kg) completed [11C]PBR28 PET imaging procedures identical to Study #1 before/after morphine (1mg/kg IM). One macaque received (+)-naloxone (1mg/kg IV) 10min prior to morphine whereas the other macaque did not receive any pretreatment. (+)-Naloxone is a TLR4 antagonist with negligible affinity for μ, Δ, or κ opioid receptors. These studies were approved by the Yale University IRB and IACUC, respectively.

Results: Study #1: A significant main effect of Time indicated that morphine increased TSPO availability across ROIs, $F(1,203) = 282.2$, $p < .001$. Reanalysis, after exclusion of one non-responder (statistical outlier), improved model fit and showed a significant Time effect, $F(1,180) = 361.7$, $p < .001$, and Dose-by-Time interaction, $F(1,180) = 13.3$, $p < .001$, which indicated that regional TSPO availability increased significantly more after the 'high' morphine dose compared to the 'low' dose (38-52% vs. 19-30%, respectively). Also, a significant Time effect indicated that morphine evoked a subjective 'high', $F(4,24) = 3.06$, $p = .036$. Controlling for morphine dose (mg), subjective 'high' at post-scan was positively correlated with the percent increase of TSPO availability in the caudate ($R2 = 0.57$, $p = .03$). Study #2: Morphine increased regional TSPO availability by 24-54% in the first macaque, consistent with our human data. Relative to the first macaque, pretreatment with (+)-naloxone attenuated morphine's effect on TSPO by 43%, on average, across ROIs.

Conclusions: First, our findings provide the first evidence that morphine evokes a neuroimmune response in people. Second, the magnitude of neuroimmune response in the caudate was linearly related to the magnitude of morphine-induced 'high' and accounted for 57% of the variance. Given the well-established role of the caudate in mesolimbic reward circuitry, our data suggest the neuroimmune signaling may modulate opioid-induced euphoria. Third, pretreatment with (+)-naloxone, a TLR4 antagonist, reduced morphine's neuroimmune effects by nearly half, suggesting that the TLR4 system is a target for medication development. Taken together, our findings highlight the role of neuroimmune signaling in opioid-induced euphoria, a process central to the development of OUD, and suggest the TLR4 system is a medication target. Future studies are needed to investigate opioid-neuroimmune relationships in OUD patients.

Keywords: Neuroimmune Activation, Opioid Addiction, Morphine, Toll-Like Receptors (TLRs), Mesolimbic Reward Circuitry

Disclosure: Nothing to disclose.

P593. Functionally and Anatomically Distinct Brain Networks Predict Stress in Subclinical Binge Drinkers

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Background: Across species, chronic alcohol consumption is associated with widespread changes in the brain and the body. These changes are especially pronounced for stress-related processes: in humans, alcohol use disorder (AUD) is associated with peripheral changes in the stress hormone cortisol as well as central alterations in the structure and function of stress-related brain networks. These stress-related alterations may play a crucial role in potentiating the development and relapse of problematic drinking. However, although peripheral stress-related alterations have also been observed in risky drinkers who do not meet criteria for dependence (e.g., Blaine and Sinha 2017), whether adaptations in stress-predictive brain circuitry are also apparent at this early stage is unknown. Here we assessed whether regular binge drinkers, an increasingly prevalent cohort at high risk for developing AUD, show distinct patterns of functional connectivity associated with stress.

Methods: We recruited 104 male and female participants ($N =$ 53 Binge drinkers/51 Light/abstinent drinkers per NIAAA criteria) to participate in a validated stress induction procedure during an fMRI scan (Sinha et al. 2016). Notably, these groups were matched in age, sex, and stress-related measures including current stress, anxiety, and depression as well as childhood history of traumatic events. We employed connectome-based predictive modeling (CPM; Shen et al. 2017) to determine whether whole-brain patterns of functional connectivity during the sustained stressor task could successfully predict subjective stress ratings in both cohorts, and developed novel analyses to nonparametrically identify differences in: (1) which brain regions contributed to successful predictions; and (2) how strongly different brain regions were associated with subjective stress. To determine the prospective validity of identified stress-predictive brain networks for Light and Binge drinkers, we collected daily diary data from our participants for 30 days following the scan to assess how engagement of these networks is associated with their subsequent real-world experiences of stress and control over their drinking.

Results: Across several models, we were able to successfully (all $p < .001$) and comparably predict subjective stress using whole-brain connectivity in Light and Binge drinkers. Despite observing no significant differences in ratings of subjective stress between groups ($p > .25$), we identified widespread group differences in the neural correlates of this stress response. All significant results were determined nonparametrically at threshold $p < .001$.

Binge drinkers engaged more connections from visual and motor regions in their stress-predictive networks, with visual and salience connections contributing significantly more to successful stress prediction in Binge than Light drinkers. At the node level,

stress-predictive networks for Binge drinkers had significantly more connections with primary visual and motor cortex, anterior cingulate, insula, and ventral tegmental area, and significantly stronger brain/stress associations in inferior temporal gyrus and cerebellum. In contrast, stress-predictive networks for Light drinkers had significantly more connections with ventromedial prefrontal cortex (vmPFC), angular gyrus, hippocampus, amygdala, and posterior cingulate, with vmPFC and angular gyrus also showing stronger brain/stress associations. Comparing the pattern of stress representations throughout the brain (following Kriegeskorte et al. 2008) revealed further group differences in cerebellar, salience, and subcortical networks.

Finally, prospective predictions revealed that stress-predictive brain networks identified for Binge drinkers (BDN) were more specialized than Light drinker networks (LDN). Engaging BDN during the fMRI scan predicted later stress levels for Binge ($b = .025$ [.008], $p = .0013$) but not Light drinkers ($p > .27$; binge vs light: $b = .02$ [.008], $p = .0041$); LDN did not show this distinction ($p > .2$). Furthermore, engaging the BDN predicted less control over future alcohol intake, whereas the LDN did not ($F1,82 = 4.78$, $p = .032$), suggesting that neural correlates of stress in Binge drinkers may be more specialized for stress-driven alcohol motivation.

Conclusions: These results suggest alcohol-induced adaptation in stress-related brain circuitry, with a shift toward overreliance on primary sensory and motor networks in predicting subjective stress responses in binge drinkers, and stronger brain/stress associations in frontoparietal and default mode networks (including vmPFC, hippocampus, and posterior cingulate) in light drinkers. These neural differences were apparent even when the subjective responses themselves (as well as the stress histories of the participants) were similar, demonstrating the utility of these analyses for uncovering group differences not apparent when examining brain or behavior alone. Interestingly, this light drinker circuitry has previously been identified as contributing to adaptive stress responses (Sinha et al. 2016; Goldfarb et al. 2020), and the differences between binge and light drinkers overlap with circuitry known to be altered in clinical AUD (e.g., Seo et al. 2013). Together, these findings indicate potential targets for early treatment intervention to mitigate the development of AUD and reveal the malleability of the neural processes that govern stress responses.

Keywords: Acute Stress, Alcohol Drinking, fMRI Functional Connectivity, Ecological Momentary Assessment, Brain Connectome

Disclosure: Nothing to disclose.

P594. Prefrontal Cortex to Ventral Tegmental Area Projection Regulates Early Social Isolation-Potentiated Heroin Seeking

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Background: Opioid use disorder 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. Stress is one of the most prominent causes of relapse. Most studies focus on stress during abstinence that triggers relapse. Less is known about how stress during early lifetime increases addiction risk and relapse vulnerability.

Adverse psychosocial factors during early childhood or adolescence compromise neural structure and brain function, inducing susceptibility for substance use disorder. Nevertheless, the mechanisms underlying early life stress-induced addiction vulnerability is still unclear, especially for opioids.

Stress and substance abuse are associated with neuroplasticity in the mesocorticolimbic pathway. For example, the maladaptation of glutamatergic projecting neurons from the prefrontal cortex (PFC) and its projecting subcortical regions (such as nucleus accumbens [NAc] and ventral tegmental area [VTA]) are implicated in both stress and addiction. Therefore, we hypothesized that ESI stress may increase addiction vulnerability via exerting pathological impairments in these key brain regions. To test our hypothesis, we used a mouse heroin self-administration model to examine how chronic early social isolation stress affects the behavioral and neural responses to heroin during adulthood.

Methods: Current study used both male and female C57BL/6J mice. Based on previous publications, early social isolation (ESI) stress was carried out after weaning from postnatal day 21 (P21) to P60 (about 5 weeks). Control mice are group housed (GH, 4-5 mice per cage). All the procedures are approved by the Institutional Animal Care and Use Committee. 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, GH and ESI mice were first trained to self-administer sucrose on fixed ratio and progressive ratio schedules. After sucrose progressive ratio test, animals underwent jugular surgery. After recovery, mice went through 10 days of heroin SA (50 ug/kg/infusion, 3 h/session), followed by progressive ratio test and dose-response test (6.25, 12.5, 25, and 50 ug/kg/infusion). Then, 14 days after the last heroin session, animals underwent 1 hour cue-induced heroin seeking test. Another set of animals were sacrificed without cue re-exposure for the study of c-Fos expression in the subregions of mesocorticalimbic system during abstinence. Circuit specific-chemogenetic tools (retrograde AAV[AAVrg]-Cre and Flex-hM3D-Gq) were used to identify the key brain pathway(s) that regulate heroin seeking under the ESI stress condition.

Results: ESI stress did not alter the acquisition for sucrose or heroin SA, nor change the motivation for sucrose on a progressive ratio schedule. However, ESI stress induced an upward shift of heroin dose response curve in female mice ($N = 11$-14/group, F1, 45 (stress) = 10.864, $P = 0.002$, F1, 45 (sex) = 8.285, $P = 0.006$), increased total responses during heroin progressive ratio test ($N = 11$-14/group F1, 45 (stress) = 15.61, $P = 0.0003$) and during heroin seeking test ($N = 7$-9/group, F1, 29 (stress) = 38.6, $P < 0.0001$) in both males and females. Furthermore, ESI stress dampened c-Fos expression in prelimbic cortex (PrL, $N = 4$/group F1,12 (stress) = 62.88, $P < 0.0001$), infralimbic cortex (IL, $N = 4$/group 62.82, $P < 0.0001$) and VTA ($N = 4$/group F1, 12 (stress) = 4.996, $P = 0.045$) after 14-day forced abstinence. To examine whether PFC to VTA projection hypofunction contributes to ESI-potentiated heroin seeking, we injected Cre-AAVrg into VTA and Cre-dependent hM3D-Gq into PrL of ESI mice during heroin abstinence. After viral expression, we injected compound 21 to repeatedly activate PFC-VTA circuit for 3 days (3 mg/kg, i.p. once per day). 24 hour after the last vehicle or C21 injection, mice underwent heroin seeking test. We found that C21 treatment significantly attenuated heroin seeking in ESI mice ($N = 7$-11/group, t13.47 = 2.5, $P = 0.02$).

Conclusions: These data indicate that ESI stress leads to increased seeking and motivation for heroin, and this may be associated with distinct changes in neuronal activities in mesocorticolimbic system, in which the PFC-VTA projection hypofunction may contribute to the ESI-potentiated heroin seeking.

Keywords: Opioid Addiction, Prefrontal Cortex, Ventral Tegmental Area, Social Isolation Stress, Chemogenetics

Disclosure: Nothing to disclose.

P595. Subtypes in Addiction and Their Neurobehavioral Impairments in Approach Behavior, Negative Emotionality and Executive Function

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Background: We applied data-driven methods to discover subtypes in individuals with past substance use disorder (SUD) from a large community sample, the NKI-Rockland Enhanced sample (NKI-RS). The NKI-RS study used a broad phenotyping approach suited to assess impairments across three broad functional domains that have been linked to addiction: 1) approach-related behavior, 2) negative emotionality and 3) executive function. To our knowledge, this is the first investigation of heterogeneity in impairments across these three domains in a sample with mixed SUD diagnoses (with different primary drugs of choice). We hypothesized to find that impairments on these three functional domains represent three independent neurobehavioral mechanisms linked to addiction, which would warrant a subtyping and personalized medicine approach for addiction treatment. Accordingly, we expected to demonstrate the existence of at least three distinct addiction subtypes, independent of the primary drug of choice: "reward users" with deviant approach-related behavior, "relief users" with high negative emotionality, and "low functioning users" with impaired executive function.

Methods: Data was collected, and ethical approval was obtained at the Nathan-Kline Institute, New York. In a subsample with complete data ($N = 644$, 66% female; 55% female within SUD; 74 assessments per individual), we first derived 12 latent phenotypic factors, using parallel analysis to determine the number of factors. Subtypes were then determined using clustering via latent profile analysis on these latent factors within those with past SUD [$N = 172$, 55% female; $N = 74$ Alcohol Use Disorder, 67% female; $N = 30$ Cannabis Use Disorder, 49% female; $N = 68$ Polydrug users, most commonly Cocaine Use Disorder, 44% female]. The optimal model was selected using the Bayesian Information Criterion. Subtypes were behaviorally characterized through their profile of impairments on these latent factors, testing for significant differences to healthy controls. Graph theory-based resting-state connectivity analyses (Brain Connectivity Toolbox) were conducted for each subtype in a subsample with complete fMRI and NIDA Quick Screen data (past SUD $N = 104$, 58.6% female; controls $N = 302$, 73.5% female). Differences in nodal global efficiency, local efficiency, and betweenness were measured for each subtype compared to healthy controls by regressing graph theory measures on lifetime number of drugs used, while covarying for past 6-month tobacco use and comorbidities.

Results: The latent factor analysis revealed the hypothesized three phenotypic domains. Individuals with past SUD demonstrated mild impairments on all three domains ($p < 0.05$, uncorrected; across all subtypes). Latent profile analysis revealed three subtypes among those with past SUD, each showing a distinct profile of impairments (all $p < 0.05$, Holm-Bonferroni corrected): (1) "Reward Users" demonstrated high sensation seeking, social risk taking, norm-breaking and openness, and decreased risk perception; (2) "Relief Users" showed high internalizing, general psychiatric symptoms and negative affect, as well as a lack of perseverance; and (3) "Lower Functioning Users" had low openness/sensitivity, crystal/fluid IQ and below-average sensation-seeking. Importantly, these three subtypes were equally distributed between individuals with different primary SUD diagnoses ($p = 0.51$), demonstrating the existence of addiction subtypes that are independent of the primary drug of choice. The resting state analysis revealed decreased connectivity (betweenness) for the supplementary motor network in reward users as compared to healthy controls, when predicting on lifetime number of drugs used (pFDR < 0.05). Relief users, conversely, showed increased connectivity (betweenness) for the attention network (7P, VIP, MIP, 6a) (pFDR < 0.05).

Conclusions: These empirical findings provide evidence for an addiction-general three-domain model, extending previous empirical work on the three-domain model in Alcohol Use Disorder to individuals with Cannabis and Cocaine Use Disorder. Crucially, our results also uncover the existence of at least three distinct "addiction subtypes" as hypothesized, demonstrating their existence independent of the primary drug of choice of an individual. The brain analysis results replicated previous findings in cocaine users (unpublished data). Overall, these results warrant future investigations into the relevance of addiction subtypes in predicting clinical outcomes and their utility in developing personalized medicine approaches. These precision medicine approaches may aid in differentially treating patients based on their individually impaired functional domains and subsequently improve treatment outcomes.

Keywords: Addiction Phenotypes, Alcohol and Substance Use Disorders, Subtypes of Substance Use Disorder, Research Domain Criteria (RDoC), Personalized Medicine

Disclosure: Nothing to disclose.

P596. Neurotensin Receptor 1 Deletion in Mice Reduces Cocaine Seeking but Not Taking

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Background: Neurotensin (NTS) is a 13 amino acid peptide that acts an endogenous modulator of brain dopamine (DA) signaling. Neurotensin modulates DA neurotransmission through action at the G protein-coupled receptor, neurotensin receptor 1 (NTSR1). The NTSR1 is expressed on DA cell bodies in the ventral tegmental area and substantia nigra, as well as on DA axon terminals in the striatum. NTSR1 signaling can be targeted pharmacologically to alter DA neurotransmission and psychostimulant (e.g., cocaine, methamphetamine)-associated behaviors. Systemic administration of a selective NTSR1 agonist is reported to transiently decrease methamphetamine self-administration in mice, and we demonstrated recently that a β-arrestin biased NTSR1 agonist can reduce cocaine self-administration. NTSR1 knockout (KO) mice are resistant to methamphetamine-induced changes in DA cell firing and exhibit reduced methamphetamine self-administration and methamphetamine-seeking behaviors. The effects of NTSR1 deletion on cocaine self-administration and cocaine-seeking behaviors, however, have yet to be determined. To this purpose, we used global NTSR1 KO mice to analyze the contribution of the NTSR1 to cocaine-induced hyperlocomotion, intravenous (iv) cocaine self-administration, and cocaine-seeking during extinction and cue-induced reinstatement using operant paradigms.

Methods: NTSR1 tm1Dgen mice (Stock #005826; Jackson Labs, Bar Harbor, ME) were acquired and bred with C57BL/6J mice. Novelty- and cocaine-induced hyperlocomotion and longitudinal iv cocaine self-administration were assessed in adult male and female mice lacking the NTSR1 (KO) and in their wild-type littermates (WT). Mice with indwelling jugular catheters were evaluated for sensitivity to novelty- and cocaine (20 mg/kg, i.p.)-induced locomotor activity (N(WT) = 25, N(NTSR1 KO) = 27). Subsequently, mice were trained to self-administer cocaine (0.5 mg/kg/infusion) paired with a cue light by lever responding in operant chambers. In once daily, 1 hr sessions, mice progressed through 2 lever and active vs. inactive lever discrimination training under FR1, FR2, and FR4 reinforcement schedules, as dictated by a contingent advancement study protocol. Once acquired, stable FR4 lever responding was assessed at 5 cocaine doses: 0.5, 0.1, 0.3, 1.0, and 3.0 mg/kg/infusion (N(WT) = 15, N(NTSR1 KO) = 20). Mice completed a single progressive ratio (PR) session with a 0.5 mg/kg/infusion cocaine dose. Dose-response and PR testing were followed by once daily 1 hr extinction sessions in which cues

were withheld and lever responses had no programmed consequences. After 20 extinction sessions, mice underwent a single cue-induced reinstatement session, in which cocaine-associated cues were presented in the absence of drug reinforcement (N(WT) = 10, N (NTSR1 KO) = 16). Mice without patent catheters and those meeting designated health endpoints were removed from the study. Two-way repeated measures or mixed model ANOVAs were used to analyze the effect of genotype on behaviors across time, drug doses, sessions, and session types.

Results: Compared to WT controls, NTSR1 KO mice exhibited reduced novelty-induced locomotor activity ($p = 0.0017$) but intact cocaine-induced hyperlocomotion. The time to reach the final criterion for the acquisition of cocaine self-administration was comparable for both genotypes (mean ± SEM, KO: 18.2 ± 1.3; WT: 16.1 ± 1.1 sessions). Notably, active lever responding was FR- and drug dose-dependent for both WT and NTSR1 KO mice. The cocaine self-administration behavior of NTSR1 KO and WT mice could not be separated based upon cocaine intake, active lever responding, inactive lever responding, latency to initiate lever responding, the fraction of responses occurring in the post-reinforcement cue period, or lever accuracy, at any of the five cocaine doses evaluated. In the PR session, WT and NTSR1 KO mice reached comparable breakpoints. Following assessment of cocaine-taking behaviors, cocaine seeking was assessed during extinction and reinstatement. Total lever responding during the 20-session extinction period was reduced in NTSR1 KO mice compared to WT controls ($p = 0.0178$). With the re-introduction of cocaine-paired cues, both genotypes displayed some degree of reinstatement of cocaine-seeking, but a genotype effect on lever responding was identified ($p = 0.0040$). In the cue-induced reinstatement session, post hoc pairwise comparisons detected a trend toward reduced active ($p = 0.0868$) but not inactive ($p = 0.7218$) lever responding by NTSR1 KO mice. No sex differences were identified, but there was a trend toward reduced cocaine intake by females as compared to males at the 0.1 and 0.3 mg/kg/infusion doses in WT mice ($p = 0.0669$).

Conclusions: NTSR1 KO mice display intact locomotor responses to cocaine and WT-like cocaine taking behaviors but reduced novelty-induced locomotion and impaired cocaine-seeking during extinction and reinstatement. In the context of the methamphetamine literature, these findings suggest the some of the mechanisms driving cocaine and methamphetamine self-administration are distinct. Our work provides further evidence that the mechanisms mediating cocaine-taking and cocaine-seeking behaviors diverge and it suggests that NTSR1 deletion selectively impairs cocaine seeking. These results may be leveraged in the development of mechanism-based therapeutics for cocaine use disorder that attenuate craving and prevent relapse.

Keywords: Cocaine, Self-Administration, Mouse Models, Neurotensin, Extinction and Reinstatement

Disclosure: Nothing to disclose.

P597. Astrocytes Participate in Amphetamine-Induced Neural Plasticity In-Vivo

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Background: Dopamine is an essential neuromodulator involved in physiological processes, such as learning and memory, motor control and reward, as well as, pathological conditions, such as Parkinson's disease, schizophrenia, and substance use disorders. In comparison to the extensive work investigating neurons, the role of astrocytes in dopaminergic signaling remains to be fully elucidated. Additionally, little is known about astrocyte involvement in plasticity effects induced by psychostimulants such as amphetamine. The current work will provide novel evidence that astrocytes are a key factor in dopaminergic signaling and psychostimulant induced plasticity and may serve as therapeutic targets for diseases with disrupted dopamine signaling.

Methods: Ethics Statement: All animal care procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC) with compliance to the National Institutes of Health guidelines for the care and use of laboratory animals. To investigate astrocyte responsiveness to dopamine in awake behaving animals we utilized fiber photometry. Both male and female adult DAT-IRES-Cre mice were used. Dual viral vector surgeries were performed to target dopaminergic neurons in the ventral tegmental area with a light activated channel (AAV5-hSyn-FLEX-ChrimsonR-tdT) and astrocytes in the nucleus accumbens were targeted with a calcium indicator, AAV5-GfaABC1D-cytoGCaMP6f-SV40. An optic fiber was placed 0.02 mm dorsal to NAc core viral infusion for calcium imaging experiments. Amphetamine locomotor experiments were performed ≥ 3 weeks after surgery. For examination of the effects of amphetamine on astrocyte responsiveness to dopamine, 2.5 mg/kg amphetamine was administered (i.p.) and astrocyte calcium elevations in response to dopamine were assessed before and after amphetamine exposure. Statistics: Data are expressed as mean ± standard error of the mean (SEM). Data normality was tested using a Kolmogorov-Smirnov test. Results were compared using a two-tailed Student's *t-test* or ANOVA ($\alpha = 0.05$). One way ANOVA with a Fisher LSD method post hoc was used for normal distributed data and Kruskal-Wallis One Way ANOVA with Dunn's method post hoc was used for non-normal distributed data. Custom MATLAB code was utilized to analyze the data.

Results: In awake behaving animals, optogenetic stimulation of dopaminergic terminals (5ms pulses for 5 s) induced astrocyte calcium elevations that were attenuated in the presence of the global dopamine receptor antagonist flupenthixol ($n = 10$, $p < 0.001$), suggesting that astrocytes respond to dopamine via dopamine receptor activation in vivo. The psychostimulant amphetamine significantly increased astrocyte responsiveness to dopamine with regards to area under the curve ($n = 15$, $p = 0.01$). Astrocyte responsiveness to dopamine was modulated by repeated exposure to amphetamine and reinstatement to amphetamine after a withdrawal period ($n = 6$, $p = 0.01$).

Conclusions: Overall, the current results indicate that astrocytes respond to dopaminergic signaling with increases in cytoplasmic calcium and participate in psychostimulant induced brain plasticity. Astrocyte calcium signaling in awake behaving animals is modulated by repeated exposure to amphetamine and by re-exposure to amphetamine after a withdrawal period suggesting that astrocytes may serve as a novel cellular therapeutic target for treatments targeting substance use disorders.

Keywords: Astrocyte-Neuron Interaction, Dopamine, Amphetamine, In Vivo Calcium Imaging, Reward System

Disclosure: Nothing to disclose.

P598. Neuron Subtype Specific Molecular Mechanisms in Fentanyl Abstinence

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Background: Opioid abuse has risen dramatically over the last decade, and potent, synthetic opioids like fentanyl are responsible

for nearly half of opioid-related deaths. Opioid withdrawal or abstinence generates a negative affective state that is thought to promote relapse. Opioid abstinence causes dendritic atrophy in nucleus accumbens (NAc) medium spiny neurons (MSNs), but the molecular mechanisms remain unknown. Our previous work demonstrated dendritic atrophy of dopamine D1 receptor expressing MSNs drives stress-like behaviors. We thus hypothesized fentanyl abstinence-induced atrophy is MSN subtype specific and blocking the molecular mediators can reverse behavioral changes caused by opioid abstinence.

Methods: Male and female mice were given fentanyl in the homecage for 5 days (10 µg/mL in drinking water), after which they underwent 10 days of abstinence. We assessed stress-like behaviors with social interaction and elevated plus maze testing in both sexes and assessed increased stress-susceptibility in males with an acute social stressor ($n = 9$-12 mice/sex/condition). To characterize structural plasticity in NAc MSNs, we used a Cre-dependent eYFP virus to sparsely label D1- and D2-MSNs in D1- and A2A-Cre mice, respectively ($n = 11$-13 cells from 4-5 mice/sex/condition). We labeled MSN subtypes for patch-clamp electrophysiology with a Cre-Off tdTomato Cre-On eGFP in D1-Cre mice ($n = 3$-7 cells/mouse, 4 mice/sex/condition). To profile molecular changes and identify molecular mechanisms of cell-type specific dendritic remodeling, we used D1- or A2A-Cre mice crossed with RiboTag mice to isolate ribosome-associated mRNA in specific cell types after fentanyl abstinence ($n = 4$ mice per sample, 6 samples/sex/cell-type/condition). We performed RNA sequencing of the D1- and D2-MSN translatome, followed by weighted correlation network analysis (WGCNA). We then further validated genes identified by RNAseq with Nanostring and RNAscope ($n = 4$-6 mice/sex/cell-type/condition). We assessed the effect of E2F1 expression on behavior, morphology, and electrophysiology with a Cre-dependent E2F1 overexpression virus in D1-Cre mice ($n = 4$-8 mice/sex/condition).

Results: Both male and female mice exhibit increased social-withdrawal and stress-like behaviors after fentanyl abstinence. (e.g. reduced open arm EPM time, Fentanyl vs water: $F(1,33) = 6.102$, $P = 0.018$). Stress-like behaviors after abstinence were associated with reduced dendritic complexity of NAc D1-, but not D2-MSNs (D1 Sholl Radius x Drug: $F(15,688) = 2.006$, $P = 0.0129$, D2 sholl radius x Drug: $F(15,688) = 0.4386$, $P = 0.97$). Using WGCNA, we identified 11 MSN subtype specific gene networks altered by fentanyl abstinence. We found a cluster of dendritic morphology genes downregulated exclusively in D1-MSNs that are transcriptionally co-regulated by E2F1. Overexpression of E2F1 in D1-MSNs protected mice from abstinence-induced D1-MSN dendritic atrophy and stress-like behaviors (E2F1-fentanyl vs E2F1-water $p > 0.05$).

Conclusions: Our findings indicate that fentanyl abstinence causes unique structural, functional, and molecular changes in NAc D1-MSNs. Further, these molecular changes can be targeted to alleviate abstinence-induced dendritic atrophy and stress-like behaviors.

Keywords: Fentanyl, RNAseq, Nucleus Accumbens, Dendritic Remodeling, Abstinence

Disclosure: Nothing to disclose.

P599. Ethanol Reward Learning Potentiates Synaptic Strength on Prefrontal Cortex Parvalbumin Interneurons

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Background: Unfortunately, the limited utility of available treatment options persists as a major hindrance in alleviating the daunting burdens of alcohol use disorders (AUD). The rational development of new pharmacological or brain stimulation-based treatment options will be driven by a better understanding of the molecular and neurocircuit mechanisms through which ethanol exposure alters brain function.

Methods: We used ethanol place conditioning to assess reward learning and aversion. PV-tdTomato fluorescent reporter mice received non-contingent vehicle or ethanol injections (2 g/kg intraperitoneal) before or after place conditioning to induce conditioned place preference or aversion, respectively. We assessed PV-IN physiology using ex vivo whole cell patch clamp electrophysiology between 3 and 7 days following ethanol exposure.

Results: We found that female mice and male mice on a C57BL/6J background display place preference or aversion to ethanol intoxication without overt differences between sexes. Ethanol place preference, but not aversion, was associated with decreased PV-IN excitability. Moreover, ethanol place preference was specifically associated with increased excitatory synaptic strength on PV-INs and the phenotype emerged during abstinence from ethanol.

Conclusions: These findings illustrate that ethanol-induced neuronal adaptations can vary based on behavioral experience. Adaptations to PFC PV-INs do not occur following any ethanol experience; rather, decreased excitability and increased synaptic strength appear to be specifically related to ethanol reward learning. These findings suggest that PFC PV-INs represent an intriguing target for modulating persistent ethanol reward memories.

Keywords: Ethanol, Parvalbumin Fast-Spiking GABAergic Interneurons, Medial Prefrontal Cortex

Disclosure: Nothing to disclose.

P600. Biased Signaling Modifies the Rewarding Properties of Mu-Opioid Receptors

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Background: Overdose deaths involving opioids have skyrocketed nationally over the last 20 years. One major difficulty in addressing this epidemic is that both the therapeutic (i.e., analgesic) and addictive properties of opioids act via mu-opioid receptors (MOPRs). However, growing evidence suggests that these different properties may be mediated by dissociable intracellular signaling mechanisms (G-protein signaling vs. beta-arrestin signaling). Recently, we have shown that MOPRs exert their rewarding, but not analgesic, effects via a lateral dorsal raphe to nucleus accumbens circuit (LDRN-mNAcSh). Here, we sought to determine how G-protein or arrestin signaling specifically control MOPR modulation of reward. By isolating these MOPR mechanisms, we can design more effective therapeutic opioids that reduce, or even avoid, their abuse potential.

Methods: Male and female adult (8-16 weeks) mice were used for all studies. Behaviorally, mice were tested (7-13/group) on a food intake task in which they were allowed to freely consume sucrose pellets for one hour. Mice were tested while ad libitum or after an acute 24-hour food deprivation. Mice were also tested on a lickometer task in which they were allowed intermittent access to a sucrose solution. Mice were tested while ad libitum or after an acute 18-hour water deprivation. Statistically, we used parametric ANOVAs/*t*-tests. Effect sizes and confidence intervals were also calculated to supplement findings. Several mice (3-6/group) were also used for anatomical validation.

Results: MOPR knockout mice (OPRM1 KO) ate and licked less after food or water deprivation compared to wildtype mice. In

contrast, beta-arrestin 2 knockout mice (Arrb2 KO) did not show overall deficits in food intake or lickometer tests, indicating that arrestin does not primarily mediate MOPR modulation of reward behaviors. Further analysis of lick microstructure showed that the reductions in licks in OPRM1 KO mice was driven by fewer initiations of lick bouts ($p = 0.018$), whereas lick bout durations were similar between KO and wildtype mice ($p = 0.231$). Arrb2 KO mice also performed fewer bouts ($p = 0.014$), but had lick bout durations that were nearly twice as long as wildtype mice ($p = 0.089$). Perhaps indicating that arrestin may play a role in how reward behaviors are expressed. To further isolate the contribution of arrestin signaling in MOPR mediated behaviors, we selectively expressed a G-protein biased mutant MOPR in OPRM1 KO mice in the LDRN-mNAcSh circuit. We found that this selective rescue partially restored food intake ($p = 0.11$) and lick behavior compared to wildtype mice. Furthermore, we found that this rescue induced a similar lick microstructure phenotype as there Arrb2 KO mice, wherein they showed increased lick bout duration but not lick bout initiation ($p < 0.001$). Ongoing studies include the development of G-protein specific CRISPR/Cas9 viral vectors for selective disruption of different G-protein subunits, as well as selective restoration of arrestin signaling in Arrb2 KO mice. Future experiments will also test how exogenous opioid rewards (e.g., morphine) are affected by G-protein or arresting signaling.

Conclusions: These results show that both G-protein and arrestin signaling contribute to MOPR mediated reward behaviors, and do so in a complementary fashion. Specifically, G-protein signaling appears to be necessary for reward consumption initiation, whereas arrestin is necessary for reward consumption cessation. These results have major implications for therapeutic opioid drug development.

Keywords: Mu-Opioid Receptors, Biased Signaling, Food Intake

Disclosure: Nothing to disclose.

P601. A "Master Regulator" of Opioid Reward and Aversion in the Ventromedial Prefrontal Cortex

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Background: The US is in the midst of an opioid abuse and overdose epidemic, which has been declared a public health emergency. Oxycodone is one of the most prescribed analgesics, is the first opioid many people experience, and has physiochemical properties that allow it to accumulate in the brain at rates higher than other opioids, perhaps explaining its considerable abuse potential. In the past two decades, a great deal of research using animal models drug addiction have focused on a small number of neurobiological systems, most notably the mesocorticolimbic dopamine system, the corticostriatal glutamate system, and the extended amygdala. Recent advances in tissue clearing and light-sheet microscopy technologies now enable high-throughput, unbiased examination of protein expression or neurocircuitry throughout the entire brain. Furthermore, the development of deep-learning based motion capture models such as DeepLabCut and behavioral segmentation/clustering allows high-throughput, unbiased analysis of behavior. By combining these techniques and taking a holistic approach to examining addictive-like behaviors, we are able to examine brain region, cell-type, and projection-specific mechanisms of behavioral control in a rigorous, unbiased manner. I hypothesize that these holistic methods may be the key to unlocking novel therapeutics for relapse prevention.

Methods: We used the iDISCO+ tissue clearing method and light-sheet microscopy to examine whole-brain c-Fos expression following experimenter-administered oxycodone, and following cue-induced reinstatement of oxycodone seeking. Using male C57Bl6/J mice we first quantified c-Fos expression following an acute injection of saline or 5mg/kg oxycodone (a dose found rewarding in CPP paradigms; $N = 6$-8 per group). We then trained Fos2A-iCreER (i.e. TRAP2) x tdTomato mice to self-administer intravenous oxycodone, with yoked-saline and sucrose self-administering control groups ($N = 9$-14 per group), and used iDISCO+ to identify neuronal ensembles activated by cue-induced reinstatement. We identified the dorsal peduncular cortex (DPC) as a highly understudied structure that showed a significant upregulation of c-Fos+ and tdTomato+ cell counts following acute oxycodone injection and cued reinstatement of oxycodone seeking, respectively. We then used single-nuclei sequencing, RNAscope, and patch-clamp electrophysiology to thoroughly characterize cell types within the DPC and their responses to opioids. To establish a functional role of the DPC in responses to opioids, we used C57, TRAP2, and MOR-Flox mice, including optogenetic and chemogenetic investigation of hedonic responses to oxycodone exposure and dependence. We used the deep-learning based Python library DeepLabCut to perform tracking of individual body parts, and a variational autoencoder (VAME) to segment behaviors and quantify time spent in each detected behavior. Finally, we performed whole-brain mapping of outputs from the DPC in TRAP2 mice using anterograde tracers in combination with iDISCO+, and identify a projection to the parabrachial nucleus that regulates opioid withdrawal.

Results: We discovered that the DPC is an aversive signaling center within the ventromedial prefrontal cortex, and contains a highly unique population of neurons that co-express vGluT2 and the μ opioid receptor (MOR). Single-nuclei sequencing, RNAscope, and qPCR all demonstrate that the DPC is heavily enriched in MOR expression relative to the nearby infralimbic cortex. Electrophysiological characterization of MOR(+) neurons showed that they have relatively depolarized resting membrane potential compared to neighboring MOR(-) neurons, showed enhanced Ih currents, and were hyperpolarized by DAMGO application, confirming the functionality of MOR on these glutamatergic neurons. Optogenetic stimulation of ChR2 in the DPC of C57 and vGluT2-Cre mice produced a real-time place aversion, and this aversion was blocked by prior administration of oxycodone. This effect was recapitulated by selectively stimulating opioid-responsive neurons in TRAP2 mice, and we show that MOR (an inhibitory GPCR) binding directly stimulates c-Fos production through ß-Arrestin2 signaling. When MOR is selectively knocked down via AAV-Cre injection into the DPC of MOR-Floxed mice, the hedonic valence of oxycodone is reversed, and a dose that is rewarding in AAV-GFP treated control mice instead produces a conditioned place aversion. In opioid-dependent mice, artificially rescuing Gi-GPCR signaling in the DPC via hM4D(Gi)-DREADD blocks the motivational symptoms of naloxone-precipitated withdrawal. Conversely, optogenetic stimulation of opioid-responsive neurons in the DPC of TRAP2 mice augments naloxone-precipitated withdrawal symptoms. Analysis of outputs the DPC of TRAP2 mice show dense projections to hindbrain regions known to regulate pain, stress, autonomic function, and aversion, including the parabrachial nucleus (PBN), and rostromedial tegmentum. Optogenetic stimulation of DPC terminals in the PBN of TRAP2 mice recapitulated the aversive effects of somatic DPC stimulation.

Conclusions: These data identify the DPC as a highly novel cortical regulator of opioid reward and aversion. Furthermore, we identify a unique cell population that co-expresses MOR and vGluT2, contradicting the canonical view that opioid action in the cortex is through inhibition of GABAergic interneurons.

Keywords: Opioid Abuse, Opioid Addiction, Addiction Circuitry

Disclosure: Nothing to disclose.

P602. Opioid Choice and Relapse are Distinct Measures of Opioid Addiction, and Both are Effectively Treated With Naltrexone

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Background: We recently developed a rodent model of opioid addiction that is capable of identifying subpopulations of heroin versus food preferring rats under conditions of mutually exclusive choice between these two rewards. The overall population exhibits ~50% choice on average between heroin and food, under conditions where reward price (FR3) and delay (0) are held constant. We applied behavioral economics principles to determine individual rats' motivation for heroin versus food and found demand for heroin to be much more inelastic than demand for food. Correlational analyses did not reveal any significant variables capable of predicting heroin choice, although predictors for relapse were identified. Furthermore, there was no correlation between choice and relapse, indicating that these are distinct measures of opioid addiction. The aforementioned findings are currently in press (Nature Communications). To further validate this new choice + relapse model of opioid addiction, we set out to reverse translate a known therapeutic for opioid use disorder, naltrexone.

Methods: Our experimental procedures followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals and were approved by the University of Colorado Anschutz Medical Campus Institutional Animal Care and Use Committee (IACUC). Male and female Wistar rats ($n = 14$) were surgically implanted with intravenous jugular catheters. After recovery, rats were trained to self-administer heroin and food simultaneously, on opposing levers with opposing cues (tone or light; cues and lever position counterbalanced). Training began on an FR1 schedule (7 days) and then progressed to an FR3 (3 days). During self-administration, both levers retracted during reward + cue delivery (5 sec). Thereafter, the choice phase began, wherein an additional 10 min time-out period (levers retracted, house light off) was imposed to restrict reward availability over the course of the session, forcing the rats to make a mutually exclusive choice for heroin versus food on each trial. After three baseline choice sessions, repeated treatment with vehicle or naltrexone began. Naltrexone (3 mg/kg, SQ) was administered 10 min prior to the start of the 150 min choice session, and again mid-way through the session, to ensure its effects persisted for the duration of each session. Treatment occurred for seven choice sessions, and the next day, a cued relapse test was conducted (~24 h after the last naltrexone injection). During the cued relapse test, both levers were simultaneously available and delivered their respective heroin or food cues (FR3), but rewards were withheld.

Results: Naltrexone treatment shifted choice away from heroin and toward food, with effects emerging on the fourth day of treatment. Choice data were analyzed using a 2-way RM ANOVA with choice session as the repeated measure within-subject and treatment group (naltrexone or vehicle) as the between-subject variable. A main effect of session [$F(9,108) = 10.11$, $p < 0.0001$] and a session x treatment interaction [$F(9,108) = 4.048$, $p = 0.0002$] were significant. Individual 1-way RM ANOVAs for each treatment group were performed to determine whether choice shifted over time. Choice shifted away from heroin (toward food) only in the naltrexone group [$F(9,60) = 1.147$, $p < 0.0001$]. Dunnett's post-hoc multiple comparisons test to the first baseline choice session (before treatment began) indicated that heroin choice was significantly reduced on the last four treatment days (p's < 0.05). Furthermore, heroin (but not food) relapse was reduced by prior naltrexone treatment. Relapse data were analyzed using a 2-way RM ANOVA with lever as the repeated measure within-subject and treatment group as the between-subject variable. A main effect of treatment [$F(1,12) = 11.76$, $p = 0.0050$] and a lever x treatment interaction [$F(1,12) = 6.522$, $p = 0.0253$] were significant. Sidak's post-hoc multiple comparisons tests indicated that naltrexone reduced responding compared to vehicle only on the heroin lever ($p = 0.0005$).

Conclusions: Despite the fact that opioid choice and relapse measure distinct aspects of opioid addiction, repeated naltrexone treatment effectively reduced both behaviors. Furthermore, the effect of naltrexone on relapse was specific to heroin and did not disrupt responding for food cues. Future studies will increase the sample size of this preliminary experiment in order to determine whether naltrexone treatment is more effective in heroin preferring rats and whether naltrexone's effects differ by sex.

Keywords: Choice, Relapse, Opioid Addiction

Disclosure: Kaleidescapes LLC: Founder (Self)

P604. Anterior Insular Cortex Activity Encodes Aversion-Resistant Alcohol Consumption

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Background: Compulsive alcohol drinking is a core facet of alcohol use disorder, where alcohol intake persists despite adverse consequences, and a major clinical obstacle to successful treatment. Recent work has shed light on underlying circuit and neurochemical mechanisms in aversion-resistant drinking, but still remain largely undefined. Previously, we have described that the anterior insular cortex, a critical mediator of emotion, motivation, and interoception, plays a significant role in promoting aversion-resistant drinking. Indeed, optogenetic inhibition of the insula-ventral striatum network significantly decreased compulsive alcohol drinking in rat as observed when the aversive stimulus was a bitter-tasting quinine or intermittent footshock. However, these same inhibitions had no effect during alcohol-only drinking, suggesting this insula-related circuit is critical specifically for aversion-resistant drinking. Additionally, heavy human drinkers show similar insula-circuit recruitment when responding for alcohol under threat of shock. Together, these studies indicate that the insula plays a significant role, in both rodent and human, when overcoming conflict (compulsion) to drink alcohol. Here, we hypothesized that alcohol intake paired with aversive challenge will increase firing levels in the anterior insula described by 32-electrode multi-wire electrophysiology implants.

Methods: Rats were first trained to consume 20% alcohol through an intermittent intake paradigm over 3 months. Quinine, a bitter additive, was given at two concentrations (10 mg/L and 60 mg/L) to assess aversion-resistant, compulsive motivation for alcohol drinking. Animals demonstrating compulsive drinking were implanted with multi-wire recording probes, allowing insula neuron activity to be recorded during alcohol-only and two levels of aversion-resistant drinking. Licking patterns were assessed concurrently with lickometry.

Results: Similar to previous findings, rats maintain longer licking bouts during alcohol-only sessions, with shorter bouts during quinine-alcohol drinking. Insular electrophysiological recording has revealed firing data of over 1200 neurons from 13 rats during alcohol-only and moderate (10 mg/L) and high (60 mg/L) quinine challenge in alcohol.

Conclusions: Ongoing analyses focus on the comparison of anterior insular neuron firing characteristics between drinking

conditions with one main hypothesis being greater insula firing scaling to the level of challenge and amount of consumption. Preliminary analysis suggests that insula firing is greater especially at initiation of intake when under negative challenge, supporting our behavioral results (Darevsky et al. 2019, 2020) that quinine-resistant alcohol intake involves earlier and more vigorous initiation of intake, relative to alcohol-only. Supported by R01AA024109.

Keywords: Compulsion, Alcohol, Anterior Insula

Disclosure: Nothing to disclose.

P605. White Matter Integrity During Early Abstinence Associated With Subsequent Treatment Outcome in Alcohol Use Disorder

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Background: Maladaptive behaviors leading to relapse in alcohol use disorder (AUD) include exaggerated appetitive drive, inability to regulate negative affect and stress, and inability to stop alcohol consumption. According to Koob and Volkow's theoretical addiction model, these maladaptive behaviors can be categorized under the following addiction domains: Incentive salience, negative emotionality, and executive functioning. Specific neural networks underlie these addiction domains. Kurtosis fractional anisotropy (KFA) is a metric derived from an extension of the more common diffusion tensor model. KFA accounts for non-Gaussian diffusion dynamics and is strongly linked to white matter microstructure. The current study directly examined whether white matter integrity, as measured by KFA within addiction networks, is associated with treatment outcome in AUD.

Methods: Multi-shell diffusion weighted magnetic resonance imaging data were collected from 49 individuals with AUD (Age: $M = 42.4$, $SD = 9.4$, 19 females) at ~2 weeks of abstinence. White matter tracts were identified in each individual subject using probabilistic tractography. KFA values were extracted from the following tracts: fornix, uncinate fasciculus, and anterior thalamic radiation. These tracts were selected because they connect regions known to mediate addiction domains including incentive salience (fornix), negative emotionality (uncinate fasciculus) and executive functioning (anterior thalamic radiation). Binary (abstained vs. relapsed) and continuous (number of days abstinent during follow-up period) treatment outcome measures were collected at 4- and 8-month follow-up periods.

Results: KFA within the uncinate fasciculus measured during early abstinence showed to be significantly (i) lower in those that subsequently relapsed during the follow-up periods vs. those that remained abstinent, (ii) associated with the number of abstinent days during the follow-up periods, and (iii) associated with an individual's self-confidence to resist the urge to drink heavily when undergoing negative emotions (as measured by the Situational Confidence Questionnaire). KFA within the fornix and anterior thalamic radiation measured during early abstinence did not show any associations with treatment outcome measures.

Conclusions: This is the first study that directly examines whether KFA within white matter tracts of theoretically defined neural networks of addiction are associated with treatment outcome in AUD. Importantly, significant associations were specific to the uncinate fasciculus, a tract that connects regions known to mediate negative emotionality in addiction. These findings highlight promising targets for neuromodulation interventions aimed at prolonging abstinence.

Keywords: Alcohol Use Disorder, Diffusion Weighted Imaging, Relapse and Treatment Outcome, Negative Emotionality

Disclosure: Nothing to disclose.

P606. A Twelve-Week Trial of Medical Marijuana Cards in Adults With Complaint of Pain, Insomnia, Anxiety or Depressive Symptoms: A Randomized, Pragmatic Clinical Trial

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Background: Despite widespread legalization of cannabis for medical concerns throughout the United States, risks and benefits of obtaining a medical marijuana card (MMC) for treatment of pain, anxiety, depression, and/or insomnia have not been rigorously evaluated. Healthcare providers are increasingly asked to advise patients who are interested in using cannabis to treat various disorders and need evidence-based studies of risks and benefits, including risks of development of a cannabis use disorder (CUD), basic use behavior, and side effect profiles of MM. In contrast to medicines that undergo Food and Drug Administration (FDA) review, patients and treaters lack basic information about cannabis safety and efficacy. Here, we evaluate risks and benefits of cannabis in adults who wish to use cannabis for pain, anxiety, depression or insomnia. Our hypothesis was that we would observe both risks and benefits of MMCs. Namely, we hypothesized that we would observe, in the MMC group, modest improvements in pain and insomnia, together with worsened CUD and depressive symptoms over 12 weeks.

Methods: We conducted a 12-week randomized, interviewer-blind, clinical trial in the greater Boston area of 269 adults aged 18 to 65 years who were light (non-daily) users or non-users of cannabis at baseline but who wished to use cannabis to treat symptoms of pain, anxiety, depression, and/or insomnia. Participants with CUD diagnoses, assessed using the DSM-5 checklist for CUD, at baseline were excluded from the trial. Participants were enrolled between August 2017 and July 2020 and randomly assigned, in a 2:1 ratio, to either obtain MMC without delay (MMC; $n = 105$), or to wait 12 weeks to obtain a MMC, a waitlist control condition (WLC; $n = 81$). Participants were stratified among 3 chief complaints; pain ($n = 37$ in MMC, $n = 24$ in WLC); insomnia ($n = 22$ in MMC, $n = 20$ in WLC); and anxiety/depression ($n = 46$ in MMC, $n = 37$ in WLC). This was a pragmatic trial in which participants in the MMC group chose their cannabis products, doses, and frequencies of use, thus allowing us to test safety and efficacy of cannabis use via the system currently in place for the recommendation, regulation, and distribution of cannabis. Primary outcomes include symptom counts for CUD, and change in self-reported pain (NRS; scale, 0-10), anxiety (HADS; anxiety subscale, 0-21), depression (HADS; depression subscale, 0-21), and sleep quality (AIS; scale 0-24), over the 12-week randomization period.

Results: 269 participants, with a mean age of 37.4 years ($SD = 14.5$; range = 18-65) and 65.1% female, were enrolled and randomized, and 186 (69.1%) completed the 12-week randomized period. Controlling for baseline cannabis use, significantly more participants transitioned to heavy use (≤3 days per week) by 12 weeks in MMC than WLC (48% in MMC vs. 8.8% in WLC, $p < 0.001$). MMC had a significantly greater increase in CUD symptoms compared with WLC, controlling for baseline CUD symptoms ($p < 0.001$). Incidence of CUD at 12 weeks was 10% in the MMC group vs. 5.4% in the WLC (OR = 2.88; 95% CI, 1.17 - 7.07; $p = 0.02$). Notably, 19.5% of the depression/anxiety symptom group assigned to MMC developed CUD versus 6.1% in the WLC group. The MMC group had greater improvement in AIS scores compared

with WLC (mean difference = -2.9; 95% CI, -4.3- -1.5; $p < 0.001$). There was no significant between-group difference in self-reported pain, depression, or anxiety ratings over the 12-week period.

Conclusions: In this randomized, pragmatic trial, obtaining an MMC was associated with significant risk for CUD symptoms over the brief 12-week intervention period. There was no significant benefit on symptoms of pain, depression, or anxiety. There was a modest but significant improvement in self-reported insomnia. The risk of MMCs for the development of CUD, as well as potential benefit of MMCs for insomnia, warrant further study. Counter to our hypothesis, this study does not support the use of MMCs for pain. These results also indicate MMCs are not indicated for treatment of anxiety and depression.

Keywords: Cannabis, Medical Marijuana, Depression, Pain, Insomnia

Disclosure: Nothing to disclose.

P607. Neural Activity Within Distinct Subregions of the Periaqueductal Gray is Associated With Social Vs. Nonsocial Stress-Induced Cocaine Seeking in Rats

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Background: Cocaine use disorder persists as a major public health issue within the US, due in part to a rise in cocaine-involved overdoses in recent years and a lack of FDA-approved medications available for its treatment. One particularly challenging feature of cocaine addiction is a high risk of relapse even after prolonged abstinence. Among the factors known to induce cocaine craving and trigger relapse is exposure to stress, with many experts arguing that the termination of perceived distress is a primary motivator for continued cocaine use. However, the precise neurobiological mechanisms by which stressor exposure produces drug seeking responses has not been fully resolved. The periaqueductal gray matter (PAG) is well-established to play a prominent role in threat response and execution of various behavioral stress-coping strategies, with functionally-distinct subdomains mediating output of active/proactive vs. passive/reactive coping behaviors. More recently, a potential role of the PAG in mediating certain aspects of alcohol intake has been reported, but no studies to date have investigated whether the PAG may be similarly involved in cocaine-seeking behaviors. The overall goal of this study was to assess neural activity within the PAG during episodes of stress-induced cocaine seeking in rats. We also examined neural activity in other brain regions that were selected on the basis of providing afferent input to the PAG and/or playing a known role in drug-seeking or stress-coping behavioral responses. Finally, because distinct neural circuits are engaged by social stressors as compared to nonsocial stressors, we examined whether observed patterns of neural activity varied when cocaine seeking was elicited by a psychosocial vs. a nonsocial stressor.

Methods: Adult male and female Long-Evans rats were trained to self-administer cocaine (0.5 mg/kg/inf, i.v.) in 2-h daily operant-behavioral sessions for 20 d. On days 11, 14, 17, and 20, a discrete tactile cue was presented in the operant chamber, and these self-administration sessions were immediately followed by exposure to psychosocial stress (social defeat, $n = 16$), nonsocial stress (footshock, $n = 12$), or a no-stress control condition ($n = 12$). Beginning on day 21, animals underwent extinction training during which lever-presses were not reinforced. Once responding was extinguished, rats were re-exposed to the tactile cue that signaled their assigned stress/no-stress condition was impending, and cocaine seeking was measured for 2 h under extinction conditions. Immediately after this reinstatement test, animals were sacrificed, and brains collected and processed for c-Fos expression as a marker of neural activation.

Results: When all three treatment groups were combined into a single experimental cohort, cocaine-seeking magnitude was found to be positively correlated with neural activity in several brain areas, including the rostral aspect of the periaqueductal gray (rPAG). Interestingly, activity within different columns of the rPAG during cocaine seeking varied as a function of the stressor employed. Specifically, activation of the dorsomedial rPAG (rPAGdm) correlated with footshock-associated cocaine-seeking behavior, while activation of the dorsolateral and lateral rPAG (rPAGdl/l) correlated with psychosocial stress-associated cocaine-seeking behavior. Moreover, activity in these distinct rPAG subdomains were differentially correlated with activation in other brain areas during cocaine seeking, as footshock-associated activity in the rPAGdm positively correlated with neural activation in the bed nucleus of the stria terminals, while psychosocial stress-associated activity in the rPAGdl/l positively correlated with neural activation in the prelimbic prefrontal cortex (plPFC). Finally, ethographic analysis of social defeat episodes revealed that plPFC activity at the time of the cocaine seeking test was positively correlated with prior display of "active-defensive" coping behaviors during social threat.

Conclusions: Our findings reveal for the first time that neural activity within the rostral PAG, a brain region that is well-characterized for its role in stress-coping behavioral responses, is correlated with stress-induced cocaine-seeking behavior. Moreover, our results suggest that psychosocial stressors may elicit cocaine seeking via a neural network that involves the dorsolateral and lateral columns of the rPAG and the plPFC. Studies are currently underway to examine the functional role of rPAG subregions in social and nonsocial stress-induced cocaine seeking behavior.

Keywords: Cocaine Self-Administration and Reinstatement, Psychosocial Stress, Periaqueductal Grey (PAG), c-Fos, Social Defeat

Disclosure: Nothing to disclose.

P608. Chronic Pain Selectively Alters the Motivation to Work for Remifentanil but Not Food Reward in Mice

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Background: Chronic pain affects a significant portion of the population, and effective pain management has proven challenging. While opioid therapeutics are potent analgesics, their use is limited by a number of undesirable side effects, including high abuse liability. Currently, both preclinical and clinical data offer conflicting views on whether chronic pain might impart resilience or vulnerability to addiction. Our current study seeks to investigate the impact of chronic pain on various aspects of the self-administration (SA) profile of remifentanil, in mice.

Methods: Remifentanil, a potent, fast-onset, short-acting μ agonist that is as rewarding as fentanyl for humans and mice, is an ideal opioid for rodent intravenous SA. Using a chronic constriction injury (CCI) model, a small cuff was placed on the left sciatic nerve. Seven days after surgery, male and female mice ($n = $~5-10 mice/sex/group) began SA of remifentanil for 2 h under a FR1 schedule. After stable responding (~5-7 days), mice were

moved to a higher demand operant schedule (FR3; ~4-6 days), followed by one day of progressive ratio (PR). Mice returned to an FR1 schedule for 3 days, and were then exposed to a reversal task, where the active and inactive pokes were reversed, to test learning. A second cohort of mice repeated the same schedule, but with the food supplement "Ensure" as a reward.

Results: We found no differences in the total number of remifentanil reinforcers earned between CCI and naïve mice during FR1 SA, although CCI mice did acquire reinforcers more rapidly within each session. However, on the higher-demand operant schedules (FR3 and PR), CCI mice showed a significant drop in reinforcers earned and a decrease in overall work effort as compared to naïve mice. This effect was not due to alterations in learning, as CCI mice showed similar performance to naïve mice on a reversal task. In contrast, CCI mice showed no differences in work effort during SA of Ensure.

Conclusions: Chronic pain significantly altered the motivation for CCI mice to work for a remifentanil reward, but only at higher-demand operant schedules. This effect was specific to the opioid and did not impact their willingness to work for a non-drug food reward (Ensure). While it is currently unclear what causes this selective switch in SA responding between schedules in CCI mice for remifentanil, it is likely due to a changing balance between the analgesic properties and hedonic value of remifentanil. Our ongoing studies will seek to parse the potential impact of time and negative affective state on this SA profile.

Keywords: Chronic Pain, Opioid Abuse, Remifentanil, Intravenous Drug Self-Administration, Operant Behavior

Disclosure: Nothing to disclose.

P609. Brevican in Nucleus Accumbens PV + Interneurons Stabilizes Synaptic Inputs and Limits the Development of Cocaine Place Memories in Adult Mice

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Background: Addictive behaviors arise in part due to drug-dependent changes in cellular and circuit function of the Nucleus Accumbens (NAc). NAc output is strongly modulated by local interneurons, and we have shown NAc fast-spiking, parvalbumin-expressing (PV +) interneurons are required for amphetamine locomotor sensitization and conditioned place preference (CPP). Others have shown that repeated cocaine exposure affects the excitability and synaptic inputs to these cells. During development, synaptic inputs to PV + neurons are regulated by perineuronal nets (PNNs), extracellular matrix structures that surround the soma and proximal dendrites. Furthermore, the PNN protein Brevican (Bcan) has been shown to be activity-regulated, and the short isoform Bcan2 to cell-autonomously control synaptic inputs to PV + cells in the hippocampus, presenting an attractive molecular target for drug-induced functional changes in NAc PV + neurons. However, whether Bcan or other PNN components contribute to addiction-relevant, psychostimulant-induced changes in NAc PV + neuron synapses is unknown.

Methods: Adult male and female Pvalb-2A-Cre heterozygous mice were used in all studies. Gene expression was measured via qRT-PCR or RNAscope fluorescent in situ hybridization (ACD). PNNs were labeled using WFA conjugated to FITC, followed by anti-FITC primary and Alexa 488 secondary antibodies to amplify the signal. To manipulate Bcan expression in NAc PV + cells, we used Cre-dependent AAV1 control (shLacZ), knock-down (shBcan), or overexpression (Bcan2-HA) viruses. For electrophysiological recordings of mEPSCs in drug-naïve mice, these viruses were mixed with AAV1-FLEX-eGFP to increase labeling visibility.

Viral constructs were validated using transfected Neuro2A cells and mice with viruses injected into NAc. For cocaine-regulated gene expression analysis, mice were put through a 3 pairing CPP paradigm (i.p. saline or cocaine, 15mg/kg), and brains were harvested 3hrs after the first drug dose or 24hrs after the test session. To assess the effect of Bcan manipulation on cocaine CPP, virally-infected mice were used in a modified CPP paradigm (i.p. saline or cocaine, 10mg/kg) with short, interleaved test sessions to measure preference development. Saline control mice were used to immunostain VGlut1/2+/PSD95 + synapses contacting somata of infected NAc PV + cells.

Cell culture: $n = 3$ wells/group
RNAscope: $n = 41$-95 cells from 3-5 mice/group
CPP: $n = 4$-11 mice/group
Synapse staining: $n = 44$-62 cells from 4 mice/group
mEPSCs: $n = 8$-13 cells from 4-6 mice/group

Data collection was performed blind to treatment or viral group, and analyzed by rmANOVA, ANOVA, Kruskal-Wallis test, Student's *t*-test, or Mann-Whitney U test with Dunn's or Bonferroni's post-hoc tests.

Results: Bcan expression in NAc cells is regulated by acute cocaine exposure. 3hrs after an acute cocaine dose (15 mg/kg), expression of Pvalb, Bcan2, and Bcan1 are induced in Pvalb+ cells (median transcripts/cell, saline vs cocaine: Pvalb 173.6 vs 271.7; Bcan2 259.6 vs 312.5; Bcan1 15.19 vs 21.5; $p < 0.05$), and Bcan1 expression is increased in Pvalb-/Bcan1+ cells. 24hrs after a CPP test session, expression of Pvalb remains up while Bcan1/2 expression returns to saline control levels. We are currently analyzing PNN staining along a time course after acute cocaine (15mg/kg) in order to map Bcan transcriptional regulation onto PNN structural remodeling.

Viruses to manipulate Bcan expression were validated in vitro and in vivo. Viral reporters colocalize with PV immunoreactivity in vivo. shBcan transfection in Neuro2A cells significantly reduced Bcan1 and Bcan2 expression compared to shLacZ, while transfection with Bcan2-HA produced a protein of the proper molecular weight (~80kD) that was immunoreactive for antibodies against Bcan and HA. In vivo knock-down was validated by quantifying total Bcan in mCherry+ cells via RNAscope.

Bcan knock-down in NAc PV + cells accelerates the development of cocaine CPP. In a 10mg/kg cocaine CPP paradigm, shBcan mice showed a significant preference for the drug-paired chamber after just one pairing; shLacZ and Bcan2-HA mice required 3 pairings ($F(15,111) = 2.806$, $p < 0.05$ for shBcan test 1/2/3 vs acclimation, shLacZ and Bcan2-HA test 3 vs acclimation).

Bcan knock-down in adult mice decreases structural and functional excitatory synapses onto NAc PV + interneurons. shBcan cells had fewer VGlut1+ and VGlut2+ synapses/μm than shLacZ cells (median shLacZ vs shBcan: VGlut1 0.149 vs 0.088; VGlut2 0.124 vs 0.071, $p < 0.001$). Electrophysiology yielded similar results, with shBcan mice showing a marked reduction in mEPSC frequency (mean shLacZ = 12.72Hz, shBcan = 3.02Hz, $p < 0.02$).

Conclusions: Bcan expression in NAc PV + cells is increased after acute cocaine exposure, and returns to control levels after a full CPP paradigm. Consistent with the idea that Bcan in NAc PV + cells plays an important role in the early stages of cocaine memory, knockdown of Bcan in these cells prior to CPP accelerates preference development, leading to a significant preference for the drug-paired chamber after the first pairing. Knock-down of Bcan in adult NAc PV + cells also decreases structural and functional excitatory synaptic inputs, showing that cell-type specific changes in Bcan expression in adulthood is capable of modulating their inputs even after circuit maturation. These data are consistent with a model where Bcan stabilizes synaptic inputs to NAc PV + cells, restricting the development of addictive behaviors.

Keywords: Cocaine Addiction, Parvalbumin Fast-Spiking GABAergic Interneurons, Perineuronal Nets, Synaptic Plasticity

Disclosure: Nothing to disclose.

P610. Interrogating the Impact of Training Prefrontal Functions on Post-Treatment Craving and Consumption: Is It Worth the Effort?

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Background: There is renewed interest in the potential benefits of treatment-based cognitive training on longer-term (i.e., post-treatment) outcomes. Recently, we completed a preliminary Clinical Trial in which treatment-seeking men and women meeting criteria for alcohol use disorder (AUD) completed treatment as usual (TAU) or a training protocol challenging neurobehavioral processes heavily reliant on the integrity of the prefrontal cortices (PFC) and their interconnections, i.e., executive functions. In addition to training gains and transfer of training on cognitive tests, we were interested in indirect outcomes such as post-treatment drinking. Participants were contacted 30 days after treatment discharge and provided information on a range of post-treatment variables including craving and alcohol use over the previous month. We anticipated that alcohol consumption would be significantly reduced at follow-up relative to pre-treatment levels. We asked if cognitive training was associated with differential reductions in drinking relative to TAU. Further, given the role of craving in relapse, we examined relationships between drinking, craving, and training condition. Recent data indicate that even if abstinence is not sustained, significant reductions may bode well for longer-term outcomes. Thus, in a secondary analysis, we examined initial and post-treatment drinking levels using the World Health Organization's (WHO) drinking levels.

Methods: Methods were approved by the UF IRB-01 and registered with ClinicalTrials.gov (NCT031376554). Participants provided written consent prior to study. Men and women seeking residential treatment for a substance use disorder (SUD), meeting criteria for DSM 5 moderate to severe alcohol use disorder (AUD), and reporting 21 to 90 days of sobriety were eligible for consideration. Individuals with significant medical or psychiatric disorders were ineligible. Following screening, Ss were assigned to treatment as usual (TAU) or cognitive training groups. Individuals in the training group were included in this analysis if they completed at least 6 (30 min. each) sessions over two to three weeks. The conclusion of the trial coincided closely with their discharge. Both groups completed a post-discharge 30-day interview. Utilizing timeline follow-back methods, respondents provided detailed information regarding their drinking patterns, permitting determination of their average consumption (quantity/frequency indices) as well as other outcomes. Post-treatment craving was ascertained via the Mini Alcohol Craving Experience (MACE) questionnaire.

Results: The conduct of the follow-up interviews was significantly disrupted by COVID-19. Data were collected on 40% (17/42; 4 women) of those completing the trial. Given the sample size and the nature of the WHO related data, we relied on non-parametric and descriptive statistics. Sex differences could not be analyzed. As expected, alcohol consumption was reduced for all respondents. Pre-treatment drinking averaged 15.2 (± 14.2) standard (std.) drinks per day for all participants. Seven individuals (41% of the sample) reported alcohol consumption during the follow-up period with identical percentages in the training and TAU groups. Days to first drink ranged from 2 – 20. The average days to 1st drink was 3 for TAU, 11 for the trained group with *n*'s too small for analysis. Among those consuming any alcohol (collapsed on training), the average number of std. drinks/day after treatment was .42 (+ .29), whereas pre-treatment estimates were 10.72 for these drinkers (Wilcoxon matched pairs signed rank test; $V = 24$, $p = .03$). Across the sample, the mean MACE score was 11. 4 (+ 9.8: range 0-26/50)). MACE scores were not related to pre-treatment drinking. Further, resumers and abstainers did not differ in their scores on any of the MACE subscales (t's < 1.12). However, for resumers, the MACE total and intrusion subscale scores were related to maximum std. drinks/day in the 30 day follow-up ($r = .67(p = .10)$;$r = .86$ ($p = .01$), respectively). The correlation between MACE intensity and average drinks/day was notable but non-significant ($r = .58$,$p = .17$).

At screening, 59% of the sample met criteria for WHO's very high-risk level (>7.1/drinks/day for men, >4.3 for women), 24% fell into the high or moderate levels (7.1, 2.9 drinks/day for men; 4.3, 1.4 drinks for women, respectively), with 8% in the low risk level (1- <2.9, 1.4 for men, women, respectively). This distribution reflects the heterogeneity in consumption observed among persons meeting criteria for AUD. It also illustrates the exceptionally high levels of drinking reported by treatment-seekers. At follow-up, all resumers were in the low-risk group.

Conclusions: Although post-treatment drinking commonly occurred, drinking levels did not exceed the WHO low risk category. Given the percentage of participants in high risk levels at screening, this outcome reflects meaningful benefit. Although small *n*'s constraint interpretation, a training related difference in days to first drink is provocative, meriting future consideration. The equivalency of craving in resumers vs. abstainers, contrasted with the relationship between craving and maximum consumption, suggests the relevance of developing multi-modal neurobehavioral training of cognitive control and response selection in future protocols.

Keywords: Cognitive Training, WHO Risk Levels, Alcohol Use Disorder, Substance Use Disorder, Craving

Disclosure: Nothing to disclose.

P611. Spatial Transcriptomics and snRNA Sequencing Identify Spatially Resolved Overlapping Prefrontal Cortex Cell-Type Specific Mechanisms Between Alcohol Dependence and Immune Activation Mouse Models

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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. We have previously shown that immune genes in brain glial cells are important regulators of alcohol drinking behavior. TLR7, key player in neuroimmune activation, is differentially expressed in mice following 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. Chronic treatment with R848, a TLR7 activator, increases alcohol consumption in mice. We aim to identify novel molecular mechanisms implicated in the escalation of alcohol consumption, which will provide the potential of therapeutics development to prevent or reverse this escalation. In this work, we identify spatially resolved overlapping prefrontal cortex cell-type specific mechanisms between alcohol dependence and immune activation mouse models.

Methods: We performed single nuclei RNA sequencing (snRNA-seq) 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 overlapping mechanisms between CIE and immune

activation model, we generated 10x Genomics Visium Spatial transcriptomics data from coronal PFC sections (10 µm) from C57BL/6J mice treated by a single injection of the R848 (50 µg, i.p.) or saline. We utilized an 'anchor'-based integration workflow introduced in Seurat on snRNA-seq data obtained from PFC micropunches (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: snRNA-seq analyses pipelines identified 24 clusters, the expression levels of canonical markers of each cell type were used to determine the clusters' cellular identity. We identified differentially expressed (DE) genes and their enriched pathways in each cluster. Astrocytic and microglial DE genes were enriched in inflammation related pathways (Il-2, CXCR4, IL-17 and chemokine signaling). Integration of snRNA-seq and spatial transcriptomics data showed increased microglial prediction scores in layers 2/3 and astrocytic prediction scores in layers 5/6, indicating location-specific cell-type specific response to R848 treatment. Additionally, we identified 19 DE gene in R848 brain slices overlapping with CIE astrocytes and microglia DE genes. Gene ontology analyses shows association of those genes to response to stress/stimuli.

Conclusions: Integration of the snRNA-seq and spatial transcriptomics datasets identifies spatially resolved transcriptomic changes induced by immune activation in the PFC mediating the escalation of alcohol consumption.

Keywords: Alcohol and Substance Use Disorders, Neuroimmune Activation, Bioinformatics, Spatial Transcriptomics, Single Cell Omics

Disclosure: Nothing to disclose.

P612. Egr3 is Essential for Opioid-Induced Regulation of Nociception and Maladaptive Motivation

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Background: Chronic pain is a debilitating condition. Profuse reliance on opioids such as oxycodone for pain management results in dependence and addiction liability due to its ability to promote long-lasting neuroadaptations in the mesolimbic dopamine system. Studies investigating the neurobiology of pain and addiction are of paramount importance since both disorders overlap in their impaired hedonic responses, compulsive drug craving, and stress. The neurobiological intersection at which opioids relieve pain and promote addiction phenotype is poorly understood and the cellular mediators that regulate these overlapping neuroadaptations are unknown.

Methods: RNA sequencing was performed on mPFC tissues obtained from rats administered with oxycodone or saline (i.p.) in the presence or absence of CFA-induced inflammatory pain. Mechanical and affective pain was assessed through VonFrey and PEAP (place escape avoidance paradigm) tests, respectively, following viral overexpression or pharmacological inhibition of Egr3 in the mPFC. Motivation to self-administer oxycodone was evaluated through a progressive ratio test in an oxycodone self-administration model following viral overexpression of Egr3 or GFP. Quantitative polymerase chain reaction and western blot were used to determine Egr3 levels and its targets altered during various treatment conditions.

Results: Pain in combination with oxycodone elicits unique transcriptional signatures when compared to pain or oxycodone alone in the mPFC. Among the various altered genes, we focused on Egr3 an immediate early gene since its expression was augmented in the oxycodone groups of both pain and no pain implicating it as a likely regulator exclusively dysregulated through opioid-induced neuroadaptations. We observed that in the presence of oxycodone, viral overexpression of Egr3 in the mPFC potentiate while pharmacological inhibition of Egr3 via cyclosporine A attenuates mechanical pain relief. Egr3 overexpression in the mPFC increase the motivation to obtain oxycodone infusions in a progressive ratio test without altering the acquisition or maintenance of oxycodone self-administration.

Conclusions: Taken together, this demonstrates the role of Egr3 as a mediator of neuroadaptations that lie at the intersection of recreational versus prescription opioid use influencing nociception and escalated motivation.

Keywords: Opioid Abuse, Pain, Immediate Early Gene, Medial Prefrontal Cortex

Disclosure: Nothing to disclose.

P613. Rapid Eye Movement Sleep Engages Melanin-Concentrating Hormone Neurons to Produce Anti-Relapse Effects After Cocaine Withdrawal

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Background: Sleep disruptions often persist long after withdrawal from chronic drug and alcohol use, which may negatively impact withdrawal symptoms and precipitate relapse. However, it is not clear which component(s) of sleep may be particularly important for this regulation, and why. Here we used a cocaine self-administration (SA) model in young adult male rats, which recapitulates the persistent sleep abnormality following long-term withdrawal. We found a collection of sleep/wake and EEG features after long-term withdrawal that correlates with future relapse-like behaviors, especially of rapid eye movement (REM) sleep features in individual rats. We then tested the potential causal relationship by selectively targeting REM sleep, integrating behavioral, chemogenetic, and optogenetic manipulations. A circuit mechanism linking REM sleep and reward network was then dissected.

Methods: Young adult male Sprague-Dawley rats were trained to self-administer cocaine using an overnight + 2 h daily x 5 d paradigm (0.75 mg/kg/infusion). Polysomnographic EEG features after 45 d cocaine withdrawal (WD) ($N = 16$) were correlated with cocaine-elicited behaviors, including SA training active nose poke# (ANP), last 3-d average cocaine infusion#, WD d1 ANP, WD d45 ANP, and incubation ratio. After REMS-cocaine behavior associations were identified, we then used three different approaches to selectively increase REMS to test potential causal relationship: environmental warming, chemo- (using AAV5-MCHp-DREADDs (Gi)), or optogenetic stimulation (using AAV5-MCHp-ChR2) of the REM-promoting melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus. We measured MCH neural activity during REM sleep under environmental warming using fiberphotometry and differentiated their activity during long- versus short-bout REM sleep. We also measured cue-induced cocaine-seeking following these manipulations and did correlation analysis. To probe for circuit mechanisms, we used slice electrophysiology to measure the synaptic accumulation of calcium-permeable AMPA receptors in the nucleus accumbens (NAc) principal neurons following all three manipulations. NAc CP-AMPARs are induced by long-term withdrawal from cocaine self-administration, which critically contribute to the progressive increase in cue-induced cocaine-seeking after long-term withdrawal (i.e. incubation of

cocaine craving). We then compared the behavioral and electrophysiological effects of REMS-manipulations to intra-NAc infusions of MCH peptide (1 µg/µl, 1 µl per side). $N = 8$-20 rats for sleep recordings, behavioral tests, and slice recordings. Two-way ANOVA or paired or unpaired *t*-tests were used as appropriate.

Results: We observed correlations between rapid eye movement (REM) sleep features and cue-induced cocaine-seeking in individual rats after long-term withdrawal from cocaine self-administration. Selectively increasing REM sleep by environmental warming ($t17 = 5.824$, $p < 0.001$) reduced cue-induced cocaine-seeking after withdrawal (control ANP, $t14 = 3.261$, $p < 0.01$; warm ANP, $t15 = 0.7947$, $p = 0.44$; incubation ratio, $t29 = 2.162$, $p < 0.05$), with the amount of long-bout versus short-bout REM sleep oppositely correlated with cocaine-seeking behaviors. Warming induced an increase in the activity of the lateral hypothalamic melanin-concentrating hormone (MCH) neurons selectively during long-REM episodes ($t5 = 4.406$, $p < 0.01$). Mimicking this effect by chemogenetic or optogenetic stimulations of MCH neurons during sleep similarly decreased cue-induced cocaine-seeking (Gq: saline ANP, $t18 = 3.437$, $p < 0.01$; CNO ANP, $t13 = 0.422$, $p = 0.68$; incubation ratio: $t29 = 1.914$, $p = 0.066$. ChR2: control ANP, $t26 = 5.249$, $p < 0.001$; stim ANP, $t24 = 1.119$, $p = 0.27$; incubation ratio, $t50 = 2.870$, $p < 0.01$). All but optogenetic stimulations also counteracted cocaine-induced accumulation of calcium-permeable AMPA receptors at NAc synapses (warming, $t26 = 5.496$, $p < 0.001$; Gq, $t10 = 3.854$, $p < 0.01$). Finally, intra-NAc infusions of MCH peptide reduced incubation of cocaine craving (aCSF ANP, $t15 = 3.626$, $p < 0.01$; MCH ANP, $t25 = 0.753$, $p = 0.46$; Incubation ratio $t14 = 2.521$, $p < 0.05$) and decreased NAc CP-AMPARs (cocaine x MCH interaction, F1, $29 = 7.116$, $p < 0.05$; cocaine SA/MCH vs. cocaine SA/aCSF, $p < 0.01$; two-way ANOVA with Tukey post hoc) without changing sleep/wake time, suggesting that MCH receptor signaling in the nucleus accumbens is sufficient to reduce cue-induced cocaine-seeking and counteract the cocaine-induced accumulation of calcium-permeable AMPA receptors

Conclusions: REM sleep may tap into individuality and produce anti-relapse effects by engaging MCH neural activity and signaling in part through the NAc.

Keywords: REM Sleep, Cocaine Addiction, Melanin-Concentrating Hormone, Relapse

Disclosure: Nothing to disclose.

P615. Sex Specific Differences in Cross-Sensitization Between THC and Heroin Seeking and Withdrawal in a Novel Model of Poly-Substance Abuse

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Background: The current opioid epidemic highlights the need for alternative pain treatment strategies and cannabis is becoming widely legalized for medical use, including pain management. Cannabis could serve as a partial therapeutic alternative to opioids, but it is not clear whether a history of chronic THC increases the risk of developing opioid use disorder (OUD). Preclinical studies using non-contingent THC injections have shown that a history of chronic THC potentiates heroin self-administration (SA). It has however been argued that since non-contingent THC is anxiogenic and can cause aversive responses, the potentiation of heroin SA may be due to the chronic stress associated with non-contingent THC injections rather than cross-sensitization between THC and heroin. Different epidemiological studies indicate that while legalizing medical cannabis decreased opioid overdose mortality rates, cannabis use also increased the risk for developing OUD.

The neurobiological underpinnings of an enhanced responsiveness to heroin after THC pre-exposure are also unknown. It has been shown that sensitization to morphine depends on MOR signaling in the Ventral pallidum (VP), however the lack of a preclinical model of chronic dual THC and heroin self-administration (SA) impedes a detailed investigation of THC-induced neuroadaptations within the reward pathway that could lead to cross-sensitization with opioids.

Here we present a rat model of dual THC and Heroin Self-Administration (SA) to test i) whether a history of THC self-administration modulates different stages of heroin intake, withdrawal and craving, and ii) whether these effects of THC pre-exposure are sex-specific and iii) to investigate neuroadaptations in brain regions involved in OUD.

Methods: Four treatment groups underwent two 10 days of THC or Vehicle SA followed by 10 days of extinction, then by heroin or sucrose SA. Different cues and active levers were used for the first and the second SA session. After the last day of SA a progressive ratio test was performed, with the response requirement increasing exponentially with each injection of Heroin. The second SA session was followed by 10 days of extinction. 24 hours after the last extinction session rats underwent cue-induced reinstatement to heroin or sucrose. We furthermore conducted an elevated plus maze (EPM) test the following day and a heroin prime reinstatement test the day after EPM. A subgroup of animals was withheld from heroin prime testing and sac'ed to harvest brain tissue for whole cell patch-clamp recordings.

Results: Our data show that a history of THC SA significantly augmented heroin intake in both males and females. Chronic THC pre-exposure seems to differentially affect cue and prime induced reinstatement in males and females. We found a strong trend towards increased cue-induced reinstatement in males but not females. Females do in general show higher prime-induced reinstatement than males and a trend towards increased reinstatement after chronic THC. We furthermore found that lever pressing in a progressive ratio test is decreased in males with a history of cannabis use whereas it is increased in females.

Conclusions: Our results indicate that chronic THC modulates the motivation for heroin and craving in a sex-specific way.

We are currently using whole cell patch clamp recordings in VP slices to investigate neuroadaptations in CB1R and MOR signaling after withdrawal from chronic THC and heroin exposure and will compare to adaptations induced by either chronic THC + Heroin or Heroin alone in male and female rats.

Keywords: Heroin Self-Administration, Sex Differences, Cannabis Use, Opioid Withdrawal, Ventral Pallidum

Disclosure: Nothing to disclose.

P616. Cannabis Exposure, Intoxication, and Mood Effects After Use of High Potency THC-Dominant vs. CBD-Dominant Cannabis Concentrates

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Background: Despite widespread availability on state legal cannabis markets, there is lack of research regarding the combined and differential effects of concentrated forms of the two most prominent cannabinoids, THC and CBD. The present study analyzes cannabinoid blood levels and subjective mood and drug outcomes after ad libitum use of legal market concentrate products.

Methods: In this cohort study with a between-groups design that was conducted in a community and university setting, cannabis concentrate users were randomly assigned to either a THC or CBD-dominant concentrate product. The THC-dominant concentrate product contained 84.99% THC and <1% CBD. The CBD-dominant concentrate product contained 79% CBD and 4.5% THC. Participants completed a baseline session, followed by an experimental mobile laboratory session consisting of 3 timepoints – before, immediately after, and one-hour after ad libitum concentrate use. Of the 81 individuals enrolled and assessed, 54 regular concentrate cannabis users complied with the study's cannabis use instructions and had complete data across primary outcomes. 28 participants (51.9%) were assigned to the THC-dominant concentrate condition (mean [SD] age 29.86 [8.7] years; 42.9%women) and 26 were assigned to the CBD-dominant concentrate condition (mean [SD] 29.88 [10.5] years; 53.8% women).

Results: As expected, the THC-dominant concentrate was associated with higher subjective intoxication effects, as well as higher ratings of drug effect and drug reward than the CBD-dominant concentrate. In terms of positive mood, both product formulations induced immediate feelings of elation that diminished over the subsequent hour. Differential negative mood was seen between products, with the CBD-dominant group seeing immediate decreases in tension and anxiety, while the THC-dominant group saw significant decreases in anxiety only after one hour. Paranoia increased immediately post-use in the THC-dominant concentrate group, which decreased back to baseline levels with an hour.

Conclusions: Overall, the positive effects of the CBD-dominant concentrate on mood accompanied a lower level of intoxication and absence of undesirable effects such as paranoia experienced with the THC-dominant concentrate. The results herein support the need for further investigation of the harm-reduction potential of concentrated CBD, both alone and in conjunction with THC.

Keywords: Marijuana, High Potency THC, Harm Reduction, Cannabidiol

Disclosure: Nothing to disclose.

P617. Nacho Deficiency Disrupts Nicotine-Evoked Behavioral Responses Mediated by Nicotinic Acetylcholine Receptors

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Background: Nicotinic acetylcholine receptors play essential roles in various cognitive and reward-related processing and have been implicated in various psychiatric diseases such as depression, schizophrenia, and addiction; as well as neurodegenerative disorders such as Parkinson's disease. Despite the widespread implication of these receptors in a wide variety of disorders, drug discovery efforts to identify targeted therapeutics have been hindered because most neuronal nicotinic receptor subtypes do not functionally express in recombinant systems. We previously identified NACHO, a neuronal endoplasmic reticulum protein, as an essential nicotinic receptor chaperone enabling recombinant, functional expression. Mice lacking the NACHO protein fail to express assembled alpha7 nicotinic receptors and show reduced expression of various other neuronal nicotinic receptor subtypes throughout the brain. Furthermore, NACHO knockout (KO) mice show abnormalities in locomotor and cognitive behaviors consistent with the lack or reduced expression of various nicotinic receptor subtypes in the brain. The high prevalence of nicotine consumption amongst patients suffering from psychiatric diseases has been attributed to patients' attempt to self-medicate, as nicotine may have stimulant and pro-cognitive effects. Here, we studied abnormalities in nicotine-evoked behavioral and physiological responses in vivo and ex-vivo in NACHO KO mice as compared to wildtype (WT) mice to gain a better understanding of how NACHO mediates nicotinic receptor function in vivo.

Methods: Nicotine-evoked dopamine release was studied in vivo (0.6 mg/kg, subcutaneous) with microdialysis in the nucleus accumbens (WT $N = 19$; KO $N = 21$) and ex vivo from striatal synaptosomes loaded with tritiated dopamine from WT ($N = 10$) and NACHO KO ($N = 10$) mice. Nicotine mediated changes in locomotor activity were measured in a chamber with infrared photobeam by quantifying X-Y ambulation 10 minutes after nicotine (0.5 mg/kg, subcutaneous) or vehicle (VEH) administration in WT (VEH $N = 15$; nicotine $N = 15$) and NACHO KO mice (VEH $N = 14$, nicotine $N = 15$). Electroencephalographic (EEG) sleep and body temperature were evaluated in baseline conditions and in response to nicotine (0.15, 0.3 and 0.6 mg/kg; subcutaneous) in WT ($N = 8$) and NACHO KO ($N = 8$) mice implanted with a telemetric device.

Results: Consistent with the role of NACHO as an essential regulator of functional neuronal nicotinic receptor expression, NACHO KO mice displayed abnormalities in various nicotine-mediated behavioral and physiological responses. NACHO KO mice displayed reduced nicotine-evoked dopamine release in vivo (20%) and ex-vivo (23%) compared to WT mice (ANOVA, $p < 0.05$). Nicotine evoked hypolocomotion in WT mice whereas the NACHO KO mice displayed hyperlocomotor activity in response to nicotine (ANOVA, $p < 0.05$). In baseline conditions, NACHO KO mice exhibited a specific decrease in REM sleep duration (6.26 minutes) during the 12-h light/rest phase and an elevated sleep fragmentation (increased microarousals) in the entire light-dark cycle as compared to WT mice (10 more in dark cycle, 16 more in light cycle; two-way *t*-test, $p < 0.05$). Wake promoting and NREM and REM sleep suppressing effects of nicotine observed in WT mice were virtually absent in NACHO KO mice (ANOVA, $p < 0.05$). Nicotine induced a dose-dependent hypothermia in WT mice, whereas this response was blunted in NACHO KO mice (ANOVA, $p < 0.05$).

Conclusions: NACHO KO mice display a variety of abnormalities in nicotine-mediated behavioral and physiological responses. These results are consistent with the role of NACHO in mediating functional expression of nicotinic receptors throughout the brain. Thus far, NACHO has not been implicated in the biogenesis of other neurotransmitter receptors, making NACHO a client-specific chaperone for neuronal nicotinic receptors. Given that the lack of NACHO mediates nicotine's behavioral effects on nicotinic receptors, this provides strong evidence that NACHO is part of the endogenous cellular pathway which leads to functional nicotinic receptor expression. As such, using NACHO as a tool to express previously inaccessible nicotinic receptors in recombinant systems can enable high throughput drug screening and allow for discovery of compounds targeting a range of neuropsychiatric and neurodegenerative conditions.

Keywords: Cognition, Nicotine Addiction, Striatum, Rodent, Nicotinic Acetylcholine Receptors, Mesolimbic Reward Circuitry, NACHO, Alpha7 Nicotinic Acetylcholine Receptor

Disclosure: Johnson and Johnson: Employee (Self)

P618. Inhibiting G9a Decreases Both Dependence- and Stress-Induced Escalation of Alcohol Drinking in Mice

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Background: Alcohol use disorder (AUD) is a chronic, relapsing disease that is difficult to treat. One reason for the pervasiveness

of AUD is that heavy alcohol drinking dysregulates the body's stress systems. This increased stress can lead to increased alcohol drinking, which can perpetuate this cycle and produce alcohol dependence. Understanding the mechanisms underlying stress-alcohol interactions is critical for the development of more effective pharmacotherapies that can target dependence- and/or stress-related excessive alcohol drinking, as well as reduce the ability of stress to trigger relapse in abstinent individuals with AUD. One target that regulates stress-induced escalation of alcohol drinking is the epigenetic modifier, G9a, which is a histone methyltransferase that regulates numerous genes important in AUD and substance use disorder. G9a levels are decreased in the nucleus accumbens (NAc) following cocaine, morphine, or alcohol exposure. Prior studies show that mimicking this reduction of NAc G9a levels decreases stress-induced reinstatement of cocaine seeking and reduces stress-induced escalation of alcohol drinking; however, the role of G9a in dependence-induced alcohol drinking has not been fully characterized. We hypothesized that reducing G9a levels in the NAc or reducing G9a activity systemically with a pharmacological inhibitor would reduce alcohol drinking in a mouse model that combines stress and chronic alcohol exposure.

Methods: All experimental protocols in these animal studies were approved by the MUSC 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.

To test the role of NAc G9a on alcohol-related behaviors, an adeno-associated virus (AAV) expressing a G9a shRNA or a scrambled control shRNA was infused bilaterally into the NAc of adult male mice aged 8-10 weeks. Three weeks later, baseline ethanol (15% v/v) intake was established under limited access conditions (1 hr/day) for 5 weeks. Mice were then treated in the CIE-FSS drinking model where repeated brief forced swim stress (FSS) increases alcohol intake in mice with a history of chronic intermittent ethanol (CIE) exposure. Mice received CIE vapor exposure in inhalation chambers (16-hr/day x 4 days) and after a 72-hr abstinence period, 5 test drinking days (as during baseline). Weekly CIE exposure cycles were alternated with 5-day test drinking periods for 3 cycles. FSS (10 min) was conducted in half the G9a shRNA and control shRNA groups 4-hr prior to the test drinking sessions.

To test the effects of a pharmacological G9a inhibitor on CIE + FSS-escalated alcohol drinking in separate groups of mice, then split into 4 groups in a 2x2 design (CIE vs AIR; FSS vs no stress). AIR alone, CIE-alone, AIR + FSS, and CIE + FSS groups received injections (i.p.) of vehicle or UNC0642 (4 mg/kg) for 15 days. Injections began during the 4th test drinking period and continued through the 5th CIE/Air exposure cycle and subsequent 5th test drinking period.

Results: In mice injected with control virus in the NAc, FSS + CIE significantly increased alcohol drinking as compared to the CIE-alone condition. In contrast, FSS did not increase alcohol drinking in CIE-exposed mice that received intra-NAc shG9a virus infusion. We also observed that repeated injections of a G9a enzymatic inhibitor, UNC0642, significantly reduced CIE-escalated alcohol drinking during the 5th test week of limited access drinking. Indeed, CIE-exposed mice consumed ~3 g/kg alcohol per day before UNC0642 treatment, but consumed only ~1.5 g/kg alcohol per day following treatment. UNC0642 treated animals also showed a significant reduction in escalated alcohol drinking in the CIE + FSS group, but without effects on body weight.

Conclusions: A NAc-specific knockdown of G9a reduced FSS-induced escalation of alcohol drinking, but had no significant effect on CIE-induced alcohol drinking. In contrast, chronic, systemic administration of a G9a inhibitor reduced both CIE-alone and CIE + FSS-induced escalation of alcohol drinking. Indeed, we observed a ~50% reduction in CIE-escalated alcohol drinking, which suggests that G9a inhibition might reduce high levels of alcohol intake associated with dependence to more moderate levels exhibited by nondependent mice. Since the NAc-specific G9a manipulation failed to reduce CIE-escalated drinking, but systemic UNC0642 did suppress CIE drinking, these findings suggest that G9a could be functioning in multiple brain regions to regulate alcohol intake. Together with our published findings, these new data provide further evidence that G9a inhibition can reduce both alcohol dependence- and stress-related alcohol drinking, and it supports the exploration of G9a inhibitors as novel therapeutics to treat AUD as well as comorbidity of stress-related disorders and AUD in humans.

Keywords: ehmt2, G9a, Alcohol Use Disorder, Novel Therapeutics, Alcohol Dependence

Disclosure: NeuroEpigenix, LLC, Founder, Self

P619. Regulation of the Neuronal Excitatory Amino Acid Transporter 3 by Methamphetamine

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Background: Amphetamine (AMPH) and methamphetamine (METH) are closely related psychostimulants that differ by only one methyl group, however their behavioral actions, therapeutic utility and potential for addiction differ greatly. The mechanisms of action of psychostimulants are thought to be primarily through the modulation of dopaminergic neurotransmission. One mechanism by which AMPH and METH regulate extracellular dopamine concentrations is through their ability to cause internalization of the dopamine transporter, DAT. We have also found that AMPH modulates glutamatergic signaling through endocytosis of the neuronal glutamate transporter, EAAT3, which results in the potentiation of excitatory neurotransmission in dopamine neurons. The sequence of events that lead to DAT and EAAT3 internalization begin when AMPH enters the cytoplasm of the cell through the DAT. Inside the cell, AMPH activates an intracellular GPCR for trace amines, TAAR1, which signals through the small GTPase RhoA and triggers the internalization of the DAT and EAAT3. Internalization of these carriers contributes to increases in extracellular dopamine and glutamate concentrations as well as enhanced neurotransmission. However, it remains unresolved whether METH activates internalization of DAT and EAAT3 through the same RhoA-dependent mechanism as AMPH.

Methods: Dopamine (DA) neurons in culture were derived from the midbrain of E15 Swiss-Webster mice. DAT activity was measured in these cultures by measuring 3H-DA uptake that was sensitive to GBR12909, a DAT-specific inhibitor. EAAT3 activity was determined by measuring sodium-dependent 3H-glutamate uptake that was insensitive to the EAAT2 inhibitor di-hydrokainate. Total internal reflection fluorescence (TIRF) microscopy was used to study the membrane localization of the fluorophore-tagged carriers in HEK293 cells in response to AMPH and METH. Forster resonanace energy transfer (FRET) sensors were used to measure the timecourse of activation of RhoA. The TAAR1-dependence of the various effects was verified in parallel experiments in HEK293 cells lacking the TAAR1 receptor. The cell surface expression of DAT and EAAT3 in acute coronal midbrain slices was determined using a cell surface biotinylation assay.

Results: In primary cultures, DAT activity was decreased in response to a thirty-minute pre-treatment with AMPH (10 uM) or METH (10 uM; $n = 6$, $p < 0.05$). However, the loss of DAT activity by AMPH could be blocked by co-application of the DAT inhibitor, cocaine, while the effects of METH were unaffected, indicating that the actions of METH are DAT-independent. Using TIRF microscopy of DAT-expressing HEK293 cells, we observed

internalization of both mCherry-DAT and GFP-EAAT3 in response to AMPH and METH. However, in HEK293 cells that were transfected with the GFP-tagged EAAT3 alone, only METH stimulated internalization of the carrier, while AMPH had no measurable effect ($n = 7$; $p = 0.0001$). METH had no effect on internalization or Rho activation in HEK2093 cells that lacked the TAAR1 receptor, indicating that TAAR1 is an obligate target for the actions of METH, as we had shown previously for AMPH. METH induced internalization of DAT and EAAT3 was blocked by the dynamin inhibitor, dynasore, and the RhoA inhibitor, C3 ($n = 12$, 9; $p < 0.05$ and 0.001). The internalization of DAT and EAAT3 by AMPH was sodium-dependent, whereas the actions of METH were sodium-independent ($n = 9$; $p < 0.0001$). Transport of radiolabled AMPH into cultured neurons was temperature dependent, while parallel experiments examining the transport of METH were not significantly affected by temperature ($n = 11$, $p < 0.01$). Application of either AMPH or METH resulted RhoA activation, however the profile of RhoA activation by METH was faster and biphasic ($n = 13$). With METH the initial of RhoA activation was observed even in cells that lacked the DAT ($n = 20$). In acute midbrain slices exposed to AMPH or METH, we observed a decrease in the cell surface expression of EAAT3. However, examination of cell-surface expression in the rostral forebrain, an area devoid of DAT, only METH caused EAAT3 internalization.

Conclusions: We found that the effects of METH on transporter trafficking are independent of DAT expression and unlike AMPH, METH can activate RhoA signaling in non-DA neurons as well as DA neurons. EAAT3 is found in many neurons and thus, the actions of METH to promote EAAT3 endocytosis could potentiate the actions of glutamate more broadly throughout the brain. These differences in the mechanisms of entry of the two drugs likely determine the distinct pharmacological profiles of the two drugs.

Keywords: DAT/NET Inhibition, Glutamate Transporter (EAAT3), Methamphetamine

Disclosure: Nothing to disclose.

P620. Effects of Low Dose THC on Delay-Phase Activity During a Working Memory Task

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Background: The acute and long-term effects of exposure to the primary psychoactive component of cannabis, delta-9-tetrahydrocannabinol (THC), are of interest due to the prospective therapeutic uses and abuse potential of the drug. Depending on the dose administered, THC exposure can produce both detrimental and beneficial effects on behavior in humans and animals. Performance on working memory tasks requires the activation of the prelimbic prefrontal cortex, and THC acts on cannabinoid receptors in this brain region. We sought to investigate the acute effects of comparatively low doses of THC on working memory task performance and associated neuronal activity in the prefrontal cortex. We hypothesized that activation of cannabinoid receptors at these doses would influence activity patterns of excitatory cortical neurons and task accuracy.

Methods: Adult male and female rats were trained on a delayed-match-to-sample working memory task. Rats first learned to complete nose-poke responses into one of 5 illuminated sample ports to receive a sucrose pellet reward. Next, rats were trained to respond into a specific illuminated sample port, wait through a variable delay phase, and then correctly choose the originally sampled port when given a choice between 3 illuminated ports. Delays between sample and choice phases ranged from 4-20 seconds. Before training, rats were injected with the genetically encoded calcium indicator AAV1.CamKII.GCaMP6f and chronically implanted with a lens probe in the prelimbic prefrontal cortex for in vivo single photon calcium imaging. Rats trained on the task until achieving at least 80% correct trials at the shortest delay before beginning imaging test sessions. During test sessions, rats were injected (i.p.) with 0.5 mg/kg THC, 0.75 mg/kg THC, 1.0 mg/kg rimonabant, or vehicle, 30 minutes prior to a working memory test session. Data was analyzed by *t*-test or one-way ANOVA ($\alpha = 0.05$ for all analyses). All experiments were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Results: Rats successfully learned to perform the delayed-match-to-sample-task, performing more correct trials at the shorter delays with reduced accuracy at longer delay lengths. Injection with 0.75 mg/kg THC 30 minutes prior to the test session reduced the number of trials initiated during the session but did not impact task accuracy. Injection with 0.5 mg/kg THC, 1.0 mg/kg rimonabant, or vehicle prior to test sessions did not significantly reduce performance. The rate of calcium events detected from individual cells ($n = 2$, 220-250 cells total) was compared across each test session and surrounding behavioral events. Initial analysis of population activity of all recorded neurons during control test sessions indicated a significant increase in activity during the delay phase preceding an incorrect response relative to activity preceding a correct response. Calcium event rates were also significantly elevated immediately prior to an incorrect trial after injection with vehicle, but this effect was no longer visible after injection with THC or rimonabant. Neither 0.5 mg/kg nor 0.75 mg/kg THC impacted the calcium event rate across the whole session relative to control test days, but there was a significant enhancement in event rate specifically during the delay phase preceding both correct and incorrect responses. Rimonabant reduced the mean calcium event rate as measured across the whole session compared to vehicle but did not impact activity during the delay phase.

Conclusions: We found that THC, at doses low enough to produce minimal behavioral effects, enhanced activity of principal neurons in the prelimbic prefrontal cortex that is specific to task performance. Further investigation of this effect can probe activity of individual neurons within and across sessions and in response to each pharmacological challenge. These results can be used to consider both the acute and long-term effects of different levels of THC exposure on cognitive performance.

Keywords: Cannabinoid, THC, Working Memory

Disclosure: Nothing to disclose.

P621. Increased Synaptic Plasticity in Paraventricular Nucleus of the Thalamus to Nucleus Accumbens Shell Projections After Prolonged Abstinence From Oxycodone Self-Administration is Sex-Dependent

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Background: Prescription opioid misuse is a national epidemic, exacerbated by the stress and isolation due to the Covid-19 pandemic. In contrast to most drugs of abuse, in which the prevalence of abuse is significantly higher in men, women are misusing prescription opioids at rates similar to or greater than men. As such, biological sex is a factor in risk of misuse and development of opioid use disorder (OUD), but the neurobiological mechanisms are not known. The high relapse rate during abstinence (Abs) is one of the main obstacles in treating OUD. Craving and relapse are often triggered by exposure to the drug

itself or to drug-associated cues. Glutamatergic synaptic transmission within the nucleus accumbens (NAc) underlies cue-driven reward-seeking behaviors, and recent evidence shows that synaptic plasticity within projections from the paraventricular nucleus of the thalamus (PVT) to the NAc shell (NAcSh) is required for the expression of morphine withdrawal (WD) in male mice. However, is unknown whether opioid self-administration (SA) results in similar PVT-NAcSh plasticity and if there is a sex difference in synaptic mechanisms in rats. Here, we determined the effects of short and long Abs from oxycodone (oxy) in male and female rats. We hypothesize that glutamatergic transmission from PVT to NAcSh will be enhanced in both male and female rats after prolonged Abs from oxy SA.

Methods: We used slice electrophysiology with optogenetics to determine the effects of both short (24 hours) and prolonged (14 days) of Abs from oxy SA on PVT-NAcSh glutamatergic transmission in males and females. We also determined the effects of short Abs on somatic WD, and of prolonged Abs on cue-reinstatement (a proxy for drug-seeking). Prior to implantation of chronic indwelling intravenous jugular catheters for self-administration behavior, rats received a unilateral injection of AAV5-CamKIIα-ChR2-EYFP into the PVT. After recovery from catheter surgery, rats underwent an 8-day short access (ShA) oxy SA training regimen (0.06 mg/kg/infusion; 1-h/d) followed by a 14d long-access (LgA) regimen (0.06 mg/kg/infusion; 6-h/d). Control rats self-administered saline (sal). All self-administration was conducted in the light phase in operant chambers equipped with two response levers (active and inactive), with cue lights above each lever and a cue house light. A fixed-ratio 1 schedule of reinforcement was used such that a press on the active lever resulted in a 4s-long oxy infusion followed by 6s timeout period. Following the last session of 14d LgA SA, rats underwent either 24h or 14d Abs periods (total # of animals: males $n = 14$ sal, $n = 14$ oxy; females $n = 15$ sal, $n = 13$ oxy). For the short Abs group, somatic WD signs were recorded after 24h, and rats were then sacrificed for brain slice preparation. The prolonged Abs group went through cue-reinstatement (2hr, pumps off) on the 14th day of Abs and were sacrificed for brain slice preparation. Vaginal lavages were conducted for all the females on the day of sacrifice. Standard procedures for slice preparation and recording were used. Briefly, we optogenetically stimulated PVT projections to the NAcSh and recorded from medium spiny neurons (MSNs) after 24hr or 14d Abs. Whole-cell patch-clamp recordings for synaptic plasticity were performed from MSNs using EPSC-10 amplifier and Pulse v8.8 software (Heka Elektronik).

Results: We compared oxy SA between males and females and did not observe significant sex differences in the number of drug infusions per session. For the short Abs group (males: $n = 5$ sal, $n = 10$ oxy; females: $n = 7$ sal, $n = 7$ oxy), rats that self-administered oxy showed somatic WD signs compared to controls (Two-Way ANOVA: main effect of drug $F(1,25) = 17.28$, $p = 0.003$), with no sex differences. Additionally, there were neither significant sex nor drug effects on NAcSh excitability or PVT to NAcSh glutamatergic synaptic plasticity after short Abs from oxy SA. This included measures of input-output curves for synaptic strength, PPR for probability of glutamate release, AMPA/NMDA ratios, input resistance, and EPSC rectification index. Preliminary data from prolonged Abs group shows that oxy females have significantly greater cue-reinstatement compared to males (Two Way RM ANOVA of Active Lever Presses: main effect of sex $F(1,13) = 9.18$, $p = 0.01$; females: $n = 5$ sal, $n = 3$ oxy; males: $n = 4$ sal, $n = 5$ oxy). In addition, female rats that self-administered oxy show an increase in synaptic strength after prolonged Abs compared to control females (Two Way RM ANOVA: main effect of drug $F(1,164) = 6.05$, $p = 0.01$), whereas males do not show any differences. Preliminary data also shows that females have enhanced synaptic strength compared to males after prolonged oxy Abs (Two Way RM ANOVA: Sidak's posthoc analysis $p = 0.001$).

Conclusions: Our preliminary findings suggest that the effects of Abs from LgA oxy SA on PVT-NAcSh synaptic transmission are time- and sex-dependent. Collectively, these data suggest that prolonged Abs from LgA oxy SA enhance PVT-NAcSh synaptic plasticity but only in female rats. In ongoing studies, we aim to determine whether NAcSh gabaergic transmission is also affected by prolonged oxy Abs and the role of the estrous cycle in females. We are also interested in looking at the effects of 24hr and 14d Abs on MSN excitability in the NAcSh of males and females. To our knowledge, this is the first and only study looking at sex differences in the PVT-NAcSh pathway and how it is affected by various timepoints of opioid abstinence, incubation of craving, and ovarian hormones.

Keywords: Sex Differences, Paraventricular Nucleus of the Thalamus, Nucleus Accumbens, Opioid Use Disorder, Synaptic Plasticity

Disclosure: Nothing to disclose.

P622. Glucocorticoid Receptor Contributions to the Disruption of Action Selection by Cocaine

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Background: Many addictive drugs, including cocaine, increase circulating corticosterone (CORT). Additionally, addictive drugs and CORT weaken the ability of organisms to select actions in a goal-directed fashion (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. The use of Thy1-driven YFP allowed for the visualization of layer V excitatory neurons in the VLO. *F*-actin cycling was manipulated in vivo to reveal effects on action selection strategies.

Results: Cocaine increased circulating CORT, and exogenous CORT exposure was sufficient to disrupt goal-directed action strategies. Cocaine had the same effects, while inhibiting CORT synthesis blocked cocaine-induced response biases. Preliminary evidence indicates that silencing Nr3c1 in the VLO also protects against cocaine-induced response biases, concurrent with dendritic spine modifications on excitatory deep-layer neurons. Finally, inducing actin polymerization in the VLO enhanced the ability of mice to update action selection strategies; upcoming experiments will use the same strategy to attempt to restore response plasticity following cocaine.

Conclusions: Our findings suggest that cocaine-induced decision-making biases are driven by repeated activation of GRs in the VLO. Future experiments will determine whether cocaine-induced CORT release disrupts reward-related decision making via the destabilization of dendritic spines on excitatory neurons in the VLO.

Keywords: Cocaine, Stress Hormones, Decision Making, Orbitofrontal Cortex (OFC), Dendritic Spines

Disclosure: Nothing to disclose.

P623. Lynx1 in the Ventral Tegmental Area Modulates Nicotine Reinforcement

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Background: Nicotine addiction is the largest cause of preventable disease and death worldwide, and current treatments are only moderately efficacious. Nicotine acts on nicotinic acetylcholine receptors (nAChRs) in the brain. Specifically, nAChRs in the ventral tegmental area (VTA) have been shown to be necessary for the reinforcing properties of the drug. Although studying nAChRs provides an understanding of the direct actions of nicotine, it does not provide a comprehensive understanding of all the mechanisms involved in nicotine addiction. Therefore, there is an urgent need to expand our understanding of nicotine's effects on the brain by assessing endogenous proteins that may mediate these processes as well. The newly discovered endogenous nAChR negative allosteric modulator, Lynx1, presents an exciting new target for modulating nicotine dependence because, unlike agonists and antagonists, allosteric modulators do not directly affect receptor function. Rather, allosteric modulators mediate the activity of the receptor in the presence of an agonist. Thus, Lynx1 is a key allosteric modulator to examine in nicotine reinforcement because it has been demonstrated to decrease nicotine mediated activity of the nAChRs.

Methods: To establish whether constitutive Lynx1 knockout alters the expression of associated proteins, we first examined baseline nAChR subunit expression using RT-qPCR. Thereafter, Lynx1 knockout mice and their wildtype littermates were examined for food self-administration to determine if there are differences in their ability to press a lever associated with reward. Following food self-administration, mice were then implanted with a catheter into their jugular vein and examined for intravenous nicotine self-administration across low, moderate, and high doses of nicotine. Next, we assessed Lynx1 expression in the VTA, since it is a brain region necessary for nicotine reinforcement. Using stereological quantification methods to examine cellular activation patterns using the c-fos marker, we also found that both male and female Lynx1 knockout mice had increased cellular activation patterns in the VTA in response to a low dose of nicotine. Finally, we used microinjection of a viral vector to knockdown Lynx1 transcripts in the VTA to examine the effects of removal of Lynx1 in nicotine self-administration. Across all studies, male and female mice were used in an independent measures design, $n = 5$-13. For the food and nicotine self-administration studies, we used between-subjects design and analyzed data using repeated measures two-way analysis of variance (ANOVA) with the Geisser-Greenhouse correction. For our multiple comparisons, we used the Sidak's multiple comparisons test, with individual variances computed for each comparison. For the c-fos and RT-qPCR studies, we used between-subjects design and analyzed data using unpaired two-tailed *t*-tests.

Results: Interestingly, we found that global knockout of Lynx1 led to increased nicotine self-administration at low doses, with male mice exhibiting this increase at a wider dose range compared to female mice. Mice that had knockdown of Lynx1 in the VTA exhibited similar behavior in nicotine intake, demonstrating that removal of Lynx1 in the VTA is sufficient to increase nicotine self-administration at the lower doses. In line with these findings, we also found that both male and female Lynx1 knockout mice had increased cellular activation patterns in the VTA following administration of a low dose of nicotine. When examining baseline subunit expression in the VTA, we found that male, but not female, Lynx1 knockout mice had increased α4 nAChR subunit expression, demonstrating a potential mechanism for the sex-specific effects. The criterion for significance was set at $\alpha < 0.05$.

Conclusions: Together, these findings indicate that Lynx1 both globally and site-specifically in the VTA plays an important role in nicotine reinforcement and cellular activation patterns. Although the net effect of Lynx1 on nicotine self-administration was similar with both sexes, we did find sex-specific effects. Importantly, to our knowledge, we are the first to demonstrate that site-specific endogenous allosteric modulation can have a significant impact on nicotine reinforcement behaviors.

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Keywords: Nicotine Addiction, Negative Allosteric Modulator, Drug Self-Administration, Self-Administration, Nicotine

Disclosure: Nothing to disclose.

P624. Role of Microglia in the Regulation of Methamphetamine Reinforcement

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Background: Repeated methamphetamine use induces long-term gene expression changes in brain regions associated with reward processing and drug-seeking behavior, and recent evidence suggests that methamphetamine-induced neuroinflammation may also be involved in behavioral and molecular responses to the drug. Microglia, the resident immune cells in the brain, are principal drivers of neuroinflammatory responses, yet the role of these cells in the regulation of methamphetamine reinforcement, particularly relapse, is poorly understood. Thus, our goal was to test if pharmacological ablation of microglia would have an effect on methamphetamine cue-induced reinstatement in a mouse model of intravenous methamphetamine self-administration (IVSA).

Methods: Methamphetamine IVSA was first established by training male C57BL/6 mice to respond for food rewards using a schedule of reinforcement in which 5 presses of an active lever resulted in delivery of a food reward and presentation of a 20-second cue light. During the presentation of the cue light active lever presses were recorded but did not contribute to earning a reward. Animals were trained in daily 1-hour sessions until criteria (>30 food rewards per session) was met for methamphetamine IVSA. Mice self-administered IV methamphetamine infusions for 5 consecutive days at a dose of 0.01 mg/kg/infusion and then the dose was increased to 0.05 mg/kg/infusion for the following 10 days. Upon conclusion of IVSA, mice were allowed to extinguish methamphetamine-seeking over 21 consecutive days in which lever pressing resulted in neither delivery of the drug nor presentation of the drug-paired cue light. Following extinction, reinstatement of methamphetamine-seeking was performed by once again presenting the drug-paired cue light and recording active lever pressing during a 1-hour session. Increase in active lever pressing during the reinstatement session over extinction response rates were interpreted as reinstatement of methamphetamine-seeking.

Pharmacological ablation of microglia was achieved by administration of the CSF-1 inhibitor, PLX5622 (1200 ppm; $n = 7$) in chow through the duration of extinction training while control mice ($n = 6$) received standard chow during this period. The effects of microglial ablation on reinstatement of methamphetamine-seeking were analyzed following completion of methamphetamine

reinstatement by comparing the percentage change in active lever pressing (extinction vs. reinstatement) between PLX5622-treated mice and controls.

Results: Pharmacological ablation of microglia in mice by PLX5622 ($n = 7$) blunted methamphetamine reinstatement when compared to mice receiving control chow ($n = 6$) ($p = 0.0101$; unpaired *t*-test). Interestingly, each mouse in the PLX5622-treated group ($n = 7$) showed a decrease in lever pressing during the reinstatement session when compared to each animal's own extinction baseline ($p = 0.0055$; unpaired *t*-test). We also did not observe any significant effect of PLX5622 on operant food training, neither in number of rewards earned nor in rate of acquisition in a separate cohort of animals ($n = 8$ per group).

Conclusions: The abrogation of methamphetamine reinstatement following microglial ablation suggests that microglia may play an important role in regulating the motivational aspects of methamphetamine-seeking. Furthermore, while preliminary and limited to only males, these results suggest that neuroinflammatory mechanisms may dictate vulnerability to methamphetamine relapse, and thus provide new therapeutic avenues for treatment of addictive disorders.

Keywords: Microglia, Methamphetamine, Intravenous Drug Self-Administration, Cue Reinstatement, Neuroinflammation

Disclosure: Nothing to disclose.

P625. Inter-Subject Correlations of fMRI Responses to Cinematic Heroin Use in Individuals With Heroin Use Disorder

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Background: Neurophysiological responses to drug cues remain poorly understood in heroin use disorder (HUD). The use of naturalistic dynamic stimuli (e.g. movies) provides several advantages over traditional fMRI tasks, including improved ecological validity, participant engagement, and estimation of functional connectivity between brain regions. Moreover, inter-subject correlation (ISC) and functional connectivity (ISFC) measures of responses to naturalistic stimuli have been successful in predicting behavioral measures like learning and memory. However, neural responses to heroin-related naturalistic stimuli have not been studied in HUD. Here we use inter-subject correlation (ISC) and functional connectivity (ISFC) methods to characterize brain responses in HUD to a movie containing naturalistic heroin use and a narrative about HUD.

Methods: We recorded BOLD activity in 30 treatment-seeking HUD (40.51 ± 9.06 years, 23 M) participants and 16 age, sex, and race-matched healthy controls (HC; 43.79 ± 10.33 years, 10 M), while watching the first 17 minutes of the movie "Trainspotting", which contains scenes of explicit heroin use, as well as scenes that are highly salient but not directly related to heroin use. Importantly, the overarching narrative structure of the clip is centered around drug-addiction, including complex contextual elements relevant to HUD (e.g. social, emotional, and economic challenges of addiction). For our analyses, the movie clip was divided into distinct scenes, which were then classified as drug or non-drug, based on whether drugs or speech about drugs appeared in the scene. After watching the movie, participants freely recalled movie details along with relevant personal reflections and rated individual scenes based on their emotional response and heroin craving. To assess neurophysiological responses, we computed whole-brain ISC and ISFC of the BOLD signal during the movie, and compared between HUD and HC groups for drug and non-drug scenes.

Results: Per the impaired Response Inhibition and Salience Attribution (iRISA) model of drug addiction, whereby excessive incentive salience is ascribed to drug at the expense of non-drug cues/reinforcers, we hypothesize increased ISC during drug-related scenes in the HUD group in reward, salience, habit, executive, self-directed, and memory networks. We also expect ISCs in reward/salience, habit and self-directed networks during drug-related scenes to correlate with craving for those scenes. Similarly, we expect scene-resolved ISCs in the salience and memory networks to correlate with scene recall. Since the movie content should preferentially evoke self-referential thought in the HUD group, we expect these subjects to show reduced ISC in primary sensory areas, but elevated ISFC in the default mode network and hippocampus that is time-locked to the explicit drug scenes. This elevated ISFC should predict the duration of self-referential speech when subjects freely recall the movie. Consistent with previous studies using engaging Hollywood movies, an initial whole-brain voxel-wise analysis of the full movie for the HUD group revealed significant ISCs in ~50% of grey matter voxels, including large regions of occipital, temporal, and parietal lobes, as well as medial and lateral frontal cortex, orbitofrontal cortex, hippocampus, amygdala, and striatum (FDR corrected, $q < 0.001$).

Conclusions: Insights from naturalistic drug cue exposure may better characterize the neural mechanisms of drug cue reactivity in heroin addiction.

Keywords: Heroin, Addiction, Inter-Subject Correlation, Naturalistic Drug Cues, fMRI

Disclosure: Nothing to disclose.

P626. Effects of Chemogenetic Inhibition of the Ventral Hippocampus to Nucleus Accumbens Projection on Sign- and Goal-Tracking Behavior in Rats

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Background: The sign-tracker/goal-tracker model provides the means to dissociate between the predictive versus incentive associations that form from Pavlovian cues. "Sign-tracking" rats (ST) reliably approach a reward-associated cue (i.e., lever) and interact with it, while "goal-tracking" rats (GT) direct their conditioned behavior away from the cue and towards the site of impending food reward (i.e., food-cup). For ST, but not GT, the cue becomes a reward in and of itself. As we could expect, ST show increased vulnerability to cue-induced reinstatement or "relapse" of drugs of abuse when compared to GT, which makes this a valuable model for understanding predisposition to addiction-like behaviors. Glutamatergic transmission in the nucleus accumbens (NAc) – a critical region of the motive circuit – seems to be necessary for the development of sign-tracking behavior and the attribution of incentive salience. Nonetheless, little is known about the sources of afferent glutamatergic neurons that regulate NAc activity and impart individual variation to the associative learning process. The NAc is densely innervated by the ventral hippocampus (vHPC), and previous work in our lab showed that lesions of the vHPC attenuate the acquisition of sign-tracking. However, whether this effect is exerted via direct modulation of the NAc is unclear. We explored the hypothesis that the vHPC-NAc projection plays a functional role in determining the ST/GT phenotype.

Methods: We used an in vivo dual-vector approach by bilaterally injecting Cre recombinase into the NAc and an inhibitory Cre-dependent, Gi-coupled designer receptor exclusively activated by designer drugs (DREADD) into the vHPC to selectively target the vHPC-NAc projection. These viral injections

were performed via stereotaxic surgery on male Sprague Dawley rats (7-8 weeks old). Five weeks after surgery, all rats underwent six daily sessions of a Pavlovian conditioning approach (PCA) procedure with clozapine-N-oxide (CNO; 3mg/kg, i.p.) or vehicle (6% DMSO, i.p.) on board, followed by a crossover treatment test session. Each session consisted of 25 presentations of a retractable lever (CS) that extended into the chamber for 10 seconds, and 25 deliveries of a sucrose-free banana pellet (US) into the food-cup. Animals were classified based on whether their conditioned responses were preferentially directed toward the lever-cue (ST) or toward the food-cup (GT).

Results: We found no significant differences in lever press and food-cup entry number, latency or probability between CNO ($n = 24$) vs Vehicle ($n = 22$) treated rats across all six training sessions, suggesting that vHPC-NAc projection does not affect the acquisition of sign- and goal-tracking behavior. Based on these six training sessions, all rats were classified as ST or GT based on their average behavior bias during sessions 5-6. We found that following disinhibition of the vHPC-NAc during the crossover treatment test session (Vehicle was given in lieu of CNO), rats that were classified as ST ($n = 4$) showed a significant decrease in lever press number compared to their last training session (Paired *t*-test: $t(3) = 14.72$, $p < 0.001$). On the other hand, rats classified as GT ($n = 7$) showed no change in neither lever nor food-cup oriented behaviors following vHPC-NAc disinhibition. In addition, inhibition of the vHPC-NAc projection seems to have to effect in the expression of sign- and goal-tracking once the behavior has been learned under normal conditions, as ST ($n = 4$) and GT ($n = 7$) that received CNO for the first time in the crossover treatment session showed no significant change in lever and food-cup oriented behaviors.

Conclusions: Our data suggest that the vHPC-NAc projection may not be necessary for the acquisition of sign- and goal-tracking behavior. However, it may have a more complex and selective role in the performance of sign-tracking behavior in ST, as chronic vHPC-NAc inhibition affected the expression/performance of sign-tracking. More experiments need to be performed to further dissect this role and shed light on the neurocircuitry responsible for biasing ST/GT behavior. Ultimately, this will lead to a better understanding of increased susceptibility to cue-driven psychopathologies such as addiction and PTSD.

Keywords: Pavlovian Conditioning, Ventral Hippocampus, Nucleus Accumbens, Sign-Tracking, Chemogenetics

Disclosure: Nothing to disclose.

P627. Influence of the Psychedelic DOI on Oxycodone-Induced Conditioned Place Preference in C57BL/6 Male Mice

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Background: Psychedelic serotonergic agonists such as lysergic acid diethylamide (LSD) and psilocybin are being increasingly studied for their potential therapeutic effects in treatment of a variety of neuropsychiatric disorders, including substance use disorder (SUD). Opioid use disorder effects over two million people in the United States with more than 120,000 deaths worldwide attributed to opioids. SUDs overall have a relapse rate of 40-60%, with current pharmacotherapies being insufficient and causing their own set of unwanted side effects. Psychedelics alter perception and cognition through activation of the serotonin 2A receptor (5-HT2AR) but have not been found to lead to dependence or lethal overdose and are considered non-reinforcing. Serotonin has modulatory effects on the mesolimbic pathway, which is implicated in the neurobiology of addiction. Clinical findings have demonstrated the ability of psilocybin to decrease alcohol consumption in heavy drinkers and increase smoking cessation in adults. Our previous findings suggested that psychedelics induce long-lasting and robust effects on synaptic plasticity in mouse frontal cortex. Aspects of SUD can be modeled in preclinical rodent models such as conditioned place preference (CPP). The present pilot study aimed to assess the ability of the psychedelic 2,5-dimethoxy-4-iodoamphetamine (DOI) to reduce oxycodone-induced CPP in adult male mice.

Methods: Use of animals has been approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Adult C57BL/6 male mice ($n = 19$-21/group) were tested in a six-day CPP paradigm. Day one consisted of a habituation session in which baseline behavior was recorded. Days 2-4 were conditioning sessions in which mice underwent two daily (AM/PM) injections (s.c.) of either oxycodone (3 mg/kg) or 0.09% saline followed by a 15-minute pre-treatment period before being placed in the drug-paired side of the chamber for 20 minutes. On day 5, mice did not receive any drug and were placed in the neutral chamber for 5 minutes followed by 15 minutes of recorded exploration to assess time spent on the drug-paired side (oxycodone CPP). After this session, mice were administered (i.p.) either DOI (2 mg/kg) or saline (0.9%) and returned to their home cages. On the final day, 24-h post-DOI injection, mice were tested again for 15 minutes to assess oxycodone CPP. Time spent on the drug-paired side and the saline-paired side on test days compared to baseline scores were used to calculate an overall preference score.

Results: As expected, all mice treated with oxycodone displayed a preference to the drug-paired side compared to vehicle (unpaired two-tailed Student's *t*-test: $p < 0.0001$). Importantly, using a paired two-tailed Student's *t*-test to assess within subject alterations in behavior, our data showed that mice that received DOI following oxycodone conditioning had a decrease in CPP ($p = 0.038$), whereas mice that received saline did not ($p = 0.208$).

Conclusions: Our results support the notion that psychedelics can alter behaviors associated with SUD in preclinical rodent models. Up to our knowledge, this study is the first to determine the extent of which a psychedelic can alter opioid-seeking behavior in preclinical models. The ability of DOI to decrease CPP 24-hours after administration suggests that psychedelics may have long-lasting therapeutic effects relevant to addiction. These findings are translationally relevant because they suggest that psychedelics may be a possible treatment for SUD after patterns of abuse have already started. Following this pilot study, future experiments will be carried out to determine if this effect is seen in female mice, with other psychedelics and whether or not this behavioral change is dependent on the 5-HT2AR. These experiments will be crucial in understanding how psychedelics modulate behaviors associated with SUD and in turn lead to better treatment for addiction.

Keywords: Psychedelics, Substance Use Disorder, Opioid Abuse, Conditioned Place Preference, Behavioral Pharmacology

Disclosure: Nothing to disclose.

P628. Chronic N-Acetylcysteine Administration Temporally Increases Matrix Metalloproteinase Activity in Nucleus Accumbens

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Background: The ongoing opioid crisis has prompted a need for further research elucidating effects of maladaptive neuroadaptations following prolonged opioid use. Activation of the tetrapartite synapse in nucleus accumbens core (NAcore), comprised of pre- and post-synapse, astrocytic processes, and surrounding extracellular matrix (ECM), has been linked to increased relapse vulnerability. This process is mediated by downregulation of glutamate transporter (GLT-1) following repeated opioid exposure, which promotes glutamate spillover into extrasynaptic compartments. Several studies focus on reversing glutamate spillover following chronic opioid use by restoring function and/or expression of GLT-1 with N-acetylcysteine (NAC), and thereby reducing heroin cue and prime reinstatement. Moreover, opioid seeking is associated with rapid and transient plasticity within each compartment of the tetrapartite synapse. In particular, matrix metalloproteinase (MMP) activity can be localized at D1 and D2-specific medium spiny neurons (MSNs), where D1 MSNs exhibit transient and reversible MMP-9 activity following cue-induced heroin seeking, while D2 MSNs exhibit increased constitutive MMP-2 gelatinolytic activity following heroin-extinction, that is transiently reduced after 15 min of cued reinstatement. While it is evident opioid relapse induces MMP activity, it remains unknown whether NAC is effective at preventing changes in MMP activity and if this is cell-type specific.

Methods: Male Sprague Dawley rats were trained to self-administer heroin, after which animals were withdrawn during extinction training, and then reinstated by heroin-conditioned cues. Brain slices of nucleus accumbens were made after extinction training or 15 min after initiating cued heroin seeking and compared to slices from rats trained as yoked saline controls. Following heroin self-administration, we measured MMP-2,9 gelatinolytic fluorescence after FITC-gelatin microinjection under extinction-related and cue-induced heroin seeking conditions with vehicle or chronic systemic high-dose NAC administration (5 consecutive days during extinction, 100 mg/kg, ip). To further evaluate NAC's effects on MMP activity under heroin-naïve conditions, systemic NAC administration was given at various timepoints and under several pharmacological conditions to determine possible mechanisms within the ECM contributing to the observed effects of NAC. Results were analyzed using one- and two-way ANOVA following by Bonferroni post hoc tests for multiple comparisons.

Results: Chronic high-dose NAC treatment (5 consecutive days, 100 mg/kg, ip) did not reduce cued heroin seeking-induced MMP-2/9 activity in NAcore of reinstating animals, despite significantly suppressed active lever pressing compared to NAC-untreated animals. Interestingly, chronic NAC treatment potentiated gelatinase activity in heroin-naïve animals, which peaked 7 days after treatment, but persisted for up to 10 days after treatment, and returned to baseline levels by 15 days. These effects were not apparent after acute NAC treatment. Interestingly, this persistent, but reversible increase was MMP-2-mediated, consistent with the constitutive functionality of MMP-2 activity. This increase was not mediated via a nitric oxide-dependent pathway, suggesting an alternative, indirect pathway activating MMP-2 activity. Further investigation is warranted to determine whether these changes are D1 or D2 MSN-specific in naïve animals and if heroin exposure disrupts the homeostatic balance of MMP activity between these cell-types.

Conclusions: These data reveal a possible therapeutic agent which can suppress heroin seeking behavior via a unique mechanism involving ECM activation. Although much work remains to fully characterize how the tetrapartite synaptic compartments interact to regulate plasticity, it is clear that each of the four compartments are altered by opioid use and therapeutic agents may play a specific role within each compartment.

Keywords: Matrix Metalloproteinase-9 (MMP-9), Heroin, N-acetylcysteine, Extracellular Matrix

Disclosure: Nothing to disclose.

P629. Histone Acetyltransferase KAT2A is a Critical Epigenetic Regulator of Cocaine Responses in the Nucleus Accumbens

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Background: Substance use disorder is a neuropsychiatric disorder most commonly characterized by persistent cycles of drug-use, abstinence, drug-seeking, and relapse. The neural basis of these long-lasting drug-associated behaviors have been linked to neural circuit function through changes in neurotransmission and receptor-based changes across the reward circuitry of the brain. However, the underlying molecular mechanisms which contribute the persistence of these drug-induced changes to neural function remain relatively poorly understood. Previous work from our lab and others has identified cocaine-induced changes to transcriptional and proteomic profiles within the nucleus accumbens (NAc). To understand how drugs of abuse, such as cocaine, generate long-lasting behavioral changes, it is critical to link between neuronal activity and changes in gene expression. One potential avenue are epigenetic adaptations, where DNA-protein interactions are modified to alter accessibility and likelihood of targeted gene expression. For example, histone acetylation is induced following various cocaine treatments, specifically in the D1-expressing subclass of medium spiny neurons (MSN) within the NAc. KAT2A is a histone acetyltransferase known to regulate activity-dependent transcription and hippocampal memory. While several KAT2A histone targets are modulated by cocaine, the role of KAT2A in cocaine-associated behaviors remains unknown. As such, we hypothesize cocaine-induced gene networks are underlied by recruitment of various histone modifications, a subset of which are regulated via KAT2A.

Methods: Mice were trained to self-administer cocaine (1mg/kg/inj) or saline under VR3 and VR5 schedules of reinforcement. Following either acute (1 day) or chronic (10 days) of cocaine self-administration, NAc tissue was collected. Using histone-specific DDA mass-spectrometry, we identified post-translational histone modifications induced by: 1) acute cocaine, 2) repeated cocaine, or 3) persisted 24 hours following self-administration. Moreover, using targeted expression of loss-of-function KAT2A variants in the D1-MSNs of the NAc, we determine if KAT2A function is necessary and sufficient for cocaine-associated behaviors and changes in D1-MSN activity during self-administration. Lastly, using calcium-dependent fiber photometry, we profile D1-MSN activity throughout the acquisition and maintenance of repeated cocaine self-administration.

Results: We identified temporally specific changes in Histone H3 post-translational modifications, including KAT2A-regulated phosphoacetylation, following chronic cocaine self-administration. Moreover, we demonstrate that loss of KAT2A function in D1-MSNs alters sensitivity and motivation for cocaine. Lastly, we generate a cocaine self-administration activity profile of D1-MSNs that is subsequently altered by modified KAT2A function.

Conclusions: The NAc has long been linked to reward and drug-associated behaviors. Various epigenetic changes within the NAc have also been linked to altered NAc response to drugs of abuse. The results of these studies contribute evidence for persistent cocaine-induced epigenetic adaptations and are the first step in generating a mechanistic link between epigenetic adaptations and changes in neuronal firing. In addition, we provide data linking these changes in epigenetic state to cocaine-seeking behavior. Future studies will identify a causal link between changes to epigenetic gene regulation and NAc circuit function during cocaine-seeking behaviors.

Keywords: Epigenetics, Cocaine Self-Administration, Nucleus Accumbens

Disclosure: Nothing to disclose.

P630. Zonisamide Treatment of Alcohol Use Disorder

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Background: Zonisamide is an anticonvulsant with a multifaceted pharmacologic mechanism of action that has shown potential efficacy in reducing drinking and treating alcohol use disorder (AUD). Zonisamide is a widely available generic medication approved by the FDA as an anticonvulsant. We evaluated the efficacy of zonisamide in a sample of subjects with AUD who reported regular heavy drinking.

Methods: We performed a 16-week randomized double-blind placebo-controlled clinical trial at three sites (two in Connecticut, and one in Virginia) in which we randomly assigned 159 subjects with AUD on a 1:1 basis to zonisamide or placebo. Zonisamide was flexibly titrated over 7 weeks to a daily dosage of 500 mg. Medical Management was used as the behavioral platform. Drinking was measured using the Timeline Follow-back method. The analysis was performed using mixed models in SAS.

Results: There was a significant effect of the medication on the prespecified primary outcome (average drinks per day) in the sample both over both the prespecified last 8 weeks of the trial [$F(1,680) = 6.20$, $p = 0.013$] and the entire 16 weeks of study participation [$F(3,1655) = 4.47$, $p = 0.035$]. Placebo patients reported an average of 0.72 [95% CI = (0.54-0.93)] more drinks per day than zonisamide patients. There was also a significant effect of sex and a sex x medication interaction effect [$F(1,680) = 5.87$, $p = 0.016$], such that men had 43% fewer drinks/week when treated with zonisamide compared to placebo, while there was no difference among women. There was also a significant main effect of the medication on heavy drinking days per week [$F(1,822) = 4.62$, $p = .032$], with the odds of a heavy drinking day with zonisamide treatment being 0.36 (95%CI = 0.14-0.92) that of placebo. There was also a significant interaction of medication x time on heavy drinking days per week [$F(3,1929) = 3.73$, $p = .011$], with the slope of effect greater in the zonisamide group than the placebo group. Finally, there was a trend for an interaction with sex on heavy drinking, with men experiencing a greater reduction in risk of heavy drinking with zonisamide compared to placebo than women (men: OR = 0.26, 95%CI = 0.07-0.94, women: OR = 0.74, 95%CI = 0.16-3.58).

Conclusions: Zonisamide is an efficacious treatment for AUD and appears to have greater efficacy among men than women, although this and other potential predictors of the response to zonisamide treatment warrant further study.

Keywords: Alcohol Use Disorder - Treatment, Pharmacotherapy, Clinical Trials

Disclosure: Nothing to disclose.

P631. Impairment from Δ9-Tetrahydrocannabinol (THC) Associated With Reduced Prefrontal Cortical Resting State Connectivity Independent of THC Dose

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Background: The primary psychoactive cannabinoid in cannabis, Δ9-tetrahydrocannabinol (THC), causes intoxication that impairs cognitive performance due largely to effects on prefrontal cortex (PFC) function. There are currently no evidence-based methods to detect cannabis intoxication or impairment. We tested whether functional near infrared spectroscopy (fNIRS) could be used to detect changes in PCF resting state connectivity (RSC) associated with impairment due to cannabis intoxication.

Methods: Study participants were healthy adults who reported at least weekly cannabis use. Participants completed two study visits at which they received a single dose of dronabinol, an FDA-approved synthetic THC ingredient in MARINOL® Capsules, 5-80 mg, or identical placebo in random order. THC was dosed according to study physicians' determination of the dose most likely to result in intoxication with no adverse events, based on the degree of expected tolerance, given participant's average dose, frequency, and type of cannabis use, and level of intoxication and any adverse events experienced with each use, sex, height, weight, BMI and baseline blood pressure. Pre-dose and every 20-25 minutes post-dose participants had heart rate and blood pressure assessed and they completed the Drug Effects Questionnaire (DEQ), a 100mm visual analogue scale, to assess the extent to which participants (1) felt any THC effect(s), and (2) felt high. Participants underwent two fNIRS sessions; one pre-dose and again approximately 100 minutes post-dose, 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). Impairment was defined by convergent classification by consensus clinical ratings and an algorithm based on post-dose tachycardia and DEQ self-rated "high," and was compared with results of extended field sobriety tests performed by certified drug recognition examiner police officers approximately 120 minutes after study medication.

Results: 181 participants were randomized, and 169 participants who completed a post-dose fNIRS scan at a study visit in which they received THC were included in this analysis. In these participants, 164 completed a placebo visit. Participants who completed a placebo visit but not a THC visit were excluded from this analysis. Following active study drug (THC), 80 participants had concordant clinical and algorithm ratings indicating that they were impaired/intoxicated following study medication (mean THC dose: 35.6mg ± 11.5); 57 of these participants had concordant ratings of not impaired (mean THC dose: 34.8 ± 16.1). Participants assessed as impaired post THC had greater subjective (DEQ) and physiologic (heart rate) signs of impairment than those who were not impaired post-THC. RSC was significantly decreased from pre-THC to post-THC in participants who were impaired post THC ($p < 0.001$; FDR- corrected). This decrease was observed in multiple channels throughout the PFC. Those who were not impaired post THC did not show a significant change in RSC. Those who became impaired from THC had significantly lower PFC RSC post-THC than post-placebo ($p < 0.01$; FDR-corrected). Those who did not become impaired post THC did not show any significant differences in RSC between post-THC and post-placebo scans, even after receiving equivalent doses of THC.

Conclusions: These findings suggest PFC RSC response can indicate cannabis impairment independent of THC dose. Future work is warranted to determine specificity to acute THC impairment and to determine whether this method can objectively detect impairment at the individual level.

Keywords: THC, Cannabis, fNIRS, Neuroimage, Resting State Connectivity

Disclosure: WO 2018/027151: Patent (Self)

P632. Examining Causal Relationships of Sleep, Mood and Alcohol Use During COVID-19

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Background: The societal changes and stresses brought on by the COVID-19 pandemic has resulted in poorer mental health and poorer health behaviors (e.g., poorer sleep, unhealthy diet, reduced exercise, greater alcohol usage). This has been well documented in multiple studies that used cross sectional surveys of subjects. However, traditional survey studies suffer from the limitations of not being ecologically valid and the analyses performed at the group-level can obscure relationships at the individual level. Both of these issues can be addressed through the use of intensive longitudinal data (ILD) which involves the collection of information in real time through active collection such as surveys and passive collection such as wearable devices. Typical data collection occurs 3-4 times a day over a 30-day period. While traditional analyses of ILD are able to identify the associations among different measures, they are not able to identify the underlying causal structure among the measures. Identifying this causal structure at the individual level is critical to developing interventions customized for the individual. Causal structure can now be revealed by using causal discovery methods, which analyze the statistical properties of observational data to construct a plausible causal structure. In this study, we apply causal discovery methods to ILD collected in a COVID-19 survey study to examine the causal contributors to drinking behavior at the individual level.

Methods: We used data from a publicly available, de identified data set from Boston College collected during the COVID-19 pandemic collected March 20 – June 23, 2020 (Cunnigham et al. SciData, 2021). Daily surveys were conducted using REDCap with a variety of questions probing mood, activities such as exercise, social interactions, alcohol intake and sleep. For this analysis, we chose 9 variables of interest: 1) total sleep time (TST), 2) Time in Bed (TIB), 3) Sleep Efficiency (SE), 4) depression (PHQ-9), 5) PANAS-PostiveAffect, 6) PANAS-NegativeAffect, 7) Worry, 8) Stress, 9) alcohol usage (alcohol_bev). The available data from 1518 subjects was filtered to those who had usable data and a minimum of 27 daily entries. This left a total of 86 subjects for analysis. For each subject, a row was created for each daily entry consisting of the 9 variables for that day. In addition, the variables from the previous day (lagged variables) were added to each row. The Greedy Fast Causal Inference (GFCI) algorithm from the py-causal python package was applied to each subject's data and the generated edges were examined. Subjects that had variables with causal relationships with alcohol usage were selected and the causes were examined.

Results: Of the 86 subjects, there were 15 subjects who had one or more variables causally related to alcohol usage. The causes included stress (6), sleep (6), negative affect (3) and depression (2).

Conclusions: Using causal discovery methods, we were able to identify variables with causal relationships to drinking behavior in 15 subjects. The identified variables included stress, sleep, negative affect and depression. These variables were consistent with those reported in the literature as contributing to drinking behavior. As these variables have been identified as causal at the individual level, they provide targets for intervention that are tailored to the individual. Future work will expand the analysis beyond direct edges to include examining the overall graph structure for each individual.

Keywords: EMA, Sleep Inconsistency, Alcohol Consumption, Stress Coping

Disclosure: Nothing to disclose.

P633. Self-Reported Symptoms and Quality of Life Among Patients Enrolled in a State Medical Marijuana Program

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Background: Quality of life (QoL) is an important metric for how the lives of patients are affected by health conditions and their treatment and includes mental health, physical health, and social well-being. Cannabis is increasingly used as a therapeutic agent for chronic and acute conditions for which traditional treatments may not be effective or well-tolerated. Previous research evaluating the effect of medical cannabis on health-related QoL has been inconclusive. Thus, the goal of the present study was to understand for what conditions patients are certified to use medical cannabis, the perceived efficacy of cannabis for mitigating symptoms, and self-reported QoL among those certified to use medical cannabis.

Methods: A total of 210 patients certified to participate in the Pennsylvania state-run medical cannabis program were recruited to participate in a 12-month study. Surveys were completed via phone at 4 time-points (baseline, 30 days, 6 months, 12 months) and included demographic information, certifying conditions, current medications, and symptoms treated. The data presented here are from the baseline assessment collected between May and October 2020. For each symptom, participants were asked to rate the severity "while not using medical marijuana" and "while using medical marijuana" on a scale from 1 (mild) to 3 (severe). QoL was assessed using the Functional Assessment of Chronic Illness Therapy-Palliative Care (FACIT-PAL) which is comprised of the original Functional Assessment of Cancer Therapy-General (FACT-G) questionnaire and the palliative care subscale. The FACT-G contains 27 items divided into four domains: Physical Well-Being (PWB), Social/Family Well-Being (SWB), Emotional Well-Being (EWB), and Functional Well-Being (FWB). The Palliative Care subscale contains 19 items that pertain to individuals with life-limiting illness. Subscale scores were added to create the FACT-G Total and the FACIT-PAL Total score (FACT-G Total plus palliative care subscale). Higher scores indicated better quality of life.

Results: Overall, the sample self-identified predominantly as White (70%), female (54.3%) and non-Hispanic/Latino (85.7%) and most participants had used cannabis prior to completing this survey (78.1%). The most common medical conditions certified for medical cannabis were pain (48.6%), anxiety (36.7%), and post-traumatic stress disorder (PTSD, 15.7%). Approximately 23% of the sample ($n = 48$) reported using a benzodiazepine, opioid and/or other anxiolytic medication. Anxiety and pain were the most commonly reported symptoms followed by sleep disturbance and depression (65.2%, 56.7%, 38.6%, and 31.4%, respectively). Unsurprisingly, anxiety was most common among those certified to use medical cannabis for anxiety and PTSD, but nearly 50% of those certified for pain also indicated experiencing anxiety. Although pain was less common among those certified for anxiety and PTSD, 30% and 21.1% endorsed pain, respectively. Compared to normative data, the current sample reported lower FACT-G Total T-score, EWB, and SWB scores (i.e., T-Scores < 45). When compared to those certified for pain, those certified for anxiety reported lower SWB and Palliative Care subscale scores (ps = 0.015 and 0.02, respectively). Likewise, those who reported use of an opioid/benzodiazepine reported lower QoL on all subscales (except SWB and FWB) compared with those who did not report use (ps<0.01). There were also significant negative correlations between each FACIT-PAL subscale and total symptom count and total number of self-reported medical conditions (ps<0.01).

Conclusions: The current data provide much needed information about the characteristics and symptom profiles of patients

enrolled in a state-run medical cannabis program. Pennsylvania is one of only a few states for which anxiety is a certifying condition for medical cannabis. Despite mixed evidence regarding the efficacy of cannabis for treating anxiety, more than a third of the current sample were certified to use medical cannabis for anxiety. Although patients reported a decrease in anxiety while using cannabis, those certified for anxiety also reported lower QoL when compared to those certified for pain, specifically SWB. Lastly, lower QoL was associated with more self-reported comorbid medical conditions, higher total symptom count, and use of an opioid and/or benzodiazepine suggesting that within this population of people certified to use medical cannabis there is a range of severity that relates to their overall well-being. The follow-up surveys will provide critical data regarding the trajectory of these symptoms as they relate to cannabis use and QoL.

Keywords: Cannabis, Quality of Life, Anxiety, Pain, Medical Marijuana

Disclosure: NovoNordisk, Inc: Grant (Self)

P634. Role of circHomer1 and Other Circular RNAs in Cocaine-Induced Synaptic Plasticity and Competing Endogenous Gene Expression Networks

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Background: Circular RNAs (circRNAs), a class of non-coding RNAs generated by pre-mRNA back-splicing, play a critical role in brain development and synapse formation. Although the function of most circRNAs is still elusive, it is known that these molecules work as sponges for sequestering microRNAs (miRNAs) and RNA-binding proteins (RBPs). We previously found that the levels of circRNAs derived from several synaptic proteins, such as circHomer1, are significantly decreased in the nucleus accumbens (NAc) of male mice that underwent cocaine-conditioned place preference. circHomer1 is localized to synapses, where it regulates HOMER1B mRNA levels, homeostatic plasticity and learning and memory. This circRNA also binds to the neuronal RNA-binding protein HuD, a protein associated with synaptic function and drug addiction.

Methods: To study the relationship between circHomer1 and incentive motivation for cocaine, male ($n = 13$) and female ($n = 13$) Sprague Dawley rats were trained to press a lever for cocaine (0.75 mg/kg/infusion, IV) and light/tone cues on a variable ratio 5 schedule or they received saline yoked to the responses of a cocaine rat. Then after either 1 or 21 days of abstinence, rats were given a 1-h cue reactivity test during which lever presses resulted in cue presentations, but no cocaine was available. Rats were sacrificed immediately after the test and circHomer1 levels were measured in synaptosomal fractions of NAc shell using RT-qPCR. As a follow-up to examine the competition of circRNAs and mRNAs for binding to miRNAs, we developed a new bioinformatics pipeline to predict mRNA-miRNA-circRNA competing endogenous RNAs (ceRNAs) in the striatum of HuD knock out mice.

Results: Lever pressing during the cue reactivity test increased from 1 to 21 days of abstinence in support of the incubation effect. In contrast, preliminary data of circHomer1 expression revealed a strong decreasing trend from 1 to 21 days of abstinence in male rats that self-administered cocaine while females showed low levels of circHomer1 at both 1 and 21 days of abstinence, suggesting that the expression of this circRNA is inversely related to motivation for cocaine only in male rats. HuD knock out mice ($n = 3$) showed altered ceRNA networks regulating genes associated with neuronal development and synaptic plasticity compared to wildtype mice ($n = 3$).

Conclusions: These findings implicate novel post-transcriptional mechanisms in the control of drug-seeking behavior.

Supported by DA048651

Keywords: Noncoding RNA, Cocaine-Seeking Behavior, Homer1, circRNA

Disclosure: Nothing to disclose.

P635. To Fight or Not to Fight: Activation of Neural Circuits in Vta-Mpfc for Aggressive Motivation and Decision Making After Ethanol Consumption in a Novel Murine Model

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Background: Assessing the neural mechanisms for the motivation to fight remains a challenge for preclinical models, particularly with inbred strains of mice. The effects of alcohol on the decision to engage in aggressive behavior in preference over sociosexual contact is hypothesized to be based on medial Prefrontal Cortex-Ventral Tegmental Area (PFC-VTA) connections that differ from those necessary for the actual execution of these pro- and anti-social behaviors.

Methods: Adult male B6 mice demonstrated sexual competence by siring offspring. During repeated confrontations with an intruder male in their home cage they exhibited pursuit, threat and attack behavior within less than 5 min. The mice were then conditioned to respond at an illuminated operandum in an experimental chamber attached to their home cage; completion of 10 responses (Fixed Ratio 10; FR10) was reinforced by access to either a female or a male intruder which were presented in the resident's home cage. Brains were harvested at the moment of choice between the concurrently available aggressive and sociosexual options. Immuno-stained c-fos in 10 brain regions of interest was analyzed by fluorescence microscopy. The effects of ethanol self-administration were examined on choice for aggressive behavior and on c-fos expression in the mPFC-VTA pathway.

Results: Six out of 50 mice chose to complete consistently the FR 10 response option that was reinforced by aggressive behavior toward a male intruder, while not responding on the concurrently available option that was reinforced by sociosexual contact with a female. Self-administered alcohol (1-1.5 g/kg) increased responding for the aggressive option in most mice. When choosing the aggressive, but not the sociosexual option, the prelimbic area of the PFC revealed more c-fos activity.

Conclusions: The new methods and results direct future inquiries into the neural networks for decision making between pro- and anti-social behavior. Gene expression data point to neural ensembles within the prefrontal cortex as relevant to aggressive choices that are sensitive to alcohol.

Keywords: Alcohol, Prefrontal Cortex, Aggression, Immediate Early Gene, Decision Making

Disclosure: Nothing to disclose.

P636. Transcriptomic Profiling of the Medial Prefrontal Cortex Reveals Specific Genes Associated With Heightening Cocaine-Seeking

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Background: The United States has suffered a three-fold increase in annual drug overdose deaths involving cocaine in the past five years alone. In the absence of overdose death, continued cocaine

misuse can progress to cocaine use disorder (CUD). This is perpetuated by the absence of FDA-approved pharmacotherapeutic treatment options for CUD. Individuals seeking to maintain abstinence from cocaine are challenged by environmental context (s) and stimuli associated with previous drug use (e.g., paraphernalia). The medial prefrontal cortex (mPFC) is neuroanatomically positioned to provide top-down control to striatal reward circuitry and regulate the incentive-motivational properties of cocaine-associated cues. However, chronic cocaine exposure elicits widespread neuroadaptations within the mPFC resulting in maladaptive executive functioning and increased relapse vulnerability. The present study was designed to identify distinctions in the mPFC transcriptome that associate with elevated cocaine-seeking behavior to identify novel gene targets for future CUD medications development.

Methods: Male, Sprague-Dawley rats trained to stably self-administer cocaine (0.75 mg/kg/infusion) were assessed for cocaine-seeking at 10-days of abstinence. Rats were phenotyped as either high cue (HC) or low cue (LC) responders based upon a median split of lever presses for previously associated-cocaine cues. Following behavioral phenotyping, we isolated the mPFC and processed mRNA for transcriptomic profiling ($n = 5$/phenotype). Interpretation of transcriptomic data was achieved using statistical gene-set enrichment methods (i.e., Cytoscape ClueGO) in which differentially expressed genes are intersected with sets of genes that are associated with a particular biological function or pathway.

Results: Comparison analyses revealed expression of 309 transcripts in the mPFC of HC rats that were significantly higher or lower relative to LC rats. Functional gene enrichment analyses identified 15 biological processes related to behavior that were overrepresented in the mPFC of HC vs. LC rats (e.g., locomotor exploration). Heatmap analysis of expression fold-change values allowed for visualization of samples with similar transcriptomic profiles, which largely clustered HC or LC rats together. Finally, we utilized Ingenuity Pathway Analysis to generate a pathway of hypothesized regulatory interactions of select genes.

Conclusions: The results of the present study identify unique differences in the transcriptomic landscape of the mPFC between high cue and low cue responders and provide prioritized candidates (i.e., HTR2C, PENK) for future pharmacotherapeutics aimed to help maintain abstinence from cocaine and prevent relapse in CUD.

Keywords: Cocaine, Transcriptome, Cue Reactivity

Disclosure: Nothing to disclose.

P637. The Effects of Cocaine Self-Administration on Astrocyte Mitochondria Morphology

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Background: Rat cocaine self-administration leads to both structural and functional impairments in astrocytes in the nucleus accumbens (NAc), a region key to the brain's reward circuitry. In particular, cocaine impairs the ability of NAc astrocytes to maintain glutamate homeostasis, the balance between extra-synaptic and synaptic levels of glutamate. This impairment is mediated in part by decreased expression of the astroglial glutamate transporter GLT-1, which is responsible for 90% of synaptic glutamate uptake. Glutamate uptake carries a high energetic cost, which is met by mitochondria. Mitochondria colocalize with GLT-1 in astrocytes and inhibition of mitochondria in astrocyte cultures reduces glutamate uptake. Moreover, degradation of astrocytic processes has been attributed to reduced mitochondrial function in cultured astrocytes. Given the cocaine-induced impairments in glutamate homeostasis and altered astrocyte morphology, and the role mitochondria may play in both of these processes, we hypothesize that astrocytic mitochondria function may be compromised following cocaine self-administration.

Methods: To investigate this hypothesis, confocal imaging of NAc core astrocytes was performed using two AAV5s: mitochondrially-targeted enhanced green fluorescent protein as well as a membrane-targeted Td-tomato fluorescent protein, both under control of an astrocyte-specific promoter in male rats, 24hrs following 12 days of short-access (2hr/day) cocaine self-administration. Morphological properties of mitochondria were assessed using the MitochondriaAnalyzer ImageJ plugin. Oxidative stress was assessed using immunohistochemistry for 8-hydroxy-2′-deoxyguanosine (8-OHdG) colocalized with astrocyte mitochondria.

Results: Results indicate that following cocaine self-administration, mitochondria in NAc astrocytes exhibit significantly increased volume, surface area, and diameter as compared those within saline self-administering animals ($p < 0.05$ by Nested ANOVA; $N =$ 10-12 rats per group; 2-6 cells per rat). This is likely indicative of oxidative stress; however, further analysis is needed. Interestingly, this effect was not observed in a separate cohort of rats trained in 12 days of self-administration and 16-17 days of extinction, suggesting this effect is associated with early abstinence, similarly as has been reported for dendritic mitochondria (Chandra et al. Neuron 2017).

Conclusions: Structural analysis of mitochondria in astrocytes indicates swelling in early abstinence, but not following a period of extinction training. While some degree of transient swelling occurs under physiological conditions, excessive swelling can be reflective of oxidative stress and compromised mitochondria function. Experiments designed to test the effect of sex on this observation, as well as expression of mitochondria stress-related genes and markers of oxidative stress in astrocytes, are ongoing.

Keywords: Astrocyte, Mitochondria, Cocaine, Nucleus Accumbens

Disclosure: Nothing to disclose.

P639. Phase 2 Study of ANS-6637, a Specific Inhibitor of ALDH2, in Treatment Seeking Individuals With Alcohol Use Disorder: A Combined Human Laboratory and Outpatient Clinical Trial

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Background: Neurobiological-based medications to treat alcohol use disorder (AUD) act on AUD-related phenotypes including craving, stress, and withdrawal. Treating AUD by altering alcohol pharmacokinetics (PK) has been less investigated. Genetic variation in the enzymes that control alcohol metabolism is associated with reduced risk for AUD. Aldehyde Dehydrogenase (ALDH) is the enzyme responsible for the conversion of acetaldehyde to acetate and has isozymes expressed primarily in the cytoplasm (ALDH1) and mitochondria (ALDH2). Disulfiram, the first drug approved to treat AUD, non-selectively inhibits both ALDH1 and ALDH2. This mechanism alters alcohol PK but also creates adverse effects attributable to peripheral acetaldehyde accumulation as a function of ALDH1 inhibition. Motivated by preclinical research demonstrating that selective inhibition of ALDH2 can prevent cue-induced reinstatement for alcohol and attenuate drug-induced stimulation of dopamine, ANS-6637 was

developed as an orally active selective and reversible ALDH2 inhibitor for investigation as a treatment for AUD and other substance use disorders. In a Phase 1 study of ethanol interactions with ANS-6637 in healthy men, the most common adverse events were flushing and increased heart rate, expected effects due to the accumulation of acetaldehyde. Participants receiving ANS-6637 also reported decreased liking of alcohol compared to those who received placebo. To further screen the potential value of ANS-6637 as a treatment for AUD, the current Phase 2 study incorporated a human laboratory study of alcohol cue-induced craving embedded within a 5-week outpatient trial in which safety and drinking behavior were assessed among treatment seeking individuals with AUD.

Methods: A 3-arm, double-blind, randomized, placebo-controlled proof-of-concept human laboratory embedded in a 5-week multi-site clinical trial tested 2 doses of ANS-6637 compared to placebo. Individuals with elevated liver function tests at screening were ineligible (AST/ALT > 2.5x ULN and/or bilirubin>1.5x ULN). Participants were treatment-seeking male and female individuals at least 21 years of age and meeting criteria for moderate-severe AUD. Eligible participants were randomized to either placebo, ANS-6637 200 mg or 600 mg daily. After the first week of study medication, participants completed a cue reactivity session in which craving was rated in response to the sight and smell of participants' typically consumed alcohol beverage and a water control condition. During the 5-week treatment period, alcohol consumption, alcohol-related negative consequences, sleep, mood and safety assessments were obtained weekly and secondary efficacy endpoints were analyzed over the last 4 weeks of treatment. 81 participants were planned.

Results: The study was terminated early following enrollment of 43 participants (ns: placebo = 15; 200 mg = 15; 600 mg = 13) due to clinically significant increases in liver enzymes in 3 women following at least 3 weeks of dosing with ANS-6637 that resolved following drug discontinuation; 2 were randomized to 200 mg and 1 was randomized to 600 mg. Elicited AEs consistent with ALDH2 inhibition were dose-dependent, including heart rate/palpitations (placebo = 6.7%; 200 mg = 67%; 600 mg =69%) and flushing sensation (placebo = 33.3%; 200 mg = 73%; 600 mg = 92%). Regarding the primary efficacy endpoint, strength of craving to the laboratory alcohol cue exposure, group differences were not statistically significant (least squares mean + se: 7.27 + 1.25 for placebo, 3.29 + 1.30 for 200mg; 6.97 + 1.56 for 600mg groups). Secondary endpoints measured during the clinical trial were also not statistically significant; however, small to moderate effect sizes across all drinking outcomes were observed for the 600 mg dose compared to placebo (e.g., % heavy drinking days effect size = 0.43; days abstinent = 0.54). Effect sizes on the PROMIS alcohol consequences scale were moderate (0.69) for the 200mg group and large (1.05) for the 600 mg group. At the end of the 5-week study, the effect size on alcohol craving was 0.42 for the 200mg group and 0.58 for the 600 mg group compared to placebo. Sleep quality was higher in the placebo group compared to ANS-6637 treatment. On the Profile of Mood States, ANS-6637 groups had greater decreases in tension and increases in vigor compared to placebo (effect sizes ranging from 0.28 – 0.71).

Conclusions: This study, which embedded a human laboratory study within the context of a 5-week clinical trial, yielded critical safety data about unexpected liver toxicity that led to early termination of the study. In addition, while the effects on alcohol craving measured in response to an alcohol cue-exposure paradigm after 1-week of dosing were small, data from the participants treated for 5 weeks provide preliminary evidence suggesting that selective ALDH2 inhibition may reduce drinking, alcohol consequences and craving. However, these results must be interpreted with caution as the effect sizes could be unreliable due to the small sample size. In summary, while the risk of liver injury limits the value of ANS-6637 for AUD treatment, as a proof-of-concept study, the results suggest that other molecules targeting inhibition of ALDH2 may warrant further investigation.

Keywords: ALDH-2, Alcohol Use Disorder - Treatment, Pharmacotherapy, Craving

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Dicerna, Alkermes, Opiant: Advisory Board (Self)

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P640. Chronic Cocaine Causes Age-Dependent Increases in Risk Taking in Rats of Both Sexes

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Background: Chronic cocaine users frequently exhibit maladaptive decision making, overweighting the rewards and underweighting the potential adverse consequences of their choices. Previous work has shown that chronic cocaine self-administration in young adult male rats causes an increase in risk taking that persists well into abstinence, suggesting that cocaine itself may cause lasting alterations in risk-based decision making. The current study set out to extend these previous findings in two directions. First, we determined whether the effects of cocaine on risk taking were due to the volitional nature of self-administration vs. the pharmacological properties of the drug (i.e., whether passive cocaine administration produces effects similar to self-administration). Second, we determined whether cocaine effects on risk taking behavior extend to female rats.

Methods: In four separate experiments, rats' preference for risk taking was assessed in operant chambers on a "Risky Decision-making Task" (RDT). In this task, rats made discrete choices between two levers, one that delivered a small, "safe" food reward and another that delivered a large, "risky" reward that was accompanied by mild footshock, the probability of which increased from 0% to 100% over the course of each test session. In Experiments 1 and 2, rats were tested on the RDT, followed by 2 weeks of passive cocaine injections or long-access self-administration, followed by 3 weeks of abstinence and re-testing on the RDT. In Experiments 3 and 4, rats underwent 2 weeks of long access cocaine self-administration or passive cocaine injections, followed by 3 weeks of abstinence and testing on the RDT. Data were analyzed via multi-factor ANOVA, with drug condition and sex as between-subjects variables and shock probability as a within-subjects variable.

Results: In Experiments 1 and 2, neither passive cocaine administration nor cocaine self-administration affected rats' risk-taking behavior. These negative results contrasted sharply with previous findings; however, cocaine administration in the current studies began at a considerably older age (15-25 weeks) than in prior experiments that employed a similar experimental design but began cocaine self-administration at an earlier age (11 weeks). Experiments 3 and 4 were conducted to address the possibility that rats are more sensitive to the effects of cocaine at earlier ages, by starting cocaine administration at 9-11 weeks. Under these conditions, risk taking was increased by both passive cocaine injections ($F(4,80) = 7.01$, $p < .01$) and cocaine self-administration ($F(4,76) = 8.04$, $p < .05$) relative to control conditions, and this effect was similar in both female and male rats. Importantly, these increases in risk taking were not attributable to cocaine-induced differences in shock reactivity or food motivation. Finally, acute

amphetamine administration reduced preference for the large, risky reward, both in cocaine-exposed and control rats.

Conclusions: The results replicate previous work showing that chronic cocaine self-administration can cause a long-lasting increase in risk taking behavior, and extend the prior results to both female rats and to passively-administered cocaine. In addition, the results suggest a limited time window during which cocaine can affect risky decision making, as initiation of cocaine exposure at 11 weeks of age or earlier caused lasting increases in risk taking, whereas initiation of cocaine exposure at 15 weeks of age or later had no effect. Finally, acute amphetamine administration attenuated cocaine-induced increases in risk taking, suggesting a route through which maladaptive risk taking in substance use disorders could be reduced.

Keywords: Cocaine, Risky Decision-Making, Development, Sex Differences, Punishment

Disclosure: Nothing to disclose.

P641. Importance of Perirhinal Projections in Recognition Memory and Methamphetamine Addiction

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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. In the first study, we evaluated activity within the reciprocal connection between the Prh and the PFC during object recognition memory testing. In the second study, we measured activation of Prh neurons during relapse precipitated either by novel or meth associated cues. 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.

Methods: To evaluate the role of the Prh-PFC reciprocal circuit in object recognition memory, male and female rats were infused with retrograde adeno-associated virus encoding GFP-tagged Cre recombinase in the Prh or the PFC. Three weeks later rats were tested for OR memory. On test day, one group explored both familiar and novel objects. A second group explored only familiar objects. Brain tissue was processed for confocal microscopy to visualize GFP and c-fos expression in either the PFC or Prh, in order to determine to which extent cells in the pathway of interest were activated during exploration of novel vs. familiar objects. To test Prh activity in response to novel cues, rats went through 1hr (short access, ShA) or 6hr (LgA) meth self-administration and abstinence. They were reintroduced into the chamber in the presence of the meth associated lever (meth cue reinstatement) or had an additional novel lever in the chamber as well as a novel cue light (novel cue group). In this study, the extent of c-fos+ cells in the Prh were quantified and compared between groups.

Results: Rats spent more time exploring novel vs. familiar objects. During novel object exploration, cortical neurons that receive input from the Prh were not activated beyond exploration of familiar objects. However, basolateral amygdala, Prh, PVT, and ventral subiculum neurons that provide input to the PFC were activated during novel object exploration, as indexed as an increase in GFP + c-fos + /GFP + cells relative to familiar object exploration. Further, the % of fos+ cells in the Prh positively correlated with recognition index (a measure of recognition memory). Following meth self-administration (ShA or LgA), rats were tested for meth seeking in response to a meth associated lever and/or a novel lever. LgA rats preferentially responded on the meth associated lever; whereas ShA meth rats responded on both the novel and meth associated lever. The induction of c-fos in general (non-output specific) Prh neurons was greatest in the the ShA rats exposed to novel cues relative to meth cues and home cage controls.

Conclusions: In conclusion, we have identified that Prh neurons that receive cortical input are most active during object recognition memory as compared to the reciprocal circuit. These data suggest a more complex circuitry governing recognition memory than previously indicated with anatomical or lesion studies. This information informs future work aimed at understanding the role of the Prh and PFC and their connectivity in meth addiction. Established findings show that exposure to novelty increases c-fos activity in the Prh, here we extend this finding to meth experienced animals. Further, we show that the salience of a novel cue is limited to ShA meth exposure rather than those that have gone through a LgA meth, which may display a more canonically "addicted" phenotype. These, studies are among the first to characterize and identify the importance of the Prh in recognition memory and meth addiction.

Keywords: Novelty Response, Novelty Seeking, Methamphetamine Seeking, Compulsive Drug Intake, Recognition Memory

Disclosure: Nothing to disclose.

P642. Modulation of the D3R-nAChR Heteromeric Complex Attenuates Nicotine Self-Administration

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Background: Tobacco dependence remains one of the largest preventable causes of disease and death worldwide. Unfortunately, currently available therapeutics are only modestly effective in assisting individuals to achieve long-term abstinence. Thus, there is a critical need to identify novel targets for therapeutic intervention. It has been recently shown that nicotinic acetylcholine receptors (nAChRs) and dopamine D3 receptors (D3Rs) form heteromeric complexes on dopaminergic neurons, and a novel compound, HyNDA-1, can enhance the interaction between the nACHR-D3R complex. Thus, in these studies, we sought to examine whether HyNDA-1 modulation of the nAChR-D3R complex could serve as a novel target for therapeutic intervention to promote nicotine cessation.

Methods: In the first study, male mice ($n = 12$) were examined for the effects of HyNDA-1 on nicotine intake with the intravenous nicotine self-administration protocol. Subjects were tested across a range of HyNDA-1 doses (0-30 mg/kg) in a within-subject Latin-square manner. Based on these findings, we next examined whether HyNDA-1 would alter general operant responding for food reward in a separate cohort ($n = 5$). Finally, a third cohort of mice ($n = 6$) were examined in the conditioned place preference protocol (CPP) to determine if HyNDA-1 infers rewarding or aversive properties at the effective dose for nicotine self-administration. Nicotine and food self-administration data were analyzed by a mixed-effects model analysis with correction for multiple comparisons, followed by a Tukey post-hoc test as appropriate. The CPP data were analyzed by a paired *t*-test.

Results: We found that pre-administration of HyNDA-1 attenuated nicotine self-administration in a dose-dependent manner. Specifically, HyNDA-1 at the 30 mg/kg dose led to a statistically significant decrease in the number of nicotine

infusions earned compared to the vehicle ($p < 0.01$) and lower doses ($p < 0.05$). Interestingly, the effective dose of HyNDA-1 was ineffective in altering food self-administration ($p > 0.05$) and did not induce a chamber preference in the CPP test ($p > 0.05$).

Conclusions: These data demonstrate that modulation of the D3R-nAChR complex by HyNDA-1 decreases nicotine self-administration. Importantly, these effects were specific for nicotine, as HyNDA-1 treatment did not alter food self-administration. Moreover, the HyNDA-1 compound does not appear to infer any rewarding or aversive properties by itself, as no differences were found with CPP. Taken together, these findings reveal that modulation of the D3R-nAChR complex has the potential to be an effective novel target for smoking cessation.

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Keywords: Nicotine/Substance Use Disorder, Dopamine D3 Receptors, Nicotinic Acetylcholine Receptors, Intravenous Drug Self-Administration, Conditioned Place Preference

Disclosure: Nothing to disclose.

P643. Intermittent Access Cocaine Self-Administration Differentially Promotes Habit-Based Versus Goal-Directed Cocaine Seeking in Male and Female Rats

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Background: Cocaine use disorder causes significant health and financial burdens to society; however, no effective treatments currently exist. While preclinical models have identified numerous interventions that can disrupt the primary reinforcing effects of cocaine to reduce self-administration, such interventions have had very limited clinical success. Some hypothesize that the compulsive nature of substance use disorders (SUDs) leads individuals to continue to use regardless of whether or not the drug is reinforcing. Thus, an over-reliance on habit-based decision-making circuitry could lead to drug use regardless of whether or not a therapy reduced the value of the drug. Thus, classic preclinical models using fixed ratio (FR) reinforcement schedules may not be effective models of the compulsive nature of the disorder found in human subjects. One model with potential for better predictive validity is the intermittent access (IA) model, which has been shown to produce increased motivation and drug seeking despite punishment in rats, features that may indicate that drug seeking has become habitual. However, this has not been tested directly, nor have comparisons in females been published. Moreover, it is not clear if IA self-administration alters the efficacy of potential treatments for SUDs.

Methods: Male and female Sprague-Dawley rats were trained to self-administer cocaine on either an FR or IA schedule of reinforcement. We tested subsets of both groups on progressive ratio (PR) and punished responding. Another group of rats was tested for habit-based decision making by infusing the dopamine receptor antagonist alpha-flupenthixol into the dorsolateral striatum (DLS) prior to a test of cue-motivated cocaine seeking. We next tested the efficacy of cue extinction learning by dividing animals under each training schedule into groups exposed to 0 or 120 cues. Cue-induced reinstatement was tested the following day. Finally, we performed exploratory analysis of dopamine release and neural activity in the dorsal medial striatum and DLS of rats across a training period that should encompass drug seeking as it transitions from goal-directed to habit-based using fiber photometry of dLight and RCaMP fluorescent indicators.

Results: Rats trained on an IA schedule exhibited escalation of intake, higher break point ratios on a PR, and increased punished responding relative to FR trained rats. We found that the increase in PR breakpoints was driven by females, while the increase in punished responding was driven by males. These results suggest males are more susceptible to habit-based drug seeking. Indeed, we also observed that drug seeking was disrupted by DLS DA receptor antagonism more strongly in males. In addition, while cue extinction was equally effective in FR and IA trained females, cue extinction was ineffective in IA trained males. Finally, our fiber photometry data suggest that increased neural activity (Ca2+ signal) is observed in response to a cocaine cue in both the DMS and DLS early in training, but that a dopamine signal is only observed in the DMS during goal-directed cocaine seeking. Detailed statistics: Data were analyzed using ANOVAs. Self-administration: we combined all cohorts for group sizes: FR male = 10, FR female = 9, IA male = 31, IA female =25. Groups were subdivided into $N = 4$ or 5 for each sex and access condition or extinction group. Analysis of DA antagonist effects were $N = 8$/group. Infusions earned: day x schedule interaction ($F(9,639) = 5.96$, $p < 0.001$), post-hoc comparison showed more infusions in IA group on day 10 than day 1 ($p < 0.0001$). PR: access model ($F(1,14) = 11.43$, $p = 0.005$), dose ($F(3,42) = 29.77$, $p < 0.001$), sex ($F(1,14) = 5.46$, $p = 0.03$), and sex x dose interaction ($F(3,42) = 4.27$, $p = 0.01$). Punished responding: effects of dose ($F(1,14) = 43.21$, $p < 0.001$), access model ($F(1,14) = 6.93$, $p = 0.02$), and an access model x sex interaction ($F(1,14) = 5.57$, $p = 0.033$). Post-hoc analysis showed higher responding in male IA rats ($p = 0.01$). DA antagonist experiments: interaction between treatment and training ($F(1,14) = 4.67$, $p = 0.049$), post-hoc analysis showed antagonist effect driven by IA males ($p = 0.026$). Cue extinction: effects of extinction ($F(1,29) = 11.61$, $p = 0.002$) and access model ($F(1,29) = 4.83$, $p = 0.036$).

Conclusions: These studies reveal that under specific schedules of reinforcement, cue motivated cocaine seeking can be controlled by goal-directed or habitual response strategies, and that IA schedules differentially affect the propensity of males and females to exhibit habit-based drug seeking. Thus, successful treatment may require different strategies tailored to individual brain circuits and signaling systems driving drug use.

Keywords: Intermittent Access Self-Administration, Cocaine, Punishment, Progressive Ratio Testing, Sex Differences

Disclosure: Nothing to disclose.

P644. The Unfolding Role of Microglia in Alcohol Withdrawal

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Background: Alcohol withdrawal is a medical emergency and a behavioral barrier to reducing alcohol abuse. Unlike withdrawal to other drugs, there is ample evidence that withdrawal to alcohol becomes worse after multiple cycles of alcohol consumption and abstinence, increasing the risks for serious adverse complications including seizures, delirium tremens, and death. Furthermore, the negative reinforcement inherent to cycles of alcohol abstinence and relapse to alcohol consumption complicates any behavioral interventions to reduce alcohol abuse.

Our hypothesis is that chronic, intermittent alcohol exposure alters the way microglia, the innate immune cells that reside in the brain, modulate neuroimmune as well as neuronal synaptic signaling.

Methods: Using the chronic intermittent ethanol vapor exposure model, we collected ribosome-associated RNA from striatal microglia using conditional transgenic RiboTag after five cycles of alcohol exposure and abstinence. We performed RNAseq

on the microglial "translatome" and analyzed the results with DeSeq2, GSEA, and WCGNA.

Results: We found that alcohol withdrawal causes oxidative stress in microglia and activates the "unfolded protein response" (UPR), a mechanism that is involved in cellular stress responses leading to neuroinflammatory signaling and derangements in microglia function that can lead to cell death. In particular, Ddit3 (CHOP), a transcription factor that is a key mediator of the UPR, was induced in microglia after ethanol withdrawal. We confirmed these observations using combined fluorescent in situ hybridization and immunohistochemistry in a second cohort.

Conclusions: Activation of the UPR can induce a strong neuroinflammatory response and can be a pivot from homeostatic to apoptotic responses to cellular injury. Recognizing the potential role of the UPR in microglial responses to ethanol tolerance and withdrawal may lead to new therapeutic targets for treating alcoholism. Our current studies are directed at determining the impact of conditional knockout of CHOP from microglia on ethanol withdrawal behaviors and the propensity to drink ethanol subsequently.

Keywords: Ethanol, RNA-Sequencing, RiboTag, Alcohol Withdrawal, Microglia

Disclosure: Nothing to disclose.

P645. Binge Intake of Alcohol Results in a Cross-Species Loss of Serotonin-Driven Inhibitory Control in the OFC

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Background: Alcohol use engenders neuroplastic changes in central serotonin (5-hydroxytryptamine, 5-HT) systems that have the potential to drive compulsive alcohol drinking and contribute to alcohol use disorder. Previous work in mice has shown that chronic alcohol exposure alters orbitofrontal cortex (OFC) activity and results in a loss of 5-HT function in this region. This is significant, as reduced forebrain 5-HT is causally linked to a loss of inhibitory control, which may drive compulsive intake. In the present experiments, we characterized binge alcohol-induced changes in OFC 5-HT signaling in mice and non-human primates and examined their role in promoting binge-like and compulsive alcohol intake.

Methods: Binge alcohol drinking was modeled in mice using a two-bottle choice drinking in the dark (DID) paradigm. Briefly, mice were given three cycles of limited access to a 20% v/v alcohol solution in addition to water. Each cycle of DID consisted of a 2h alcohol access period every Mon-Wed and 4h access every Thur. In rhesus macaques (Macaca mulatta), reliable self-administration of alcohol (4% w/v) was established using schedule-induced polydipsia. Following induction, alcohol consumption occurred under 12-month open access (22h/d) conditions. In this procedure, non-human primates (NHPs) develop distinct drinking typologies and self-select into distinct, stable drinking categories (e.g., low drinking, LD, and binge drinking, BD). Whole-cell patch clamp electrophysiology was used to assess alcohol-induced plasticity in 5-HT signaling in the OFC in mice and NHPs. Fluorescence in situ hybridization was used to quantify 5-HT1A and 5-HT2A mRNA expression levels in the OFC in mice. To conditionally delete 5-HT1A receptors in OFC pyramidal neurons (PN), AAV5-CaMKIIa-GFP-Cre (or GFP control) was injected into the OFC of Htr1a floxed male and female mice. In addition to DID, mice were tested for compulsive or aversion-resistant alcohol-drinking. Here, alcohol was adulterated with the bitter tastant quinine (100μM) and mice were given 4h access to this solution. To test the specificity of 5-HT1A deletion on alcohol intake, mice were challenged with a 4h sucrose (3%) preference test.

Results: In OFC PNs, repeated cycles of DID induced plasticity in 5-HT signaling at both acute (24h) and protracted (7-10d) withdrawal timepoints, leading to a profound loss of 5-HT's inhibitory control over the OFC. In water control mice, bath application of 5-HT robustly hyperpolarized OFC pyramidal neurons, but not in alcohol-exposed DID mice (total change in membrane potential (mV) induced by 5-HT in 24h and 7-10d withdrawal mice vs H2O mice, $p < 0.01$). Similarly, 5-HT-induced hyperpolarization of OFC PNs was absent in binge-dinking compared to low-drinking rhesus macaques ($p < 0.05$ LD vs BD). In male and female mice, we found that Htr1A mRNA expression was significantly reduced after DID, whereas 5-HT2A mRNA was unaltered. These findings suggest that the loss of 5-HT-induced inhibitory signaling in the OFC is due to 5-HT1A downregulation. Next, we sought to directly test the role of 5-HT1A in driving excessive alcohol intake by selectively and conditionally deleting 5-HT1A in OFC PNs. We found that deletion of 5-HT1A in OFC PNs increased binge-like alcohol drinking in male (alcohol intake (g/kg/4h) and preference, $p < 0.05$ Cre vs GFP) but not female mice and promoted aversion (quinine)-resistant alcohol drinking in male and female mice (alcohol intake (g/kg/4h), Cre vs GFP, $p < 0.05$ both sexes; alcohol preference, Cre vs GFP: $p < 0.05$ M and $p < 0.01$ *F*), but did not affect sucrose intake.

Conclusions: Our cross-species physiology demonstrates that there is conserved plasticity in orbitofrontal cortical 5-HT signaling induced by binge alcohol intake. Specifically, we show that binge alcohol drinking results in a loss of 5-HT-mediated inhibitory control over the OFC in mice and NHPs. Downregulation of 5-HT1A receptors may serve as one potential mechanism for this, as in mice binge alcohol robustly reduced mRNA expression levels of this receptor. Further, our work demonstrates that loss of 5-HT1A in OFC PNs promotes compulsive and binge-like alcohol drinking. Thus, it is likely that binge alcohol-induced reductions in 5-HT1A may serve to support and maintain an individual's alcohol misuse, and thereby increase their risk of developing alcohol use disorder. Overall, our work highlights the therapeutic potential of targeting OFC 5-HT1A signaling for the treatment of binge drinking and alcohol use disorder.

Keywords: Serotonin, Serotonin 1A Receptors, Orbitofrontal Cortex (OFC), Rhesus Monkeys, Binge Alcohol Use

Disclosure: Nothing to disclose.

P647. Microbiome Knockdown Causes a Dose-Dependent Shift in the Reinforcing Efficacy of Fentanyl in Male Rats

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Background: There is growing evidence indicating that a diverse gut microbiome is necessary for good mental health. Maladaptive alterations of the gut microbiome have been demonstrated in multiple psychiatric conditions including autism spectrum disorder, depression, and substance use disorders. Mechanistic studies in animal models have shown that alterations to the microbiome result in altered behavioral and synaptic plasticity in multiple domains. Recently, animal studies have indicated that drug reward and intake can be affected by reduction of the microbiome with antibiotics. In preclinical tests of opioid reward, mice with a reduced microbiome have attenuated morphine locomotor sensitization and conditioned place preference. In addition to its effects on opioid reward, microbiome knockdown drastically altered gene transcription in the nucleus accumbens

(NAc). The current study assessed the effects of microbiome depletion on opioid intake in a more translationally relevant self-administration paradigm. Additionally, we utilized global proteomic analysis of the NAc to determine microbiome effects on opioid-induced protein expression.

Methods: Adult male Sprague-Dawley rats (weighing ~250 g) were pair-housed upon arrival. Cages were randomly assigned to normal drinking water (H2O) or antibiotics (Abx; containing vancomycin, neomycin, bacitracin, and pimaricin) to knockdown the microbiome. Rats received their respective drink solutions 2 weeks before the start of behavior and remained on their drink solutions for the entirety of the study. Rats were run in three independent cohorts with different experimental aims for each. For all experiments, rats were implanted with a jugular catheter one week before the experiment began and were trained to self-administer fentanyl or saline on an FR1 schedule for 10 days before continuation of procedures as outlined below. Experiment 1 measured overall intake during acquisition of high-dose (5 mcg/kg) fentanyl self-administration (H2O $n = 6$, Abx $n = 6$). For Experiment 2, rats were trained at a lower dose of fentanyl (2.5 mcg/kg) before undergoing an increasing FR study over 6 days (2 days at FR2, 2 days at FR3, and 2 days at FR5), and concluding with two days of progressive ratio (H2O $n = 6$, Abx $n = 7$). For Experiment 3, rats were trained at 2.5 mcg/kg before assessment of their dose-response curve. Rats administered two consecutive days at each of the following doses: saline, 0.025 mcg/kg, 0.25 mcg/kg, 0.79 mcg/kg, 7.9 mcg/kg, or 25 mcg/kg. Doses were presented in randomized order (H2O $n = 7$, Abx $n = 7$). Finally, to gain insight into the neurobiology underlying differences in motivation between H2O and Abx rats, the NAc of rats from Experiment 2 were dissected and flash frozen. Unbiased data-independent acquisition using mass spectrometry was used to determine differences in protein expression in the NAc of rats with an intact and a depleted microbiome. All animal procedures were approved by the Mount Sinai IACUC and conformed to the "Guide for the Care and Use of Laboratory Animals".

Results: For Experiment 1, microbiome depletion by Abx reduced high-dose fentanyl intake during acquisition (linear mixed effects analysis; significant main effects of session ($p < 0.001$) and drink type ($p = 0.0053$)). However, in Experiment 2 there was no difference in acquisition or administration of 2.5 mcg/kg fentanyl (linear mixed effects analysis; significant main effect of session only ($p < 0.0001$)). In contrast, Abx rats worked harder to obtain fentanyl in the increasing FR and progressive ratio tasks (increasing FR: linear mixed effects, main effects of session ($p = 0.0006$), drink type ($p = 0.0104$), and a significant interaction ($p < 0.0001$); progressive ratio: independent samples *t*-test, $p = 0.012$). Since Abx rats administered less fentanyl than H2O rats at a high dose and equal amounts of fentanyl at a lower dose, Experiment 3 assessed the effects of Abx on rats' fentanyl dose-response. Microbiome knockdown produced a leftward shift in the dose response curve (linear mixed effects analysis, significant main effect of dose ($p < 0.001$) and a significant interaction ($p = 0.0109$)). Finally, proteomic analysis of the NAc indicated that pathways relating to G protein signaling, cAMP signaling, opioid receptor signaling, and nitric oxide production were upregulated in Abx rats only, and synaptogenesis pathways which were upregulated in H2O rats only.

Conclusions: The current study determined that alterations to the microbiome by oral Abx influence fentanyl self-administration by inducing a leftward shift in the dose-response curve and cause an increase in motivation for fentanyl at a moderate dose. Proteomic analysis of NAc indicate that microbiome depletion affects several pathways related to drug-taking behavior. Future studies will explore the link between altered expression of proteins in these pathways and the altered opioid self-administration induced by microbiome depletion.

Keywords: Fentanyl, Gut Microbiome, Self-Administration
Disclosure: Nothing to Disclose.

P648. An Exploratory Analysis into the Role of Abnormal Peripheral Metabolism in Individuals That Consume Alcohol

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Background: Peripheral metabolic biomarkers play a role in energy homeostasis and feeding behavior. It has also been proposed that they may modulate central reward pathways important for alcohol and drug seeking behaviors. Dysregulation of metabolism may result in changes in reward pathways and alcohol-related behaviors. Increases in metabolic markers such as triglycerides have been associated with changes in reward related neural regions, decreases in dopamine turnover in reward neurocircuitry, and reductions in reward related behaviors. Our recent findings revealed levels of triglycerides and HDL cholesterol significantly affected neural activity in the anterior insula during anticipation of potential monetary loss and this effect was not mediated by alcohol use.

Methods: Preliminary analysis consisted of sixteen male and female participants from a previously collected dataset. Participants were treatment seeking, detoxified individuals with AUD ($N = 7$), non-treatment seeking heavy alcohol drinkers ($N = 3$) and controls without AUD ($N = 6$), with clinically abnormal levels of at least 3 of the following: blood pressure, triglyceride, glucose, HDL, and BMI Participants completed a neuroimaging version of the Monetary Incentive Delay task (MID). Mediation models used metabolic biomarkers to predict neural activation in ROIs during the anticipation phase (e.g., high gain, high loss, low gain, low loss, outcome: gain, missed gain, loss, avoided loss) and AUDIT were entered as a potential mediator of this relationship.

Results: Abnormal metabolic status had a significant effect on ACC activation during the anticipation of gaining a high value reward (b = -.945, 95%, CI = -1.96- (-.14) $p = .02$), high value loss (b = -2.656, 95%, CI = -3.44 - (-1.87) $p = .01$) with the no effect of alcohol use. Alcohol use mediated the anticipation of losing a low value reward (b = -85.48, 95%, CI = -178.230 - (-7.90) $p = .03$). Abnormal metabolic status also significantly affected right anterior insula (AI) activation during the anticipation of losing a high value reward (b = -2.158, 95%, CI = -2.73 - (-1.58) $p = .01$) and NAc during the anticipation of losing a high value reward and this effect was mediated by alcohol (b = -60.18, 95%, CI = -112.81 - (-7.55) $p = .02$).

Conclusions: We found abnormal metabolism had a significant effect on reward related pathways, however alcohol use only mediated that relationship in the anticipation of losing a low value reward. We previously reported that normal triglyceride and HDL levels had a significant direct effect on neural activity in the left AI in response to the anticipation of potential losses however this was not affected by alcohol use severity. These preliminary findings highlight the potential role of both normal and abnormal metabolism in reward and motivation pathways in an alcohol drinking population and suggest the interplay of alcohol use, peripheral metabolism and reward process may be more pronounced in individuals with clinically abnormal metabolic function. Additional analysis is currently under way to expand the sample.

Keywords: Alcohol Use Disorder, Metabolism, Reward Processing
Disclosure: Nothing to disclose.

P649. A Prelimbic Cortex to Nucleus Accumbens Core PACAPergic Projection Promotes Excessive Alcohol Drinking

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Background: Prefrontal cortex projections to the striatum have been proposed to play a key role in the pathological excessive drinking which characterizes alcohol use disorder, with these inputs to the striatum becoming strengthened over the course of alcohol exposure. Here, we investigated the role of the neuropepeptide pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptor, PAC1R, on excessive alcohol intake in male and female mice in a prelimbic cortex (PrL) to nucleus accumbens core (NAcc) circuit. We hypothesized that overactivation of this system in this circuit mediates excessive drinking following alcohol exposure.

Methods: We first characterized the PrLPACAP population and its projection targets, using in situ hybridization ($N = 2$) and viral tracing ($N = 3$), respectively. Following 8 weeks of alcohol or water exposure using the Intermittent Access to Two Bottle Choice (IA2BC) method, we quantified the amount of PACAP and PAC1R, and the activity of cells expressing these markers in the PrL and NAcc, as measured by FosB co-expression ($N = 4$-6/group). We then used a cre-dependent, caspase-mediated viral approach to ablate PrLPACAP neurons and measured the effect on alcohol drinking in male and female mice ($N = 11$/group, 5-6/sex). We extended these results by acutely inhibiting these PrLPACAP neurons using inhibitory DREADDs, following 8 weeks of IA2BC exposure ($N = 14$, 7/sex). We next investigated the role of NAccPAC1R by measuring ethanol intake following administration of PA-8, a small molecule antagonist of PAC1R ($N = 18$, 9/sex), followed by a more chronic manipulation, short-hairpin RNA (shRNA) knockdown of the receptor in the NAcc ($N = 9$-11/group). Last, we used channelrhodopsin-associated circuit mapping (CRACM), a combination of optogenetic excitation of incoming PrLPACAP neurons and whole-cell patch clamp recordings of NAccD1R +/- neurons, to confirm functional connectivity between the two populations ($N = 4$).

Results: Using fluorescent in situ hybridization, we identified the PrLPACAP population to be glutamatergic long-range projecting cells in layer 2/3. We characterized the projections of PrLPACAP neurons and observed visible fibers in NAcc and other downstream areas relevant to alcohol drinking. We then used the Intermittent Access to Two Bottle Choice (IA2BC) method to model chronic alcohol drinking. In mice exposed to eight weeks of alcohol, we found increased co-localization of FosB and PACAP in the PrL ($t(7) = 4.159$, $p \leq 0.01$). There was also increased overall PACAP ($t(7) = 3.118$, $p \leq 0.05$) and PAC1R ($t(4) = 3.664$, $p \leq 0.05$) in the PrL and NAcc, respectively, implicating activation of this neuropeptidergic system following alcohol exposure (8 weeks of IA2BC). When we used a cre-dependent caspase virus to selectively ablate PrLPACAP neurons prior to alcohol exposure, we observed a decrease in prolonged alcohol consumption that was specific to male mice (Virus X Time X Sex: $F(13, 221) = 2.83$, $p \leq 0.001$; Males - Virus: $F(1,8) = 5.65$, $p \leq 0.05$, Virus X Time: $F(13,104) = 1.96$, $p \leq 0.05$, Females – Virus: $F(1,9) = 0.24$, n.s., Virus X Time: $F(13,117) = 1.74$, n.s.). We then used a designer-receptor approach to study the effect of inhibiting PrLPACAP neurons on alcohol consumption in male and female mice with 8 weeks of IA2BC alcohol exposure; we found that inhibition of PrLPACAP neurons decreased alcohol drinking, again only in male mice (Dose X Sex: $F(2,32) = 45.9$, $p < 0.001$; Males - Dose: $F(2,16) = 6.505$, $p \leq 0.01$, Dose X Time: $F(4,32) = 2.656$, $p \leq 0.05$. Females – Dose: $F(2,16) = 0.16$, n.s., Dose X Time: $F(4,32) = 0.27$, n.s.). Studies using a floxed PACAP mouse model are ongoing to determine if PACAP in PrL neurons is specifically responsible for increased alcohol consumption. Additionally, we are currently using fiber photometry to investigate whether alcohol exposure modulates the endogenous activity of PrLPACAP neurons. Pharmacological antagonism of PAC1R, via the PA-8 drug administered systemically to alcohol exposed animals, also decreased alcohol intake, again only in male mice (Dose X Sex: $F(2,36) = 2.15$, n.s. [$p = 0.13$], Males - Dose: $F(2,22) = 27.88$, $p \leq 0.001$, Dose X Time: $F(4,44) = 0.57$, n.s., Females – Dose: $F(2,16) = 0.25$, n.s., Dose X Time: $F(4,32) = 0.76$, n.s.). We followed up on this finding by assessing whether PAC1R knockdown would block excessive drinking by injecting a short hairpin RNA targeted to the PAC1R receptor via viral vector into the NAcc of male mice prior to alcohol exposure; we found that the PAC1R knockdown decreased ethanol consumption, compared to a control virus (Virus: $F(1,18) = 11.34$, $p \leq 0.01$, Virus X Time: $F(6,108) = 1.45$, n.s.). Channelrhodopsin-Assisted Circuit Mapping (CRACM) experiments have demonstrated functional connectivity at PrLPACAP to NAccD1R +/- synapses; electrophysiology experiments are currently underway to determine the effect of ethanol exposure on this connection, as well as the possible modulation of glutamatergic transmission at these synapses by PACAP application, and PAC1R antagonism.

Conclusions: Overall, these data describe a projection from PACAP-expressing cells in PrL to the NAcc Core, and show that these neurons play a key role in aberrant alcohol drinking in a mouse model of chronic alcohol exposure, which appears specific to male mice.

Keywords: PACAP, Alcohol Use Disorder, Nucleus Accumbens Core, Prelimbic Cortex

Disclosure: Nothing to disclose.

P650. Reduced Dopamine Terminal Function in the NAC Following Heroin Self-Administration

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Background: Recent evidence has illustrated that heroin produces its rewarding and addictive effects, at least partially, through activation of the mesolimbic dopamine system. Notably, it has been shown that heroin seeking can be attenuated by systemic administration of antagonists of negative regulators of the dopamine system, such as kappa opioid receptors (KOR) or presynaptic D3 receptors (D3R), thus increasing dopamine levels. Therefore, it is likely that withdrawal from chronic heroin exposure drives a state of hypodopaminergia in the nucleus accumbens (NAc), as previously observed following withdrawal from chronic stimulant and alcohol use. To this end, this study aimed to (1) investigate alterations in the dopamine terminal function following heroin self-administration in male and female rats and (2) identify a mechanism for hypodopaminergia following chronic heroin self-administration.

Methods: Adult male and female Long Evans rats were trained to self-administer heroin (0.05 mg/kg/inf) and then placed on long access (LgA; FR1, 6-hr session, unlimited infusions, 0.05 mg/kg/inf) heroin self-administration paradigm to induce escalation of heroin intake. Following LgA, rats were utilized for neurochemical analyses – in vivo microdialysis and ex vivo fast-scan cyclic voltammetry (FSCV) in the NAc.

Results: The results support previous literature in that following LgA, male and female rats had decreased basal extracellular levels of dopamine as well as a reduced dopaminergic response to a

heroin challenge (0.1 mg/kg/inf, IV) in the NAc during withdrawal from heroin self-administration. FSCV results revealed that heroin exposed rats have reduced dopamine release during single-pulse stimulations but increased phasic-like dopamine release during stimulation trains (5 pulses, 5-100Hz) when compared to their heroin naïve counterparts. In addition, we found that presynaptic D3R and KOR activity in the NAc was increased following LgA in male and female rats.

Conclusions: These results reveal a marked reduction in dopamine system function following heroin exposure and identify a potential mechanism in the NAc that may drive the hypodopaminergic state observed during heroin withdrawal.

Keywords: Heroin Self-Administration, Dopaminergic System, Fast Scan Cyclic Voltammetry, Microdialysis, Opioid Abuse

Disclosure: Nothing to disclose.

P651. Vaporized Δ9-Tetrahydrocannabinol (THC) Produces Conditioned Rewarding Effects in Male and 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. Development of animal models using vapor exposure is important for increasing our understanding of the rewarding and aversive effects of vaped cannabinoids. Currently there are limited data on the conditioned rewarding effects of THC vapor in rats, and no studies to date examining sex differences.

Methods: Male and female Sprague-Dawley rats ($N = 96$; 12 per sex/drug condition) underwent place conditioning after exposure to THC vapor (3 exposure conditions) or vehicle (propylene glycol, PG) for two sets of 8 daily sessions (16 days total). THC vapor-conditioned rats received vehicle vapor (PG) and one of 3 THC vapor exposure conditions on alternate days (Conditions: 5 puffs of 100 mg/ml THC, 5 puffs of 200 mg/ml THC, or 10 puffs of 200 mg/ml THC). Vehicle-conditioned rats received PG vapor exposure each day. Rats were passively administered vapor for 30-min immediately before daily, 30-min conditioning sessions. Place preference tests occurred after the 8th and 16th conditioning sessions, and continued daily until extinction occurred. Following extinction, rats underwent a drug-primed reinstatement session.

Results: Male and female rats showed an exposure-dependent preference for the THC vapor-paired chamber. The lowest THC vapor exposure condition tested (5 puffs of 100 mg/ml THC) did not produce conditioned place preference. The highest THC vapor exposure condition tested (10 puffs of 200 mg/ml) produced place preference in both males and females (p's < 0.05). Sex differences were observed, where males showed a preference for the THC-paired chamber when exposed to fewer puffs (5 puffs of 200 mg/ml THC). This exposure condition was not sufficient to produce conditioned place preference in females (Sex × THC interaction; $p < 0.05$). Preference for the THC-paired chamber (10 puffs of 200 mg/ml THC) extinguished more quickly in males than in females. THC vapor re-exposure (i.e., drug-prime) did not result in reinstatement for either sex.

Conclusions: This is the first study to observe conditioned rewarding effects of THC vapor in both male and female rats, as well as the first to examine extinction and reinstatement of CPP of THC vapor. Conditioned place aversion was not observed at any of the THC vapor conditions tested. The use of vaporized THC in preclinical models is important for translational value and informing our understanding of the rewarding effects of cannabis constituents and consequences of vapor exposure.

Keywords: THC, Vapor, Conditioned Place Preference, Reward and Aversion, Sex Differences

Disclosure: Nothing to disclose.

P652. Dose-Response in Modulating Brain Response to Transcranial Direct Current Stimulation: An Exploration in Four Levels

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Background: Non-invasive brain stimulation methods for modulating brain activity via transcranial technologies are increasingly prevalent to investigate the relationship between modulated brain regions and stimulation outcomes. As one of the most frequently used technologies, transcranial direct current stimulation (tDCS) has shown promising results to modulate brain activity/connectivity. However, the inter-individual variability to tDCS has made it challenging to detect intervention effects at the group level. Animal and human studies has demonstrated significant variability in the electrical dose that different brain regions receive at the individual level during a fixed stimulation protocol. Collecting multiple modalities of magnetic resonance imaging data (i.e., structural and functional MRI) helps to investigate how dose-response ultimately shapes brain function in response to tDCS. In this study, we present our pre-registered trial data (NCT03382379) on the association between electric fields (estimated with subject-specific computational head models) and brain response (changes in BOLD signals) in a group of participants with methamphetamine use disorders (MUDs). Here, we propose that the dose-response relationship could be investigated in at least 4 different levels.

Methods: We collected data in a randomized, triple-blind, sham-controlled trial with two parallel arms. Sixty participants with MUD were randomly assigned to sham or active tDCS ($n = 30$ per group, 2 mA, 20 minutes, anode/cathode over F4/Fp1). Structural and functional MRI (including high-resolution T1 and T2-weighted MRI, resting-state fMRI and methamphetamine cue-reactivity task with meth versus neutral cues) were collected immediately before and after tDCS. T1 and T2-weighted MRI data were used to generate head models for each individual to simulate electric fields. Associations between electric fields (dose) and changes in brain function (response) were investigated at four different levels: (1) voxel level; for each of the voxels a general linear model was used to model brain activation in response to meth>neutral contrast (2) regional level (atlas-based parcellation); Brainnetome atlas was used for cortical parcellation of the head models and task-based functional maps, (3) cluster level (active clusters in the contrast of interest); significant clusters' masks, obtained from time by group interaction analysis of meth>neutral contrast were used to determine associations (post- and pre-stimulation functional activity were compared by using LMEs (3dLME, AFNI). Family-wise error (FWE) was found by Monte Carlo simulation-based (3dClustSim, AFNI) multiple comparison correction with alpha<0.1. $P < 0.005$ and cluster size>40), and (4) network level (both task-based and resting-state networks); independent component analysis was used to extract large-scale resting-state networks' masks, and regional homogeneity (ReHo) was calculated for each individual. Normalized mean ReHo and averaged electric fields were extracted from networks. Frontoparietal task-based connectivity (generalized psychophysiological interaction) within the executive control network was also calculated. A sample size of 30 participants per arm provided 80% power to detect an effect

size (Cohen's d) of 0.74. Pearson correlation coefficient with FDR correction was used to investigate the associations.

Results: Our results showed that (1) at the whole brain voxel-level, block designed analyses have not found any significant correlation between electric fields and BOLD signal change—post minus pre stimulation (p corrected>0.05; 9.74% of the voxels with small effect size ($0.1 < |r| < 0.3$), 1.36% with medium effect size ($0.3 < |r| < 0.5$), and only 0.09% with large effect size ($0.5 < |r| < 1$), total number of voxels were 8530021 and Pearson correlation coefficient ($|r|$) was considered as a measure of effect size). (2) at the whole brain regional level, no significant correlation survived FDR correction (24.29% of the regions with small effect size, and 3.33% with medium effect size, total number of regions were 210 cortical regions). (3) at the cluster level, our results showed no significant correlation between changes in functional activity and electric fields within the clusters (p FDR corrected>0.05; 40% of the clusters with small and 40% with medium effect size; total number of clusters were 5). (4) at the network level, a significant negative correlation was found between electric field and ReHo in default mode network ($r = -0.46$ (medium effect size), p corrected $= 0.018$). For the network-level analysis of task-based fMRI data, frontoparietal connectivity showed a positive significant correlation with electric field in the frontal stimulation site ($r = 0.41$ (medium effect size), p corrected $= 0.03$).

Conclusions: The proposed pipeline provides a methodological framework to analyze tDCS effects in terms of dose-response relationships at four different levels to directly link the electric field (dose) variability to the variability of the neural response to tDCS. The results suggest that network-based analysis might be a better approach to provide novel insights into the dependency of the neuromodulatory effects of tDCS on the brain's regional current dose in each individual. Dose-response integration can be informative for dose optimization/customization or predictive/treatment-response biomarker extraction in future brain stimulation studies.

Keywords: Transcranial Direct Current Stimulation, Computational Modeling, Functional MRI (fMRI), Substance Abuse Disorders, Methamphetamine

Disclosure: Nothing to disclose.

P653. Amygdala Transcriptome Profile After Daily Δ9-Tetrahydrocannabinol (THC) Administration to Adolescent Nonhuman Primates: Modulation by Co-Administration of Cannabidiol

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Background: Marijuana is among the most widely used drugs globally, especially among adolescents and young adults. Repeated long-term marijuana use can lead to marijuana (cannabis) use disorder or CUD, cognitive impairment, anxiety and psychosis in susceptible individuals. The magnitude of neuropsychological consequences is associated with dose, frequency, duration of use and age of onset of use, with adolescent-onset use related to higher risk. Recent trends in youth marijuana use, consumption of higher potency marijuana strains or marijuana-based concentrates, and more daily use amplify these risks. The adverse consequences of marijuana are attributed to THC, the most prominent phytocannabinoid in the marijuana plant. Although both THC and cannabidiol (CBD) are present in marijuana plant strains (e.g. Cannabis Sativa), the pharmacological effects of THC and CBD differ markedly, as CBD displays none of the pharmacological actions of THC (addictive potential, psychosis, intoxication, anxiety, cognitive deficits). Combined with THC, CBD reportedly mitigates, improves, attenuates, or is without effect on THC-induced memory impairment, anxiety, psychosis, or neuroadaptation. As the full spectrum of behavioral and molecular consequences to adolescents exposed daily to high THC levels remain unknown, we previously investigated the effects of THC administered alone or combined with CBD in adolescent primates, using behavioral and pharmacokinetic endpoints. The THC phenotype was nominally attenuated by CBD in nonhuman primates, and unlikely related to CBD disruption of THC metabolism or brain entry. The current study concentrated on molecular changes in brain elicited by daily THC or THC + CBD administered for four months to adolescent nonhuman primates. Our initial focus was the transcriptome profile of the amygdala, a region of interest implicated in anatomical, functional, and behavioral changes reported in human marijuana users.

Methods: Twelve male adolescent squirrel monkeys (2.3 – 2.5 years) were divided into three treatment groups: vehicle control, THC, or THC + CBD ($n = 4$/group). Initially they were treated with low doses of THC or THC + CBD weekly for four weeks. Thereafter, at the onset of the testing regimen, animals received THC (1 mg/kg) or THC + CBD (1 mg/kg+3mg/kg) daily for 4 months. mRNA levels in selected brain regions were assessed by bulk RNAseq and candidate markers were confirmed by RT-qPCR. Gene ontology analysis using the Ingenuity Pathway Analysis Software interrogated whether genes regulated by THC or THC + CBD compared with controls, were implicated in pathways associated with specific brain diseases or brain function. Candidate markers were confirmed using qPCR and statistical differences between treatment groups were computed using two-way ANOVA.

Results: RNA Sequencing revealed that more than 1,000 genes were differentially expressed between each treatment group (control vs THC, control vs THC + CBD, THC vs THC + CBD). Of these, 81, 92 and 32 genes were altered by >1log2 fold. Down-regulated genes were associated with pathways implicated in GPCR, CDK5, synaptogenesis, dopamine, opioid, endocannabinoid, netrin, ephrin signaling pathways, and others. THC down-regulated genes implicated in schizophrenia, suicidality, bipolar disorder, emesis, with CBD attenuating some of the regulated genes. Of interest to adolescent development, D2 dopamine receptor down-regulation was confirmed by qPCR ($p = 0.05$). Patterns of gene expression differed in amygdala and hippocampus, with CBD attenuating THC regulated changes in gene expression more robustly in hippocampus.

Conclusions: Adolescent primates exposed to a daily high dose of THC revealed altered mRNA expression of genes implicated in key neuronal pathways and in psychiatric disorders. Our findings provide leads to investigate the relevance of adaptive changes to functional and pathological consequences of frequent youth use of potent marijuana. Ongoing research is applying this methodology to other brain regions implicated in adolescent brain development, such as the prefrontal cortex.

Keywords: Marijuana, Amygdala, Adolescence

Disclosure: Nothing to disclose.

P654. Linguistic Analysis of Psilocybin Session Narratives From a Double-Blind, Methylphenidate Comparison Study

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Background: Interest in classic psychedelics has grown in recent years, in part due to their potential use as therapeutics. Some of this work has shown how the nature of the subjective drug experience relates to short and long-term health outcomes. These

studies have broadly found that greater mystical experiences (i.e., a construct reflecting unity, sacredness and noetic, positive mood, transcendence of time and space, and ineffability) are associated with greater positive clinical benefit. Prior research has relied on self-reported, standardized scales to evaluate subjective experience and the extent to which similar relationships may exist using alternative modes of assessment is unclear. The purpose of this secondary analysis was to apply a linguistic analysis to compare narrative drug experiences completed after double-blind administration of psilocybin (a classic psychedelic) and an active drug comparator, methylphenidate. A secondary aim was to evaluate the relationship between linguistic features and traditional subjective effect scales.

Methods: Healthy participants ($N = 36$) completed a double-blind, crossover study in which oral psilocybin (30 mg/70 kg) or visually matched oral methylphenidate (40 mg/70 kg) were administered in a counterbalanced order. Standardized questionnaires of subjective drug effects including mystical experience and challenging effects were completed. Participants were also asked to complete an open-ended narrative describing the session. Computerized text analysis was conducted using Linguistic Inquiry Word Count (LIWC) 2015 software with outputs included linguistic processes (total word count, words/sentence), and word categories including psychological processes (affective language), and higher-order linguistic domains (analytical thinking, clout, authentic, and emotional tone). Analyses first compared psilocybin and methylphenidate sessions using within-subjects *t*-tests with effect sizes summarized as Cohen's dz. Textual features identified as significantly different between groups were then compared using one-sample *t* tests to normative expressive writing data samples (6,179 files containing 2,526,709 words) collected in (non-psychedelic) experimental studies in which participants were asked to write about deeply emotional topics (e.g., a personally upsetting experience). Finally, significant textual features were evaluated for their association with mystical and challenging experience questionnaires using Spearman correlations.

Results: Narratives were longer for psilocybin sessions (mean word count = 1784 words) than for methylphenidate sessions (mean word count = 1149 words) ($p = .004$, dz = 0.52). Comparisons by condition indicated that psilocybin narratives contained lower scores on the higher-order construct of "Analytical Thinking" ($p = .002$, dz = -0.57). Psilocybin narratives also had a higher occurrence of impersonal pronouns (e.g., it, it's; $p < .001$, dz = 0.82), auxiliary verbs (e.g., will, have; $p = .003$, dz = 0.53), and certainty language (e.g. always, never; $p < .001$, dz = 0.78), and lower occurrence of relativity language (e.g., exit; $p = .003$, dz = -0.53), work language (e.g., job; $p = .009$, dz = -0.46), and leisure language (e.g., movie; $p < .001$, dz = -0.70). Comparisons of psilocybin values to normed "expressive writing" samples indicated the former had higher analytic writing scores ($p < .001$, $d = 1.02$), and fewer auxiliary verbs ($p = .002$, $d = -0.55$), relativity language ($p = .003$, $d = -0.53$), and work language ($p < .001$, $d = -3.51$). Higher challenging experience scores were related to lower Analytical Thinking ($r = -.46$, $p = .01$), less relativity language ($r = -.37$, $p = .04$), and more impersonal pronouns ($r = .40$, $p = .03$). Mystical experience scores were not significantly associated with linguistic features (absolute *r* values = .05 to .33).

Conclusions: Qualitative narratives of psilocybin experiences contained linguistic features that departed from both a drug comparator (methylphenidate) and large sample normative data. Notably, these linguistic features showed only modest overlap with standardized, quantitative scales suggesting they index unique aspects of the subjective drug experience. Future analyses may use this framework of narrative analyses as a tool to understand aspects of psychedelic experiences to evaluate potential mechanisms underlying positive psychological effects of psychedelics towards a broader goal of optimizing treatment settings and novel drug design.

Keywords: Psychedelics, Psilocybin, Methylphenidate, Language

Disclosure: Nothing to disclose.

P655. Accumbal D1 and D2 Medium Spiny Neurons Encode Valence-Independent Associative Learning Parameters

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Background: Value-based decision-making is at the core of nearly all motivated behaviors and requires the ability to associate outcomes with specific actions and make adaptive decisions about future behavior. At the core of value-based decision-making and reinforcement is the nucleus accumbens (NAc) which is integrally involved in learning, selecting, and executing goal-oriented behaviors. The NAc is a heterogeneous population primarily composed of D1 and D2 medium spiny projection (MSN) neurons that are thought to have opposed roles in behavior, with D1 MSNs promoting reward and D2 MSNs promoting aversion. However, this framework is largely based on ex vivo recordings showing cell-type specific plasticity after reward/drug exposure. Here we focused on defining the temporal dynamics of D1 and D2 MSNs in response to a variety of stimuli across contexts to define how information is processed in these populations.

Methods: We tested the role of D1 and D2 MSNs in behavioral paradigms that require processing of stimulus valence, salience, prediction, and timing using optogenetics, patch-clamp electrophysiology, fiber photometry, and cellular resolution calcium imaging. First, we tested whether activation of D1 and D2 MSNs is reinforcing using an optogenetic intra-cranial self-stimulation task. Then, we recorded cellular activity at the population and single neuron level during operant and Pavlovian learning tasks with rewarding and aversive outcomes. Additionally, we examined how inhibition of these two populations at temporally specific time points (cue versus outcome presentation) affects learning. Finally, we tested if aversive learning induces plasticity on both D1 and D2 MSNs using patch-clamp electrophysiology.

Results: First, we found that optical stimulation of both D1 and D2 MSN populations supported intracranial self-stimulation. Next, using patch-clamp electrophysiology, we discovered that both D1 and D2 MSNs underwent plasticity following aversive learning demonstrating that plasticity within these populations was not determined by the valence of the experience. To define the information that is encoded within these populations, we recorded their in vivo activity during reinforcement schedules and Pavlovian learning paradigms that dissociate stimulus value, outcome, cue learning, and action from one another. We demonstrated that D1 MSNs responded to the presence and intensity of unconditioned stimuli – regardless of valence. Conversely, D2 MSNs responded to the presentation of predictive cues independent of whether these cues signaled positive or negative outcomes. We also found that learning was disrupted when D1 MSNs were inhibited at the time of the outcome, and D2 MSNs were inhibited during cue presentation – showing a causal role of these signals in learning.

Conclusions: Overall, these results provide foundational evidence for the discrete information encoded within D1 and D2 MSN populations in the NAc. The information encoded within these populations goes beyond simply valence encoding and shows that these populations do not have opposing actions. These results will significantly enhance our understanding of the involvement of the MSN sub-populations within the NAc in both

basic learning and memory as well as how these neurons contribute to the development and maintenance of substance use disorders.

Keywords: Medium Spiny Neuron, Nucleus Accumbens, Valence, Associative Learning

Disclosure: Nothing to disclose.

P656. Effects of Aromatase Inhibition During Cocaine Extinction on Drug Seeking-Behavior in Male Rats

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Background: The role of endogenous estrogens in the male brain in connection to drug addiction is unknown. Females are more vulnerable to cocaine misuse, resulting in an increased rate of cocaine consumption, and in initiation of drug use earlier in adolescence, relative to males. In females, estrogens facilitate extinction of cocaine-seeking behavior, suggesting that estrogens could play a central role in mediating extinction learning. Males also utilize estrogens within the brain through aromatase conversion of circulating androgens. However, it is unclear whether estrogens mediate extinction in males, as they do in females. We hypothesize that blocking estrogen synthesis in male brains should result in an impairment of extinction learning, leading to increased cocaine seeking behavior.

Methods: We employed two different cocaine addiction paradigms, cocaine-conditioned place preference (CPP) and self–administration, followed by extinction and reinstatement. Following CPP conditioning (12 days) for 20 min. daily (6 cocaine, 6 saline), male rats were injected with either the potent aromatase inhibitor, fadrozole (FAD, 0.5, 1 or 2.5mg/kg), or with vehicle, before each CPP extinction days (30 min daily). We also examined the effects of FAD on extinction and reinstatement of cocaine SA. During 10 days of short access (2 hours) cocaine self-administration sessions with two levers, one active and one inactive, rats learned that the active lever was paired with cues, such as light and tone, and with a cocaine infusion. After the last self-administration session, rats were separated into three groups, and male rats were injected with either FAD (1 or 2.5mg/kg), or with vehicle, 30 min. before each extinction. In the extinction phase, rats were exposed to the same operant chamber as before, but without any cues or cocaine infusion, two hours daily, for 15 days. Subsequently, the drug-seeking memory was recalled using a cue- and cocaine primed reinstatement.

Results: Our data shows a dose-dependent effect of FAD during extinction from cocaine CPP, as 1.0 mg/kg facilitated extinction from cocaine CPP, and 2.5 mg/kg impaired it, compared to the vehicle group. On another hand, preliminary data shows no effect of FAD on cocaine extinction from SA. FAD also shows a dose-dependent effect on cue-reinstatement; 1.0 mg/kg decreased active lever presses, while 2.5 mg/kg produced a similar number of presses, compared with vehicle group. Interestingly, both doses of FAD decreased active level presses in cocaine-induced reinstatement. Moreover, no effect was observed on food reinstatement with a dose of 1.0 mg/kg, but a dose of 2.5 mg/kg impaired it.

Conclusions: Our findings suggest that estrogens modulate extinction of cocaine-CPP in a dose-dependent manner. Preliminary data also show that a lower dose of FAD during SA extinction attenuates cue- and cocaine-induced reinstatement, suggesting that aromatase inhibition strengthens extinction memory, and thus decreases cocaine-seeking behavior. These findings suggest that estrogens play a role in modulating extinction learning in male rats. Another experiment is currently underway, adding more rats to all groups to ensure statistical validity.

Keywords: Cocaine, Aromatase, Self-Administration, Conditioned Place Preference

Disclosure: Nothing to disclose.

P657. Transferring Extinction Memories to Oppose Renewal-Induced Relapse

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Background: Renewal of drug-seeking behaviors is often due to persistent memory retrieval of conditioned responses previously associated with drug contexts, and this maladaptation becomes evident as the subjects fail extinction-based therapies. Thus, the inability of transferring and applying the learned extinction as subjects revisit the original drug context is associated with alterations in the brain reward circuit including the nucleus accumbens (NAc). Unfortunately, extinction and its adjunct medications have shown limited success in the clinic, perhaps due to a lack of understanding of the neurobehavioral mechanisms governing the transfer of the extinction memory to drug-associated contexts.

Methods: Here, we used a rat model of contextual drug self-administration (SA; conditioning) (AAA or ABA), where rats acquire cocaine or saline (controls) SA in context A, then extinguish in the same (A) or a different context (B), followed by a re-exposure test in context A (renewal). The percent change from conditioning to extinction/renewal tests is used as an indication of extinction learning. We are combining this behavioral paradigm with the use of the novel transgenic rat lines expressing Cre recombinase in medium spiny neurons expressing dopamine 1 or 2 receptors, chemogenetics, and genome-wide transcriptomic profiles of NAc subregions to understand the impact of extinction/renewal at the behavioral, cellular, and transcriptional levels.

Results: As expected, we first observed that 100% of the rats exposed to the AAA paradigm showed extinction learning, however, a heterogeneous distribution (>50% extinction and >40% renewal) was observed in the ABA group. Along with this, a group of rats not receiving extinction learning (home-cage withdrawal) resulted in >70% of renewal. Ongoing transcriptomic experiments on these resulting phenotypic groups will reveal so-called hub genes encoding specific extinction or renewal-associated phenotypes in the NAc subregions (core vs. shell). Complementary to this dataset, we used RNAscope, slice electrophysiology, pharmacology, and behavior in the D1- and D2-Cre rats. Preliminary results validate cell-type-specific Cre expression and recombination in the two rat lines. Furthermore, we found that chemogenetic inhibition of D1 cells in the NAc decreased the renewal-induced relapse, suggesting a key role of D1 cells in renewal and the feasibility of these rats as a tool for cell-specific characterizations (i.e., circuit and transcriptional).

Conclusions: Together, these approaches will provide behavioral and molecular evidence of how contextual extinction, withdrawal, or renewal reprogram the transcriptome of the NAc to develop novel molecular venues to facilitate extinction transfer and prevent renewal/relapse.

Keywords: Drug Relapse, Cocaine Addiction, Extinction, Transcriptomics

Disclosure: Nothing to disclose.

P658. Sex-Specific Cholinergic Regulation of Dopamine Release Mechanisms Through Nicotinic Receptors in the Nucleus Accumbens

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Background: The mesolimbic dopamine system is involved in the expression of sex-specific behavior and is a critical mediator of many psychiatric diseases such as Schizophrenia, anxiety, depression, and Substance Use Disorder (SUD). In the field of SUD research specifically, work has focused on sex differences in the anatomy of dopamine neurons and relative dopamine levels between males and females. Interestingly, 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 nicotinic acetylcholine receptors (nAChRs). In the NAc dopamine is released in tonic (slow and regular) and phasic (short, burst/spikes) patterns that are subject to heavy modulation by cholinergic (ChAT) interneurons signaling through α4β2* containing nicotinic receptors located directly on dopamine terminals. However, our understanding of the interaction between the ChAT and dopaminergic systems has been completed almost entirely in male subjects and our data show that this interaction is fundamentally different in females.

Methods: Using ex vivo fast-scan cyclic voltammetry combined with pharmacological antagonism of α4β2*-nAChRs with Dhβe in NAc slices from males ($n = 7$ – 10 slices), naturally cycling (intact, $n = 7$ - 10) and ovariectomized females ($n = 7$ – 10), we measured sub-second dopamine release after a series of tonic (1 pulse and 5 pulses at 5 Hz) and phasic (5 pulses at 10, 20, and 100 Hz) stimulations. Further, we assessed the interactions between 17β-estradiol (E2) effects and Dhβe effects on α4β2*-nAChRs by measuring the potentiating effects of E2 on dopamine release mechanisms with and without application of Dhβe in NAc slices. Lastly, we defined the sex-specific effects of ChAT interneuron activity on reward-seeking behavior using Gq-DREADDs injected into the NAc of male ($N = 13$) and female ($N = 15$) ChAT-cre +/- mice to selectively activate ChAT interneurons during positive reinforcement operant conditioning with sucrose.

Results: We find that dopamine release regulation through α4β2*-nAChRs is not present in female mice under most conditions. Deficits in nAChR modulation of dopamine release in intact females were not affected by the estrous cycle; however, they were rescued by ovariectomy – indicating that ovarian hormones play significant a role in this process. Critically, we find that E2 increases dopamine release acutely, an effect that is blocked by antagonism of α4β2*-nAChRs. Finally, the Gq-DREADD behavior studies revealed that male mice learned at a faster rate than intact females when ChAT interneurons were activated indicating that ChAT regulation of dopamine release and reward learning are enhanced in males with little to no effect in females.

Conclusions: Overall, we show that circulating ovarian hormones alter the ability of α4β2*-nAChRs on dopamine terminals to modulate dopamine release in the NAc suggesting that sex differences in ChAT regulation of dopamine neurotransmission underlies sex-dependent differentiation in reward learning. Moving forward it will be critical to directly link these sex-differences to reward processing and reinforcement learning for the development of sex-specific pharmacotherapies to treat SUD as well as a variety of psychiatric disease states.

Keywords: Dopamine, Nicotinic Acetylcholine Receptors, Sex Differences, Fast Scan Cyclic Voltammetry

Disclosure: Nothing to disclose.

P659. Orexin/Hypocretin Signaling in the Medial Prefrontal Cortex in Rodent Models of Cocaine Addiction

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Background: The orexin system is a promising target to treat substance abuse disorders. A major component of these disorders is relapse, which can result from impaired executive control originating from brain regions like the prefrontal cortex. Orexin neurons project to the medial prefrontal cortex (mPFC), but little is known about orexin signaling in this region during substance addiction. Prior studies found that mPFC orexin receptor 1 regulates alcohol intake, as SB334867 microinfusion into this region attenuates excessive alcohol consumption as well cued reinstatement to ethanol seeking in mice. However, it is unknown if mPFC orexin signaling promotes cocaine self-administration, escalation of intake, or motivation for drug.

Methods: Male rats underwent jugular vein catheterization, followed by training on operant cocaine self-administration procedures. Briefly, we trained rats to self-administer cocaine on an FR-1 schedule, followed by behavioral economics demand curve baseline assessments, then short- and intermittent access self-administration training. Finally, we re-assessed rats on the behavioral economics paradigm to characterize changes in demand elasticity, an inverse measure of motivation for drug. Following behavioral assessments, brain tissue was collected and processed for immunohistochemistry and in-situ hybridization analyses of the mPFC.

Results: Consistent with prior studies, preliminary data show that intermittent access cocaine self-administration significantly decreases demand elasticity ($t6 = 3.5$, $p < 0.05$), indicating an increased motivation to respond for drug. Tissue analyses show that intermittent access training increases the expression of orexin-a fibers in the mPFC compared to naïve rats ($t4 = 2.23$, $p < 0.05$). Our ongoing studies are also examining whether there are changes in orexin receptor expression in mPFC or in the numbers of orexin neurons that project to this region after cocaine self-administration.

Conclusions: These results indicate that cocaine addiction increases orexin signaling to the prefrontal cortex, which may promote relapse. We are examining how chronic cocaine self-administration changes orexin signaling in this brain region and if there is a causal role for the plasticity of cortical orexin innervation in addiction endophenotypes. These studies will provide new perspectives on the role of orexin signaling in the brain during substance addiction.

Keywords: Medial Prefrontal Cortex, Orexin, Cocaine Addiction, Orexin Receptor, Intermittent Access Self-Administration

Disclosure: Nothing to disclose.

P660. Differential Effects of Nicotine Delivery Rate on Abuse Potential and Urges to Smoke: A Human Laboratory Study With Implications for Tobacco Regulatory Science

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Background: Convergent evidence has shown that rapid delivery to the brain enhances the abuse potential of drugs of abuse. Nicotine delivery rate is a key feature of the design of electronic cigarettes – which can be used either recreationally, for their stimulatory and

pleasurable effects, or therapeutically, to alleviate urges to smoke. Still, the tradeoff between the abuse potential and therapeutic effects of nicotine, as a function of delivery rate, remains poorly understood. We developed a novel human laboratory paradigm that involves administering nicotine intravenously at distinct rates, aiming to determine the dose-effect curves for abuse potential and suppression of urges to smoke. We hypothesized that the abuse potential of nicotine would gradually increase with faster delivery rates, but that the effects of delivery rate on suppression of urges to smoke would be minimal.

Methods: This human laboratory study included twenty-six non-treatment seeking, overnight abstinent smokers (50% female, aged 28 ± 3.7 years) who smoked ≥ 5 cigarettes per day for the past year. The participants completed five test sessions, in which they were randomly assigned to receive either a saline infusion, or a 1 mg per 70 kg body weight dose of nicotine – delivered over 1, 2.5, 5 or 10 minutes intravenously, at rates of 1, 0.4, 0.2, or 0.1 mg/min, respectively. Biomarkers of nicotine use, including serum cotinine and nicotine levels, were measured at baseline, to confirm smoking status and overnight abstinence, respectively. Primary outcomes were the abuse potential of nicotine, measured with the Drug Effects Questionnaire (DEQ) stimulatory and pleasurable effects subscales; and urges to smoke, measured with the Factor 1 (desire to smoke) and Factor 2 (withdrawal relief) of the Questionnaire of Smoking Urges – Brief (QSU-B). Each measure was analyzed using a mixed effects model, with within subject factors of delivery rate, time and the interaction between delivery rate and time.

Results: At baseline, participants had an average serum cotinine level of 203 ± 137.2 ng/ml, consistent with daily smoking, and a pre-session average serum nicotine level of 2.9 ± 3.7 ng/ml, confirming overnight abstinence from smoking. Preliminary results show a main effect of nicotine delivery rate on DEQ stimulatory ($F(4,67.1) = 8.46$, $p < 0.0001$) and pleasurable effects ($F(4,89.9) = 5.35$, $p = 0.0007$), such that the fastest delivery rate produced higher stimulatory ($d = 0.6$) and pleasurable effects ($d' = 0.5$), compared to the slowest delivery rate – indicating higher abuse potential. For Factor 2 of the BQSU, there was no significant main effect of delivery rate; however, urges to smoke greater under placebo, without significant differences among nicotine conditions ($F(4,77.3) = 3.00$, $p < 0.05$) ($d' = 0.5$) – indicating that the nicotine delivery rate did not influence its ability to suppress urges to smoke.

Conclusions: We have characterized the delivery rate-response curve for a nicotine dose that is roughly the amount of nicotine delivered by smoking one standard tobacco cigarette. Our findings reinforce the importance of delivery rate when evaluating the tradeoff between abuse potential and therapeutic effects of nicotine. Future studies should investigate whether the delivery rate in electronic cigarettes can be optimized (e.g., puff duration and power) to reduce their abuse potential, while maintaining their ability to suppress of urges to smoke. This strategy holds promise to favor smoking cessation over the reinforcing effects of nicotine, thereby reducing the morbidity and mortality associated with nicotine addiction.

Keywords: Nicotine Addiction, Electronic Cigarette (e-cigarette), Abuse Liability, Harm Reduction, Nicotine Withdrawal

Disclosure: Nothing to disclose.

P661. The Protective Effect of Operant Social Reward on Cocaine Self-Administration, Choice, and Relapse is Dependent on Delay and Effort for the Social Reward

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Background: Social-reinforcement-based treatments are effective for many, but not all, people with addictions to drugs. We recently developed an operant rat model that mimics features of one such treatment, the community-reinforcement approach. In this model, rats uniformly choose social interaction over methamphetamine or heroin. Abstinence induced by social preference protects against the incubation of drug seeking that would emerge during forced abstinence. Here, we determined whether these findings generalize to cocaine and whether delaying or increasing effort for social interaction could reveal possibly human-relevant individual differences in responsiveness.

Methods: We trained male and female rats for social self-administration (6 days) and then for cocaine self-administration, initially for 2-h/day for 4 days, and then for 12-h/day continuously or intermittently for 8 days. We assessed relapse to cocaine seeking after 1 and 15 days. Between tests, the rats underwent either forced abstinence or social-choice-induced abstinence. After establishing stable social preference, we manipulated the delay for both rewards or for social reward alone, or the response requirements (effort) for social reward.

Results: Independent of cocaine-access conditions and sex, operant social interaction inhibited cocaine self-administration and prevented incubation of cocaine seeking. Preference for social access was decreased by delay of both rewards or social reward alone, or by increased response requirements for social reward, with notable individual variability.

Conclusions: This choice procedure can identify mechanisms of individual differences in an animal model of cocaine use and could thereby help screen medications for people who are relatively unresponsive to treatments based on rewarding social interaction.

Keywords: Animal Models, Social Behavior, Drug Relapse

Disclosure: Nothing to disclose.

P662. Distinct Coordination of Dorsomedial and Dorsolateral Striatum Encoding Social and Exploratory Behaviors

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Background: Social behaviors are critical for animals to sustain healthy living patterns. Deficit or dysfunctional social behaviors are correlated with several psychiatric disorders including addiction. The dorsal striatum (DS) has been considered a key brain area controlling context-dependent decision-making processes including social behaviors. In the DS, the striatonigral and striatopallidal circuits orchestrate the go and no-go pathway and yield biased behavior in exploratory tasks according to the environments such as the existence of approach and avoidance conflict. Interestingly, the two distinctive regions in the DS, dorsomedial and dorsolateral striatum (DMS and DLS) have been spotlighted due to their different roles in context-dependent reward-seeking behaviors. However, although recent studies suggest that the DS presents a variety of task-related temporal cellular signals and alcohol withdrawal induces abnormal DS activities, leading to the impairment of social behaviors, it is still elusive how the coordinated activities in the DMS and DLS encode social behaviors and whether repeated alcohol exposure affects this coordination.

Methods: Using multi-regional fiber-photometry calcium imaging, we examined the spatiotemporal activities of the neurons and astrocytes in the DMS and DLS simultaneously that represent context-dependent signatures in the environments with different social conditions and their contributions to alcohol-induced impairments. We selectively expressed the GCaMP6s, a genetically encoded calcium-dependent fluorescent indicator, in direct-pathway striatonigral medium spiny neurons (dMSNs), indirect-

pathway striatopallidal medium spiny neurons (iMSNs), and astrocytes of transgenic male mice expressing both Cre-dependent GCaMP6s and D1R-Cre, A2AR-Cre, or ALDH1L1-Cre. With mice expressing enhanced green fluorescent protein (eGFP) in the iMSNs, we confirmed the fluorescence changes in the GCaMP6s mice are unlikely to reflect motion-related artifacts.

The brain calcium imaging was acquired and matched with the locomotion speed during voluntary movements in three-chamber social approach tasks. The mice were exposed to the chamber with three different social conditions: without any social factors, with a strange mouse, or with a strange mouse and a familiar mouse. "Social preference" was measured as the comparison between the time the experimental animal spent with a stranger mouse and a novel object (sociability) or a familiar mouse (social recognition).

We also compared whether the cellular profiles could be affected by the exposure to chronic intermittent ethanol (CIE) paradigm. Briefly, mice were exposed to air or vapored ethanol in vapor inhalation chamber for five weeks. Each daily cycle consisted of ethanol vapor for 16h followed by 8 h of abstinence in their home cage. This was repeated each day for 4 consecutive days, followed by 3 days of abstinence.

Results: In freely moving mice showing GCaMP6s expression in the dMSNs of DS, we commonly observed sustained locomotion-related increase in Ca2+ signaling in the DMS independent to the existence of a stranger mouse. Interestingly, during the locomotion activity in the environment with a stranger mouse, reduction of Ca2+ signaling in the DLS was predominantly evoked and those opposite cellular Ca2+ influx changes in the DMS and DLS during exploration with a stranger mouse were significantly decreased when the mouse was exposed to the choice condition with a stranger and familiar mouse together. Moreover, machine-learning analysis through support vector machine (SVM) demonstrated that the coordinated temporal dynamics of dMSNs in the DMS and DLS predicted which social environments the mice are exploring. The Ca2+ influx changes in iMSNs and astrocytes were also observed during the voluntary movements, but those were not fully dependent on the social environment factors.

We also observed that, at 3 days withdrawal from repeated alcohol exposure, the dMSNs' activities were significantly dampened in the DMS, but not the DLS. Furthermore, the withdrawal from repeated alcohol exposure interfered with the speed prediction by the DMS dMSN's temporal dynamics. When we inhibited the dMSNs' activity in the DMS of alcohol naïve mice using chemogenetic approaches without any changes in locomotion, the effect of alcohol withdrawal was recapitulated by the reduction of social preference.

Conclusions: Our results demonstrate that the coordinated striatonigral neuronal activities in the DMS and DLS are the main distinguishable signatures for a social-context dependent locomotion and alcohol-withdrawal disrupts these coordinated neuronal activities. These findings provide new insights how striatal cellular activities temporally coordinate the social context-dependent behaviors and can be a computation model to predict the types of the social behaviors.

Keywords: Social Behavior, Dorsal Striatum, Neural Coordination, Alcohol Withdrawal

Disclosure: Nothing to disclose.

P663. An Integrated Amygdalo-Fronto-Striatal Network Governs Causal Learning and Memory

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Background: Goal-directed decision making (i.e. performing actions based on their expected outcomes) is a fundamental adaptation for navigating day-to-day life, failures of which are thought to represent a core feature of many neuropsychiatric disorders including addiction. Adaptive action selection requires the concerted integration of disparate cognitive processes across multiple time scales, including the flexible encoding, stable representation, and retrieval of learned action-outcome associations (contingencies), in order to guide future choices. The orbitofrontal cortex (OFC) supports outcome-guided decision making across a diverse array of behaviors. However, the coordinated neural circuitry and cellular mechanisms by which OFC networks enable causal learning remain elusive.

Methods: We sought to identify OFC circuits that mediate causal learning and memory by studying the ability of mice to select actions based on their consequences using a food-reinforced instrumental choice task. We utilized viral-mediated, projection-selective chemogenetic manipulations to examine the role of OFC circuits during the encoding of new contingency information and the subsequent retrieval of that learning during choice epochs ($n = 108$). Next, we utilized activity-dependent neuronal manipulations to determine whether a memory trace for learned decision variables is stored by specific neuronal ensembles within the OFC ($n = 43$). Further, we developed a trans-synaptic anterograde relay mapping method to identify OFC neurons based on their specific di-synaptic network connectivity properties and used this to quantify circuit-specific dendritic spine dynamics associated with contingency learning ($n = 24$). Finally, we employed a molecular-functional disconnection strategy to assess the role of neurotrophin tone within the OFC for coordinating neural circuit function across memory encoding and retrieval epochs ($n = 63$). Male and female mice were used for this study. Biological sex was included as a covariate, which did not reveal statistically significant effects of sex.

Results: We first demonstrate that chemogenetic inhibition of afferent basolateral amygdala (BLA)- > OFC projections disrupts the encoding, but not retrieval, of new contingency memories. We further show that chemogenetic stimulation of BLA- > OFC projections is sufficient to enhance contingency learning, thus revealing that these projections bidirectionally control contingency memory encoding. Next, we show that chemogenetic inhibition of efferent OFC- > dorsomedial striatum (DMS), but not OFC- > BLA, projections disrupt contingency memory retrieval. Thus, we hypothesized that OFC neuronal ensembles stably represent learned contingency information across time and facilitate the retrieval of that learning during choice epochs. Here, we demonstrate that the reactivation of OFC neurons that respond to novel, but not familiar, contingency information is necessary for the retrieval of newly learned contingencies to guide choice behavior. Finally, we reveal that contingency learning triggers dendritic spine plasticity specifically within a BLA- > OFC- > DMS network, and that neurotrophin tone within BLA- > OFC and OFC- > DMS circuits are required for new contingency learning.

Conclusions: We describe the directional transmission of learned causal information within a coordinated amygdalo-fronto-striatal network across time, whereby contingency memories are encoded by BLA- > OFC inputs, represented within OFC ensembles, and retrieved via OFC- > DMS outputs during future choice. We also reveal that the integration of these separable learning and memory processes requires circuit-specific neurotrophin tone and neuronal structural plasticity within the OFC. Thus, we identify the OFC as a critical locus within a distributed amygdalo-fronto-striatal network, providing the temporal link between memory encoding and retrieval, thereby bridging the initial learning of new information with its future application.

Keywords: Orbitofrontal Cortex (OFC), Instrumental Learning, Memory Encoding and Retrieval, Dendritic Spines

Disclosure: Nothing to disclose.

P664. Neuronal Metabolic-Epigenetic Exchange in the Regulation of Behavior – Implications for Alcohol Use Disorder and Beyond

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Background: Recently, we have shown that alcohol metabolites contribute to brain histone acetylation by the direct deposition of alcohol-derived acetate on histones. This reaction is catalyzed by metabolic enzyme acetyl-CoA synthetase 2 (ACSS2), which is nuclear and chromatin-bound in neurons. In ongoing studies, we now explore the role of this pathway during voluntary alcohol intake as well as during prenatal alcohol exposure.

Methods: We use in vivo stable isotope labeling in mice to determine the contribution of alcohol metabolites to histone acetylation in adult and fetal brains. We also employ an array of molecular (RNAseq, ChIPseq) and behavioral experiments to show that this deposition has important functional and behavioral consequences.

Results: We show that alcohol exposure leads to lasting impairments of histone acetylation, which might underlie alcohol use disorder and related comorbidities, including cognitive decline and neurodegeneration. In addition, we show that in pregnant mice, exposure to alcohol results in the incorporation of alcohol-derived acetate into gestating fetal brains. The deposition of this metabolite is dose-dependent and restricted to developmental time points where ACSS2 is expressed and chromatin-bound. This novel mechanism thus defines a unique window of epigenetic sensitivity to alcohol metabolites in the gestating brain, which might underlie fetal alcohol spectrum disorder.

Conclusions: We recently discovered a novel aspect of alcohol's effects on the brain, driven by the direct incorporation of alcohol metabolites into histone acetylation. We now show that this pathway plays an important role in fetal alcohol spectrum disorder, alcohol use disorder and related comorbidities such as neurodegeneration and cognitive decline. Targeting this pathway could thus be a promising new therapeutic avenue.

Keywords: Alcohol, Drug Metabolism, Epigenetics, Fetal Alcohol Spectrum Disorder, Cognitive Decline

Disclosure: Nothing to disclose.

P665. Altered δ Opioid Receptor Expression and Function Mediate Opioid Addiction Vulnerability After Early-Life Adversity

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Background: Early life adversity (ELA) is associated with vulnerabilities to reward-related problems, such as addiction to pro-hedonic opioid drugs. Women may be particularly vulnerable, suggesting a sex-specific derangement of reward circuit maturation by ELA. However, the mechanisms by which this occurs are poorly understood. To test the hypothesis that ELA perturbs the normal maturation and function of endogenous opioid systems, we employed a naturalistic model of ELA, in which bedding and nesting materials are limited during the first week of life, and examined the impacts of ELA on expression and function of opioid ligands and receptors and on opioid drug seeking behaviors in female rats.

Methods: Adult female ELA-experienced rats were tested for aspects of opioid addiction-like behaviors including free consumption and economic demand elasticity to measure motivation for opioid drugs ($n = 12$/group). To gain insight into ELA-induced molecular changes in reward-related regions, we employed RT-qPCR for a suite of molecular candidates ($n = 8$-9/group). Following on our intriguing molecular findings, we pharmacologically manipulated endogenous opioid signaling during opioid self-administration to test the mechanisms of ELA-enhanced opioid seeking ($n = 12$/group).

Results: ELA led to enhanced motivation for opioid drugs in female rats, in accord with our prior findings ($t(22) = 3.620$, $P = 0.0015$). RT-qPCR revealed a selective reduction in delta opioid receptor expression following ELA in basolateral amygdala ($t(17) = 3.197$, $P = 0.0053$), and no change in μ or κ receptor, nor in the endogenous ligands. Preliminary results from pharmacological manipulation of δ opioid receptors in BLA during opioid self-administration suggest a possible mechanism by which ELA may cause vulnerability to addiction.

Conclusions: ELA causes enduring changes in δ opioid receptor expression in amygdala, which may underlie the sex-specific pro-addiction phenotype in female rats. Unlike μ and κ, which are expressed at adult levels in neonatal rats, δ expression matures later, potentially rendering the receptor vulnerable to adverse early-life experiences. Understanding the mechanisms by which ELA promotes vulnerability to opioid use disorder is critical for identifying those at high risk for addiction and developing effective interventions for preventing opioid-related morbidity and mortality.

Keywords: Early-Life Adversity, Opioid Addiction, Delta Opioid Receptor, Basolateral Amygdala, Nucleus Accumbens

Disclosure: Nothing to disclose.

P666. Somatostatin Neurons in the Prelimbic Cortex Control Binge Drinking

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Background: Somatostatin (SST) neurons have been implicated in a variety of neuropsychiatric disorders such as depression and anxiety, but their role in substance use disorders, including alcohol use disorder (AUD), is not fully characterized. In our previously published work (Dao et al. 2021 Neuropsychopharmacology) we found that repeated cycles of alcohol binge drinking via the Drinking-in-the-Dark (DID) model led to hypoactivity of SST neurons in the prelimbic (PL) cortex by diminishing their action potential firing capacity and excitatory/inhibitory transmission dynamic. We discovered that by chemogenetically manipulating these neurons, we could reduce binge alcohol consumption in both male and female mice. Ongoing work in our lab seeks to define the role that SST peptide signaling itself plays in this circuit-mediated change in alcohol consumption.

Methods: All experiments were conducted with approval by Penn State's Institutional Animal Care and Use Committee. Using a combination of behavioral pharmacology, we have worked to identify the role of SST peptide signaling in the PL cortex. For electrophysiology, naïve male and female C57BL/6J mice were used. Mice were sacrificed under isoflurane anesthesia and coronal sections of the PL cortex were prepared. SST (1uM) was bath applied following establishment of stable resting membrane potential for each cell. For cannula experiments, male and female C57BL/6J mice were anesthetized under isoflurane anesthesia, and unilateral cannulas were implanted targeted to the PL cortex (Plastics One, provided by Dr. Patrick Drew, Penn

State). Ongoing experiments are using bilateral cannulas. Mice were allowed to rest for one week before being assessed for anxiety-like behavior. Statistical analyses were conducted in GraphPad Prism (*t*-tests and ANOVAs as outlined below; mouse numbers/cell numbers included below for individual experiments).

Results: First, we investigated SST peptide effects on pyramidal neurons in the PL cortex. Pyramidal neurons (identified by membrane properties and gross morphology) were patched in male and female mice and following stable properties in current clamp, 1uM SST was bath applied for 10 min ($n = 15$ cells for females, 19 cells for males). Paired *t*-tests were used to account for pre- and post- SST bath application for RMP and rheobase, and ANOVAs were used for voltage x current plots. SST significantly hyperpolarized the resting membrane potential in both sexes (RMP, females: $t\ 14 = 2.544$, $p = 0.0234$; males: $t\ 18 = 3.171$, $p = 0.0053$) as well as properties such as the rheobase (pA, females $t\ 14 = 5.059$, $p = 0.0002$; males $t\ 18 = 5.259$, $p < 0.001$). SST reduced the number of action potentials evoked at various current steps in both sexes as well (females ANOVA, SST effect $F\ 1,18 = 10.71$, $p = 0.0042$; current x SST interaction $F\ 20,276 = 9.51$, $p < 0.0001$; males ANOVA SST effect $F\ 1,18 = 9.660$, $p = 00.61$; current x SST interaction $F\ 20,360 = 7.772$, $p < 0.001$). These effects were consistent at the common holding membrane potential of -70 mV as well. In a separate cohort of mice, retrograde tracers were injected into downstream regions (the bed nucleus of the stria terminalis and the nucleus accumbens, respectively), and confirmed SST modulation of these output pathways. Ongoing slice experiments include assessing whether these changes are reversible (using the SST antagonist cyclosomatostatin).

In order to understand the functional significance of SST peptide signaling in this region, mice were implanted with unilateral cannulas (Plastics One) targeted at the PL cortex. In a preliminary mixed-sex cohort, mice received either aCSF vehicle control ($n = 4$ animals) or Octreotide, an SST analogue (0.1 ug administered in sterile aCSF, $n = 5$ animals) 15 min prior to behavior. Octreotide administration led to an increase in distanced traveled in the open field test, as well as an increase in open arm entry frequency in the elevated plus maze ($t\ 6 = 2.578$, $p = 0.0419$). Ongoing experiments are replicating this work in cohorts with bilateral cannulas. In particular, we are exploring the role of SST signaling/Octreotide administration in anxiety- like behavior in naïve mice, as well as its effect on alcohol consumption.

Our work builds upon our previously published paper to establish a role for SST peptide signaling in the PL cortex. We show that bath application of SST reduces multiple measures of pyramidal neuron excitability, including hyperpolarization of the membrane potential and reduced action potential firing. We translate this effect to in vivo behavior by administering an analogue of this peptide directly to the PL cortex. We found that unilateral administration had an anxiolytic effect in behaviorally-naïve (importantly, stress naïve) mice. Based upon our previously published work showing downregulation of SST neuronal function following binge drinking, we expect these effects to be even further pronounced following exposure to alcohol or procedures such as chronic stress.

Conclusions: Somatostatin neurons, and SST peptide itself, serve as a promising therapeutic target for multiple neuropsychiatric disorders. Here, we attempt to elucidate the mechanism by which SST alters neuronal signaling in the PL cortex (by reducing the overall excitability of pyramidal neurons, a key output population known to play a role in substance use disorders). In addition, we provide preliminary evidence for the use of an SST-like compound (Octreotide) for anxiety- like behaviors and potential substance use.

Keywords: Somatostatin, Prelimbic Cortex, Alcohol

Disclosure: Nothing to disclose.

P667. Opioid, Cup of Coffee, or Antidepressant? The Perceived Functions of Kratom Among Current and Former Users

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Background: Mitragyna speciosa ("kratom" in the US) is a plant with over 40 bioactive alkaloids, some which act at μ-opioid receptors as partial seemingly "biased" agonists with dose-dependent stimulant and analgesic effects. Kratom alkaloids also have non-opioid mechanisms of action. Surveys of current users indicate kratom is primarily used to self-treat symptoms of pain, depression, and fatigue, and to relieve/avoid opioid withdrawal. Surveys of current users, however, may overrepresent those with favorable experiences (or those unable to stop). We surveyed former and current users. One of several study aims was to collect formative data for a momentary-assessment study, which requires knowledge of the temporal dynamics of use (e.g., whether each kratom dose has acute effects or is used more like a maintenance medication).

Methods: Amazon Mechanical Turk was used to crowdsource participation between September 2020-March 2021. Of 2,615 substance-using respondents, 289 had ever used kratom. All were recontacted between April-May 2021 for this kratom survey, comprising updated items from previous surveys and pilot questions.

Results: Of 289 respondents with lifetime kratom use, 134 (48%) completed our kratom survey. They were 34.8 ± 8.4 years old, female (52%), white (69%), high school or college educated (100%), employed (63%) and reported first using kratom at 29.9 ± 8.8 years of age. Most (60%) had used kratom >100 times and reported having ever used kratom >4 times per week, for an average of 61.9 ± 104.3 weeks (81%). Using a DSM-5 checklist, 23% qualified for remitted kratom use disorder and 30% qualified for past-year kratom use disorder. DSM criteria most frequently endorsed reflected tolerance or use to avoid withdrawal, compared to disruption of roles or obligations less frequently endorsed. Additionally, 33% of respondents reported withdrawal symptoms from discontinuation of >1 day; 18%, from missing 1 regular dose.

Most ($N = 103$, 80%) had acute subjective effects from each kratom dose. Among them, 54% said the effects helped them meet daily obligations; 29% said effects were compatible with (not necessarily helpful for) daily obligations. Less than 4% said the effects undermined their daily obligations. Between 40-50% reported using kratom to generally improve daily quality of life, increase energy, or to address anxiety, depression, fatigue, pain; 24.5% used as an opioid substitute.

Under half (42%) considered themselves "regular" kratom users. Self-reported regular doses (in respondents' preferred units) were 5.4 ± 4.8 capsules, 4.6 ± 3.6 grams, 2.5 ± 2.7 spoonfuls, 2.1 ± 1.0 tablespoons, or 1.6 ± 1.1 cups of tea. On days of use, respondents dosed 2.6 ± 2.4 times on average, with this routine stable for 65.0 ± 112.9 weeks. A sizeable minority (41%) reported taking more kratom during the first waking hour than at other times during the day; 54% reported that they preferred their first daily dose of the morning to other doses. Changes in dosing routines during periods of use occurred "occasionally" (33%) or "not often" (29%). Since first using kratom, participants reported that doses had: increased (26%), remained unchanged (23%), decreased (19%), or ceased (21%).

No participant reported feeling kratom effects within seconds, whereas 83% reported typically feeling effects within minutes; 12% within hours. A majority (92%) reported that they typically

stopped feeling kratom's effects within hours. Only 2 participants reported feeling that effects stopped within minutes; 7% were unsure as they took more kratom before effects dissipated.

The mean "too low" dose (unable to elicit desired effects) was 3.96 ± 4.95 capsules ($N = 50$), 2.64 ± 2.44 grams ($N = 45$), 1.37 ± 0.96 spoonfuls ($N = 19$), 2.2 ± 2.17 tablespoons ($N = 5$), or 1.57 ± 0.98 cups of tea ($N = 7$). The mean lower-threshold effective dose was 4.13 ± 3.31 capsules ($N = 45$), 3.19 ± 2.25 grams ($N = 43$), 2.33 ± 2.20 spoonfuls ($N = 24$), 2.00 ± 0.89 tablespoons ($N = 6$), or 1.3 ± 0.67 cups of tea ($N = 10$).The mean upper-threshold doses (no unwanted effects) was 5.88 ± 4.02 capsules ($N = 43$), 6.85 ± 4.58 grams ($N = 40$), 2.87 ± 1.58 spoonfuls ($N = 23$), 2.5 ± 1.58 tablespoons ($N = 10$), or 2.25 ± 1.16 cups of tea ($N = 8$). The mean "too high" dose ("a bit too much") was 7.25 ± 4.24 capsules ($N = 40$), 8.68 ± 4.38 grams ($N = 37$), 3.39 ± 1.66 spoonfuls ($N = 27$), 3.57 ± 1.72 tablespoons ($N = 7$), or 3.44 ± 2.01 cups of tea ($N = 9$).

The average perceived effectiveness of kratom across all reported use indications (VAS, 0-100) was 72.8 ± 16.7. For unwanted effects of cessation, the average rating was 53.0 (±24.1). After standardizing dose amounts by within-unit z-scores, we found that greater severity of cessation effects was predicted by greater weekly use (β = 5.24, 95% CI = -0.52, 11.0; $p = 0.07$), more weeks of regular use (β = 6.74, 95% CI = 0.88, 12.60; $p = 0.02$), and having decreased doses (β = 20.88, 95% CI = 5.75, 35.99; $p < .001$). Having quit kratom was the strongest predictor of lower ratings of desired effects (β = -16.45, 95% CI = -25.9, -6.90; $p < .001$). Amount consumed weekly tended to be positively associated with beneficial-effect ratings, but not significantly (β = 2.19, 95% CI = -1.52, 5.89; $p = 0.25$); likewise for weeks of use (β = 0.90, 95% CI = -2.98, 4.78; $p = 0.65$).

Conclusions: Kratom was typically associated with acute effects rather than chronic effects (differentiating it from most psychiatric or maintenance medications), but the acute effects were usually compatible with, or helpful for, daily obligations (differentiating it from many illicit drugs).

Keywords: Kratom, Partial Agonist, Mu-Opioid Receptor Agonist, Opioid Side-Effects

Disclosure: Nothing to disclose.

P668. Depressed Mood and Cognitive Impairment are Associated With Striatal Dopamine Dysfunction and b2*-nAChR Availability in Recently Abstinent Tobacco Smokers

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Background: In animal studies, nicotine, the primary addictive component of tobacco smoking, binds to and activates b2-subunit containing nicotinic acetylcholine receptors (b2*-nAChRs) on ventral tegmental area neurons, which acutely facilitates dopamine release in the ventral striatum and reinforces nicotine use. Chronic nicotine exposure, however, desensitizes and increases the number of b2*-nAChRs and reduces the dopaminergic response. b2*-nAChR upregulation and lower striatal dopamine D2/3 receptor availability have been reported in 7-day abstinent and current smokers, respectively, compared to nonsmokers. Less is known about striatal dopamine function in current or quit-attempting smokers. Furthermore, cognitive deficits and mood changes are hallmarks of nicotine withdrawal linked with the cholinergic and dopaminergic systems. Difficulty in quitting smoking may be attributed to cholinergic and dopaminergic dysfunction and concomitant changes in cognition and mood. The goals of this positron emission tomography (PET) imaging study were to examine b2*-nAChR availability and striatal dopamine function in recently abstinent smokers and nonsmokers, and to explore cholinergic and dopaminergic correlates of cognition and mood.

Methods: Twenty-seven tobacco smokers (9F; aged 38 ± 10 years) and 28 nonsmokers (11F; aged 30 ± 11 years) participated. Smokers had moderate nicotine dependence (Fagerström Test for Nicotine Dependence: 5.9 ± 2.1) and smoked 16 ± 7 cigarettes/day for 18 ± 10 years. Smokers received cessation counseling and contingency management for ~2 weeks. [18F]Flubatine PET scans, which measure b2*-nAChR availability, were acquired 90-120 minutes after bolus-infusion of 254.9 ± 44.9 MBq [18F] Flubatine (Kbol = 360 min) from 14 smokers at 6 ± 2 days of abstinence and 19 nonsmokers. Dopamine function was assessed in 19 smokers at 11 ± 9 days of abstinence and 18 nonsmokers with two PET scans after bolus injections of the dopamine D2/3 receptor agonist radioligand [11C]PHNO (scan 1, 443.1 ± 156.6 MBq; scan 2, 424.6 ± 168.4 MBq): one "baseline" scan before and one scan 3 hours post-amphetamine administration (0.5 mg/kg, PO). Subjects performed the 1-back working memory task (CogState) and completed the Center for Epidemiological Studies Depression scale (CES-D) on [18F] Flubatine and [11C]PHNO scan days, respectively. PET measures included free fraction corrected distribution volumes (VT/fp) of [18F]Flubatine in frontal and striatal regions estimated with equilibrium analyses, baseline [11C]PHNO non-displaceable binding potential (BPND) (dopamine D2/3 receptor availability) in the ventral striatum estimated with the simplified reference tissue model (cerebellum as reference), and amphetamine-induced percent change in [11C]PHNO BPND (stimulant-induced dopamine release) in the ventral striatum. Group differences per PET estimate were evaluated with two-sample tests. Correlation coefficients (r) were computed to examine associations of PET estimates with cognitive and mood measures. Significance was $p < 0.05$.

Results: In this preliminary sample, there were no significant group differences in b2*-nAChR availability in the frontal cortex, caudate, or putamen (ps>0.05). Higher b2*-nAChR availability in the frontal cortex was significantly associated with higher mean reaction time for correct responses on the one-back task (i.e., worse working memory performance) in recently abstinent smokers ($n = 13$, $r = 0.68$, $p = 0.01$), but not in nonsmokers. Recently abstinent smokers exhibited less amphetamine-induced percent change in BPND in the ventral striatum than nonsmokers (19 NS: 26.5 ± 6.5%, 18 AS: 19.6 ± 10.0%; $p = 0.02$). Higher scores on the CES-D (worse mood) were associated with less amphetamine-induced dopamine release in the ventral striatum in recently abstinent smokers ($n = 15$, $r = -0.63$, $p = 0.03$), and not in nonsmokers. Analyses are ongoing to determine within-subject relationships between b2*-nAChR availability and dopamine function.

Conclusions: In this preliminary data set there were no group differences in b2*-nAChR availability. However, higher frontal cortex b2*-nAChR availability was associated with worse working memory in recently abstinent smokers, highlighting a potential neural correlate of cognitive impairment during early abstinence. Abstinent smokers had less amphetamine-induced dopamine release in the ventral striatum than nonsmokers, the extent of which was linked with worse mood. This is consistent with evidence of 'blunted' dopamine release in individuals with other substance use disorders and suggests that anhedonia in abstinence is related to dopamine dysfunction. Work to fully elucidate cholinergic-dopaminergic interactions during attempts to quit smoking is ongoing.

Keywords: Smoking Cessation, PET Imaging, a4b2 Nicotinic Acetylcholine Receptors, Dopamine Function, Mood and Cognition

Disclosure: Nothing to disclose.

P669. Elucidating Automatic and Controlled Processes in Addiction to Identify a Potential Treatment Target

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Background: Dysregulated circuits in addiction implicate several cognitive functions related to automatic and controlled processes. Separating these processes could benefit in developing targeting interventions related to these processes and the underlying dysregulated circuits identified in substance use disorders (SUDs). Both processes are elicited after a commission error during a Go/NoGo task which results in event-related potential (ERP) components termed the error-related negativity (ERN) and error positivity (Pe). The ERN first indexes the initial (automatic) processing of the error (e.g., identification of a salient error-event) followed by the Pe which indexes post-error processing (e.g., top-down control to increase accuracy on subsequent trials). Both theta and delta frequency power contribute to this ERP component that is measured from fronto-central electrodes. Theta power indexes the initial automatic process and has been source localized to the anterior cingulate cortex (ACC). A secondary effortful process post-error elicits the Pe, which is broad and diffuse, compared to the ERN, and is measured from central-parietal electrodes and is largely explained by delta power. Frequency analyses often disentangle ERP components by identifying specific contributions of theta and delta which help elucidate the underlying sequence of cognitive functions captured with ERPs. With dysregulated circuits related to these automatic and controlled processes, we hypothesize the SUD sample to exhibit lower ERN theta power (a measure of automatic processing) and Pe delta power (a measure of controlled processing), relative to HC, suggesting substantial impairment in identifying and regulating behavioral errors.

Methods: Individuals with ($N = 71$) and without ($N = 82$) a SUD completed a Go/NoGo task while recording ERPs. We used a 64-channel BioSemi ActiveTwo system collect EEG data and Matlab, EEGLab, Psychophysiology Toolbox, and *R* to analyze these data. Mean amplitudes were calculated within the ERN window, -30 to 150 ms, and the Pe window, 125 to 450 ms, relative to a commission error. Time-frequency surfaces were computed for both (3-9 Hz) and delta (below 3 Hz) filtered range and principal components (PCs) were computed. Nine electrodes were extracted and averaged for the ERN and theta power (F1, Fz, F2, FC1, FCz, FC2, C1, Cz, C2) and Pe and delta power (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2) analyses. These averages were compared between groups with a *t*-test. To better characterize the relationship between ERN amplitude and theta and delta power, correlation and regression analyses were computed to explain shared and unique, respectively, contributions of ERN and Pe amplitude by theta and delta PCs.

Results: Compared to the HC group, the SUD group exhibited lower amplitude in the Pe window $t(1,151) = 4.17$, $p < .01$ but no difference in the ERN window $t(1,151) = -0.75$, $p > .45$. Supporting our hypothesis, we identified the SUD group, compared to the HC group, to have less theta power measured during the ERN, $t(1,151) = 4.37$, $p < .01$, and delta power measured during the Pe, $t(1,151) = 5.28$, $p < .01$. Theta power was negatively correlated with ERN amplitude for both the HC, $r(80) = -.28$, $p = .01$, and SUD groups, $r(69) = -.33$, $p < .01$, and positively correlated with Pe amplitude, [HC] $r(80) = .34$, $p < .01$, [SUD] $r(69) = .32$, $p < .01$; delta power was negatively correlated with ERN amplitude, in the SUD group, $r(69) = -.40$, $p < .01$, but not the HC group, p's > .24; two delta PCs were correlated with Pe amplitude in both groups, [HC] $r(80) = .78$, $p < .01$, $r(80) = .71$, $p < .01$; [SUD] $r(69) = .55$, $p < .01$, $r(69) = .46$, $p < .01$. Interestingly, significant regressions testing unique contributions of theta and delta power explaining ERN amplitude in HC, $R2 = .16$, $F(5, 74) = 2.47$, $p = .04$, identified only a theta PC, $p < .03$ and in the SUD group, $R2 = .21$, $F(5, 65) = 3.52$, $p < .01$, identified only a single delta PC, $p < .02$.

Conclusions: ERP neural correlates of automatic and controlled processes elicited during a Go/NoGo task were identified to be dysregulated in a SUD group relative to a HC group. Supporting our hypothesis, we measured less ERN theta and Pe delta in the SUD group relative to the HC group. Also, unique contributions by theta and delta to ERN amplitude differentiated groups. The HC group exhibited a traditional theta power relationship and the SUD group exhibiting unique delta contributions to ERN amplitude. Clearly, the groups were differentiated by theta power and its relationship to ERN amplitude which likely influenced the subsequent controlled process indexed by Pe. That is, dysregulations identified in the Pe window for the SUD group (i.e., less delta power) begin with reduced theta power related to the ERN reflecting dysregulation of the initial automatic process that cascades to dysregulation of the secondary controlled process. These findings suggest that strengthening automatic processing, and thus increasing theta power, with specific interventions holds potential for future treatments of SUDs. Using a circuit-based intervention approach, the theta generating ACC could be targeted with cortical transcranial magnetic stimulation to modulate theta power and potentially the automatic process dysregulated in SUDs. Studies of the specific mechanisms of neuromodulation targeting this circuit are necessary to evaluate this as a potential intervention.

Keywords: Substance Use Disorder, Event-Related Potentials, Time-Frequency, Treatment Targets

Disclosure: Nothing to disclose.

P670. New Mechanistic Understanding of Acute and Extended Nicotine Abstinence: Evidence From a Smoking Cessation Protocol

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Background: Even though almost 70% of smokers express a desire to quit smoking, less than 10% will successfully quit (1). These poor smoking cessation outcomes have been linked to the Nicotine Withdrawal Syndrome (NWS). Multiple brain circuits and cognitive constructs have been implicated in the NWS, including cognitive, affective and reward processing (2-4). Although behavioral evidence suggest that NWS symptoms improve during the first week of withdrawal and decrease thereafter, mechanistic understanding of extended nicotine abstinence are not available.

Methods: As part of a longitudinal study aimed to investigate changes in cognitive control and affective processing as a function of duration of abstinence, we hypothesized cognitive control processing would decrease, and affective processing would increase as a function of abstinence. We also hypothesize that NWS symptoms would increase during acute and improve during extended abstinence. Our analysis plan is pre-registered on the Open Science Forum (https://osf.io/xy26j/).

A total of 101 smokers were enrolled in the study protocol. Functional MRI data were acquired during both a Parametric Flanker Task (PFT) and an Emotional Faces Task (AMY) at three timepoints: ad libitum–sated smoking (T1), acute abstinence (~48 h, T2), and extended abstinence (~30 days, T4). T4 was only gathered on participants who enrolled in the smoking cessation arm and received weekly CBT-based counseling. NWS symptoms were measured by the Wisconsin Smoking Withdrawal Scale (WSWS) at each timepoint, along with other psychometric

measures including the Positive and Negative Affect Scale (PANAS).

Results: We first conducted an exploratory analysis on a subset ($n = 68$) of dropouts and non-treatment seeking participants who did not remain in the study after T2. A voxel-wise linear mixed-effects modeling analysis did not find any main effect of smoking status on either AMY or PFT -induced activity. We then extracted 6 task-based ROIs from the AMY (Visual Areas, bilateral Fusiform gyrus, right Inferior Frontal Gyrus, bilateral Amygdala) and 5 from the PFT (bilateral Superior and Inferior Parietal Cortex (SPC, IPC), right Middle Cingulate Cortex (MCC)) maps, and calculated the effect size of the contrasts of interest in each ROI to set the boundaries of the Smallest Effect of Interest (5).

In a discovery sample, including all the subjects who underwent evaluation at T4 ($n = 19$), we tested both for the presence and the absence of an effect of abstinence on task activation in these pre-obtained ROIs using one-sided *t*-tests and Two One-Sided *T*-test procedures to test for, respectively, statistical difference and equivalence between T2 and T1 and T4 and T1 (9). Non-parametric Friedman and Wilcoxon signed-rank Tests were used to evaluate abstinence-induced changes in PFT behavioral performance and WSWS scores.

PFT elicited brain activity in left SPC and IPC, and MCC significantly decreased in acute abstinence (p's = 0.039, 0.0003, 0.009), and was statistically equivalent to the sated state in two right parietal ROIs. After extended abstinence, PFT activation in four out of five ROIs was statistically equivalent to ad lib smoking and were neither significantly different nor equivalent in the right IPC. Accuracy for Congruent, Low and Medium Demand PFT trials significantly decreased in acute (p's = 0.012, 0.05, 0.04) but not extended abstinence, while no difference was found for High Demand trials at any timepoint. Moreover, the percent error of omissions across cognitive demand levels significantly increased following acute abstinence ($p = 0.0045$) and tended to continue increasing at T4 ($p = 0.07$).

Statistical equivalence was shown in AMY task activation between both T2 and T1 and T4 and T1, in 4 of 6 ROIs. Although, neither significant difference nor equivalence was seen in bilateral Amygdala at any timepoint. However, positive affect, measured by the PANAS decreased at T2 ($p = 0.004$), with a trend towards reduction observable at T4 ($p = 0.06$).

Craving did not differ between T1 and T2, though a significant reduction was reported at T4 ($p = 0.0005$). Other NWS symptoms increased at T2 as expected: anger ($p = 0.041$), concentration difficulties ($p = 0.008$), sadness ($p = 0.047$), and sleep disturbances ($p = 0.014$); none differed between T1 and T4.

Conclusions: These preliminary findings indicate that acute abstinence-induced changes in cognitive processing tend to be restored after extended abstinence, which is also accompanied by a reduction in NWS symptoms and craving. Further analyses will evaluate the effects of extended nicotine abstinence on affective processing.

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References:

1.Center for Diseases Control and Prevention, 2020. Health Effects of Cigarette Smoking

2.Ashare RL et al. 2014. Cognitive function during nicotine withdrawal: Implications for nicotine dependence treatment. Neuropharmacology 76: 581-91

3.Fedota JR et al. 2021. Time-Varying Functional Connectivity Decreases as a Function of Acute Nicotine Abstinence. Biol Psychiatry Cogn Neurosci Neuroimag 6: 459-469

4.Koob GF (2009) Dynamics of neuronal circuits in addiction: reward, antireward, and emotional memory. Pharmacopsychiatry 42 Suppl 1: S32-S41

5.Lakens D et al. 2018. Equivalence Testing for Psychological Research: A Tutorial. Advances in Methods and Practices in Psychological Science 1: 259-269

Keywords: Nicotine Addiction, Cognitive and Affective Neuroscience, Flanker Task, Facial Emotional Processing, Smoking Cessation

Disclosure: Nothing to disclose.

P671. Exploring a Role for Organic Cation Transporter 3 in Ethanol and Cocaine Co-Abuse

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Background: Concurrent use of cocaine and alcohol is a major cause for emergency hospitalization, underscoring a vital need to understand the mechanistic basis of this highly addictive, and dangerous drug combination. Both cocaine (blocker of dopamine, norepinephrine and serotonin transporters, DAT, NET and SERT, respectively) and ethanol (EtOH) increase extracellular dopamine (DA), norepinephrine and serotonin. However, we and others, find no evidence for EtOH interacting with DAT, NET or SERT, suggesting that EtOH may be acting elsewhere to inhibit uptake of these monoamines. Organic cation transporter 3 (OCT3) is emerging as an important player in regulation of monoamine signaling. Nonetheless, whether OCT3 contributes to actions of abused drugs that act primarily by increasing extracellular levels of monoamines, remains unclear.

Methods: To establish proof-of-principle for this idea, we conducted studies to determine the ability of EtOH to inhibit uptake of the prototypical cation [3H]1-methyl-4-phenylpyridinium ([3H]MPP +) into cultured HEK293 cells stably overexpressing human OCT3, and found the IC50 to be 4 mM, supporting a role for OCT3-dependent actions of EtOH, and showing that concentrations of EtOH reaching brain following behaviorally relevant doses may be sufficient to engage OCT3. Previously we found that cocaine does not have activity at OCT3 (Mayer et al. 2018, PMID: 29773909) raising the possibility that EtOH may interact with OCT3 to inhibit uptake of monoamines, potentially increasing the addictive properties of cocaine and propagating the concurrent use of these drugs. To this end we used high-speed chronoamperometry to interrogate the effects of local application of EtOH, cocaine, and their combination on clearance of exogenously applied DA from extracellular fluid in striatum of male and female constitutive OCT3 + / + and OCT3-/- mice in vivo. In addition, we conducted behavioral experiments to assess conditioned place preference in response to administration of EtOH, cocaine, or both, to determine if coadministration of EtOH and cocaine enhanced place preference compared to the administration of each drug individually. 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: We found that in both male ($n = 11$-12/dose) and female ($n = 11$-12/dose) OCT3 + / + mice, EtOH inhibited DA clearance and enhanced the ability of cocaine to inhibit DA clearance, effects that were lost in OCT3-/- ($n = 8$-10M and 8-10F/dose) mice. Behavioral data in male ($n = 16$/dose) OCT3 + / + mice reveal that the combination of EtOH (1000 mg/kg) and cocaine (3.2 mg/kg), doses which by themselves did not induce conditioned place preference (CPP), produced robust CPP. OCT3 + / + mice, EtOH did not produce CPP, whereas cocaine did. Moreover, cocaine CPP in females was potentiated by co-administration of EtOH. CPP studies in OCT3-/- mice are ongoing ($n = 7$M and 7F/dose), but so far demonstrate no development of CPP, regardless of treatment.

Conclusions: Taken together, results suggest that potentiation of the neurochemical and behavioral effects of cocaine by EtOH are OCT3 dependent. OCT3 may be a putative target for therapeutic intervention in the treatment of EtOH and cocaine co-abuse.

Keywords: Alcohol and Substance Use Disorders, Cocaine Use Disorder, Organic Cation Transporters

Disclosure: Nothing to disclose.

P672. Amygdala-Cortical Circuit Determinants of Social Isolation-Induced Alcohol Consumption

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Background: An individual's standing in a social hierarchy is inversely related to alcohol consumption in rodents and non-human primates as well as problematic drinking in humans, highlighting the conserved impact of subordination on motivation for alcohol. Social rank also influences how individuals respond to challenges such as social isolation, a particularly profound stressor with increasing human relevance. However, the neural circuit mechanisms by which social stress experiences engender aberrant alcohol drinking remain largely unknown.

Methods: In this study, we combined behavior, ex vivo whole-cell patch-clamp electrophysiology, in vivo cellular resolution calcium imaging, and machine learning to identify circuits underlying social rank and isolation effects on alcohol drinking.

Results: We found that social rank of adult, male mice correlates with alcohol intake ($r2 = 0.25$ by Pearson's correlation; $^{**}p = 0.01$; $n = 26$), and social isolation increases alcohol intake ($^{***}p < 0.001$ by paired *t*-test; $n = 14$). Notably, we identified a previously unknown relationship between prior social rank and social isolation-induced escalated alcohol drinking, where subordinates display a greater magnitude increase in drinking compared to dominants ($^{**}p < 0.01$ by unpaired *t*-test; $n = 3$/group). These data suggest behavioral factors, emerging from dominance hierarchies, can predict vulnerability to social isolation-induced escalated alcohol drinking. Using ex vivo whole-cell patch-clamp electrophysiology, we found that social isolation increases basolateral amygdala (BLA) excitability ($^{*}p < 0.05$ by two-way ANOVA; $n = 13$-14 cells), highlighting the vulnerability of the BLA to social isolation. Unilateral photoactivation of ChR2-expressing BLA terminals in the mPFC increased alcohol (YFP: 138.5 licks and ChR2: 392.5 licks; $^{***}p < 0.001$ by two-way ANOVA post hoc; $n = 6$/group), but not sucrose or water, drinking, suggesting that social isolation may escalate alcohol drinking through BLA projections to the mPFC. Indeed, using single-cell calcium imaging, we found that the BLA encodes alcohol ($n = 180$-109 cells), and the percentage of alcohol-encoding neurons is increased following social isolation (from 9.5% to 18.3%). Using a SVM classifier, we found that population-level BLA activity preceding a drinking bout accurately (>70%) decodes whether alcohol or water was consumed, further supporting a role of the BLA in encoding alcohol.

Conclusions: Together, these findings suggest that low social rank may be a potent risk factor for increased alcohol drinking following a social isolation challenge, which may be mediated by amygdala-cortical circuits.

Keywords: Alcohol, Social Isolation, Circuits

Disclosure: Nothing to disclose.

P673. Pallidal Perineuronal Nets Regulate Opioid Relapse

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Background: Opioid use disorder remains a major health challenge worldwide, necessitating the development of more effective treatment strategies and a better understanding of the neural circuits that drive opioid use and relapse. Neuronal activity in the ventral pallidum (VP) is critical for opioid reward, and for driving relapse to opioid seeking, but the precise neuronal mechanisms that mediate these behavioral states are incompletely understood. A major population of VP neurons linked to drug relapse is characterized by expression of the calcium binding protein parvalbumin (PV). Throughout the brain PV neurons are ensheathed by specialized extracellular structures known as perineuronal nets (PNNs). However, despite the dense expression of PNNs in the VP, the role of these structures in VP neuronal physiology and opioid seeking remains unknown.

Methods: To investigate the role of PNNs in the VP on opioid relapse, male and female mice ($n = 17$) were surgically implanted with indwelling jugular vein catheters, and chronic guide cannulas above the VP. Mice were trained to nose poke for heroin (and a simultaneously delivered heroin cue light) on a fixed ratio schedule of reinforcement (FR1, 8d), then progressed through variable ratio schedules (VR3 and VR6, 3d each) to reliably assess acquisition of heroin taking. Afterwards mice underwent extinction training, followed 24h later by a microinfusion of the PNN-depleting enzyme chondroitinase ABC (ChABC) or vehicle (0.1% BSA) into the VP. The next day, relapse to drug seeking was assessed using a cue-induced reinstatement test, after which mice were perfused for verification of VP PNN depletion. Experimental procedures followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals and were approved by the University of Colorado, Anschutz Medical Campus Institutional Animal Care and Use Committee (IACUC).

Results: Within VP the majority of PNNs are localized around PV neurons, but a subset of PNNs are associated with distinct, as of yet uncharacterized neurons. ChABC microinjection into VP completely abolished PNNs in the proximity of the microinjector needle. Compared to vehicle, microinfusion of ChABC in VP attenuated cue-induced reinstatement of heroin seeking by selectively reducing active nose pokes for heroin cues (two-way ANOVA, main effects of reinstatement: $F(1,15) = 20.33$, $p = 4.16 \times 10$^-4, treatment: $F(1,15) = 5.833$, $p = 0.029$, and reinstatement x treatment interaction: $F(1,15) = 5.837$, $p = 0.029$), but produced no change in inactive pokes. Ongoing experiments are characterizing the role of PNNs on the physiology of VP neuron subtypes, including the activation of PV neurons during relapse with or without PNN depletion.

Conclusions: These results affirm that the VP is a critical regulator for opioid relapse, and expand our knowledge of the opioid addiction circuitry by showing that heroin seeking requires intact PNNs in the VP. Given the importance of PNNs for memory maintenance and behavioral plasticity, therapies aimed at modifying PNN structure might provide a useful novel avenue for treating opioid addiction.

Keywords: Heroin, Ventral Pallidum, Perineuronal Nets, Parvalbumin Neurons, Relapse

Disclosure: Kaleidescapes, LLC: Founder (Spouse)

P674. Irritability in Cocaine Use Disorder: The Roles of Childhood Trauma and Immune Dysregulation

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Background: Irritability, a low threshold for anger frustration in response to stress, is a common yet understudied feature of

several psychiatric disorders, including cocaine use disorder (CUD). Recent reports have linked irritability to social stress and poorer psychosocial functioning, lower quality of life, and persistently elevated suicidal ideation in adults with mood and/or substance use disorder. However, little is known about the impact of childhood trauma on symptoms of irritability amongst individuals with CUD (iCUD). In addition, childhood trauma and CUD are both associated with heightened immune states involving neuroimmune interactions with long-term influence on psychopathology, yet how this dysregulation relates to symptoms of irritability remains poorly understood. We aimed to characterize irritability in CUD and to elucidate the relationship between irritability, childhood trauma, and immune dysregulation in this population.

Methods: Clinical data were compared between 31 iCUD and 32 healthy controls (HC) not differing on age, gender, race, and IQ, along with blood serum for analysis of inflammatory cytokines, chemokines, and growth factors. A novel composite irritability measure, capturing both the trait and state dimension of irritability, was created using irritability-related items from the Difficulty in Emotion Regulation Scale, Cocaine Selective Severity Assessment, and Perceived Stress Scale. Psychometric properties of this measure were tested with exploratory factor analysis, and convergent validity via the association between measures of state and trait anger, anxiety, and depression. In univariate analyses, we evaluated the association between this novel measure of irritability and measures of childhood trauma, life experiences, and levels of immune markers.

Results: In exploratory factor analysis, the five irritability-related items were loaded on a single factor (first eigenvalue = 2.44, rest <0.16). The composite measure of irritability was strongly correlated with measures of anxiety (rs = 0.61, $p < 0.0001$) and trait anger (rs = 0.56, $p < 0.0001$). Levels of irritability were significantly higher in iCUD versus HC (Cohen's $d = 0.59$; $p = 0.02$). Early life adversity in the form of childhood emotional abuse, sexual abuse, and physical neglect were significantly associated with irritability in both groups (rs = 0.34-0.41, all $p < 0.01$). A differential association between irritability and total negative life experiences was noted among HC (rs = 0.40, $p = 0.028$) but not in iCUD (rs = -0.01, $p = 0.98$). Among immune factors assays, Macrophage-Derived Chemokine (MDC/CCL22) was associated with higher levels of irritability (rs = 0.28, $p = 0.026$) as well as with sexual abuse (rs = 0.244, $p = 0.058$, trend) across groups.

Conclusions: Using our composite measure of irritability, we found that childhood trauma and CUD were both significantly associated with irritability. However, a differential association between irritability and total negative life experiences was only observed in HC, with the mechanisms behind this to be explored in future analyses. Individuals with CUD had a heightened immune activation state of MDC/CCL22, a proinflammatory cytokine implicated in post-traumatic states, which is associated with irritability. These findings indicate a potential relationship between immune, social factors, and psychopathology in cocaine addiction, providing a foundation for future causal testing. Delineating the peripheral underpinnings of stress-related inflammatory signatures in relation to psychosocial states and psychiatric symptomatology in CUD could advance the development of novel treatment to enhance efficacy and recovery.

Keywords: Irritability, Cocaine Use Disorder, Immune Biomarkers, Childhood Maltreatment Exposure, Neuroimmune Interaction

Disclosure: Nothing to disclose.

P675. Cingulate Thickness in Cocaine Use Disorder: Mediation Between Early Life Stress and Lifetime Cocaine Consumption

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Background: Early life stress (ELS) is an important environmental risk factor for cocaine use disorder (CUD). Due to the high plasticity of the brain during early development, exposure to ELS might lead to long-lasting alterations in brain structure and enhanced vulnerability for CUD later in life. These appear to be more pronounced in relevant brain regions for motivation, impulsivity, and inhibitory control, such as the cingulate areas. Thus, we investigated the effect of CUD on thickness of 4 cingulate areas in both hemispheres and tested whether a history of ELS could influence CUD effects.

Methods: The participants were 78 patients with CUD and 53 healthy participants (HC) without CUD nor any neuropsychiatric diagnosis. The magnetic resonance imaging (MRI) examination of cingulate thickness (rostral anterior, caudal anterior, posterior, and isthmus regions) occurred 2 weeks after admission to the detoxification unit on a 3 T Signa GE scanner. The Childhood Trauma Questionnaire (CTQ) was used to assess ELS, and the Addiction Severity Index (ASI) was performed to evaluate drug use patterns (alcohol, tobacco, cannabis, and cocaine). The cingulate regions with significant group differences and with significant associations with CTQ score, were inserted in a mediation model in which lifetime cocaine consumption, CTQ score, and cingulate thickness were, respectively, the independent variable, intermediary variable, and outcome. All statistical analyses were controlled for age and sex.

Results: All cingulate areas in both hemispheres had significantly lower thickness in the CUD group compared with the HC group (p-values < 0.05). Also, a higher CTQ score in the CUD group than in the HC group was found. Significant negative correlations were found between CTQ score and isthmus cingulate (right hemisphere), and with rostral anterior cingulate (left hemisphere). In the mediation analysis, we found a negative significant direct effect of cocaine consumption on isthmus cingulate (b = -0.005, 95IC [-0.008, -0.002]) and an indirect effect of cocaine consumption on isthmus cingulate mediated by CTQ score (b = -0.001, 95IC [-0.002, -0.0001]), suggesting partial mediation. Specifically, 17.5% of the model total effect was explained by the indirect effects mediated by ELS. Also, a marginal sex interaction effect was observed ($p = 0.055$). Regarding the rostral anterior cingulate, ELS did not exert a significant indirect effect on thickness (b = -0.0004, 95IC [-0.001, 0.0004]).

Conclusions: We demonstrated that ELS is related to CUD and conjunctly mediate the effect of CUD on decreased isthmus cingulate thickness in the right hemisphere. This supports the role of ELS as a key environmental stressor that influences brain developmental trajectories associated with drug addiction.

Keywords: Cocaine Use Disorder, Brain Structure, Early Life Stress

Disclosure: Nothing to disclose.

P676. Extinction Attenuates Hyperalgesia During Withdrawal From Self-Administered Heroin: Role of the PVT → NAc Pathway

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Background: The paraventricular thalamus (PVT) has been recently identified as a key component in the neural circuitry of drug addiction in both humans and rodents. The PVT receives

inputs from cortical brain regions and has extensive connections with subcortical regions. In particular, the PVT is a major source of glutamatergic inputs to the nucleus accumbens (NAc), an area known to play a crucial role in relapse to drug seeking. One of the most interesting roles of the PVT → NAc pathway is its ability to drive the aversive somatic states experienced during opioid withdrawal, which can drive relapse. We recently found the PVT → NAc pathway to be necessary for heroin relapse after abstinence, but not after extinction training. We hypothesized that this pathway may thus be a substrate by which cognitive behavioral therapy (e.g., extinction training) might alleviate opioid withdrawal and thereby reduce heroin seeking. First, we tested the hypothesis that acute withdrawal from heroin self-administration induces mechanical hyperalgesia. Next, we investigated whether hyperalgesia is alleviated by extinction training. We then tested whether chemogenetic inhibition of the PVT → NAc pathway could simulate extinction-induced analgesia during heroin withdrawal. We showed that extinction, but not abstinence, from heroin self-administration causes a loss of synaptic plasticity in the NAc D1-, but not D2-expressing, medium spiny neurons (MSNs) receiving inputs from the PVT. Moreover, chemogenetic inhibition of the PVT → NAc pathway reduced heroin relapse after abstinence, but not after extinction training. Accordingly, we applied an in-vivo long-term depression (LTD) protocol using optogenetic stimulation of PVT terminals in the NAc in an effort to recapitulate the extinction-mediated loss of synaptic plasticity in this pathway and thereby reduce heroin relapse in abstinent rats. Fiber photometry was used to validate the in-vivo LTD protocol by simultaneously stimulating PVT terminals and recording calcium activity in the NAc from the same fiber, in the same animal.

Methods: Our experimental procedures followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals and were approved by the University of Colorado, Anschutz Medical Campus Institutional Animal Care and Use Committee (IACUC). For chemogenetic experiments, the NAc of male and female rats ($n = 23$) was bilaterally injected with an AAVrg-Cre and the PVT was injected with an AAV2-hSyn-DIO-hM4D(Gi)-mCherry. Other behavioral data from this cohort are in press at Cell Reports (Giannotti et al. 2021, in press). A different cohort of male and female rats ($n = 5$) was used for the in-vivo LTD experiments. The NAc was bilaterally injected with an AAV9-hSyn-GCaMP6f and the PVT was injected with an AAV9-hSyn-ChrimsonR-tdT; fiber optic cannula were bilaterally implanted in the NAc. Rats underwent 12 days of heroin self-administration and hyperalgesia was assessed after 14d of withdrawal (7 d of extinction or 14 d of home cage abstinence) by using an electronic Von Frey apparatus, 15 min after J60 (0.1 mg/kg) or vehicle (H2O) pretreatment. A different cohort of rats ($n = 5$) received a bilateral in vivo optogenetic LTD protocol stimulation (638 nm, 10 mW; 1 Hz - 15 min), or no-stimulation, after 14 d of home cage abstinence, 45 min before the cued relapse test. Optogenetic evoked calcium events (638 nm; 20 Hz - 1s) were recorded every 5 min before (pre-LTD baseline; 20 min) and after (post-LTD; 40 min) the in-vivo optogenetic LTD protocol. Chemogenetic experiments were analyzed using a 2-way RM ANOVA followed by Fisher's LSD post hoc test. Fiber photometry data were analyzed using a two-tailed paired *t*-test.

Results: Withdrawal (14d) from self-administered heroin results in mechanical hyperalgesia (2-way RM ANOVA; treatment: $F(2,42) = 59.79$, $p < 0.001$; treatment x withdrawal modality: $F(2,42) = 3.437$, $p = 0.0415$) in both extinction and abstinence groups compared to pre-heroin baseline (p's < 0.001). However, extinction training significantly attenuated heroin withdrawal-induced hyperalgesia compared to the abstinence group (abstinence vs. extinction: $p = 0.0447$). Finally, chemogenetic inhibition of the PVT➡NAc pathway after abstinence reduces hyperalgesia to levels similar to those observed after extinction training (abstinence vs. extinction: $p = 0.8311$), suggesting that extinction may alleviate opioid withdrawal by reducing activity in this pathway. Moreover, our fiber photometry data indicate that the in-vivo LTD protocol reduces peak amplitude ($p = 0.0055$; paired *t*-test) and the area under the curve (0-2s; $p = 0.0093$; paired *t*-test) of optogenetically evoked calcium responses in NAc MSNs. Importantly, these are preliminary data given the sample size ($n = 5$) and pending histology. Thus, we cannot conclude whether this in-vivo LTD protocol is capable of reducing relapse rates in heroin-withdrawn, abstinent rats.

Conclusions: Here we demonstrated that the PVT → NAc pathway promotes heroin withdrawal-induced hyperalgesia after abstinence, but not after extinction training. Moreover, we successfully employed fiber photometry to validate the in-vivo optogenetic LTD protocol in the PVT → NAc pathway, as several parameters of the calcium transients pointed to reduced synaptic activity within this pathway. Future studies will increase the sample size of this preliminary experiment and will investigate whether the same in vivo LTD protocol can attenuate heroin withdrawal-induced hyperalgesia and subsequent relapse.

Keywords: Heroin Self-Administration, Opioid Withdrawal, Hyperalgesia, Chemogenetics, Fiber Photometry

Disclosure: Nothing to disclose.

P677. Identification of Individuals With Resistant, Mild, Moderate, and Severe Cocaine Addiction-Like Behaviors in 500 + Heterogeneous Stock Rats

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Background: A key question for cocaine addiction research remains why casual drug consumption escalates to problematic use associated with high motivation and compulsivity typical of substance use disorder in some individuals, but not others. A better characterization of individual differences in the propensity to develop addiction-like behaviors could help identify new pharmacological targets, biomarkers, gene variants, and facilitate medication development. However, most studies have used small sample size ($N <$ 8-20), limited access self-administration paradigms, or animal models with limited individual differences. Such limitations reduce the translational relevance of the results and limit the reproducibility and replicability of these studies. To address this issue, we characterized addiction-like behaviors using an advanced model of extended access to cocaine self-administration in large cohorts of heterogeneous stock (HS) rats, a unique outbred strain of rats with large individual differences.

Methods: HS rats ($N = 567$ with 275 females and 292 males) were allowed to self-administer cocaine through the model of extended access (2 weeks of short 2 h/daily, 3 weeks of long 6 h/daily and 1 week of additional testing). Animals were also screened for compulsive cocaine use using progressive-ratio (PR) responding and responding despite adverse consequences (contingent foot shocks), and withdrawal-induced irritability-like behavior (bottlebrush test). Large cohorts ($n =$ 46-60) were used to minimize cohort-specific effects and the level of responding was normalized within cohorts. *T*-tests and ANOVA were performed to determine significant effects. With this many animals, small effect sizes (Cohen's $d = 0.2$) can be detected with high power (1-B = 0.95, a = 0.05).

Results: Behavioral characterization showed large inter-individual differences in addiction-like behaviors, with small intra-individual differences. While large sex differences were observed, with females having a higher responding under fixed ratio (FR) (escalation of intake, $p < 0.01$) and responding despite foot shock (compulsivity, $p < 0.001$) than males, there were considerably larger differences between individuals than just between sex. Final cocaine intake showed a bimodal distribution, with 80% vulnerable rats escalating their cocaine intake over the extended access protocol and 20% resilient rats, who maintain low intake levels. Besides escalation ($p < 0.001$), these groups also showed significant differences in responding under PR (motivation, $p < 0.001$) and compulsivity ($p < 0.001$), but not in withdrawal-induced irritability. Principal component analysis of intake, motivation, compulsivity, and withdrawal showed escalation, motivation, and compulsivity clustering together on the first principal component (PC1), which explained 48% of the variance (eigenvalue >1, $r = 0.52$ to 0.60). Irritability-like behavior was orthogonal to PC1 ($r = 0.008$). An addiction index was calculated by averaging the Z-scores of the three dependent variables as a proxy of PC1 for a comprehensive evaluation of compulsive cocaine use. The addiction index was further used to classify vulnerable animals as having resistant, mild, moderate, or severe addiction-like behaviors with significant differences in escalation ($p < 0.001$), motivation ($p < 0.001$), and compulsivity ($p < 0.001$). The addiction index could not be predicted by the behavioral measures obtained during the short access sessions (FR, PR, time out response, loading phase intake (first 15 min), titration phase intake (last 60 min), and inter-injection interval, all $R < |0.12|$, $p =$ ns). The behavioral measures during the extended access sessions progressively predicted the addiction-like phenotype starting on day 5 for drug intake ($R = 0.18$, $p <$ 10-5) until day 14 ($R = 0.47$, $p <$ 10-6). PR responding ($R = 0.62$), loading phase ($R = 0.49$), titration phase ($R = 0.57$), inter-injection intervals ($R =$ -0.25), and number of responses during the shock session ($R = 0.22$) also predicted the addiction index (all $p <$ 10-6).

Conclusions: This is to our knowledge the largest intravenous cocaine self-administration study in rats. We identified individuals with resistant, mild, moderate, and severe cocaine addiction-like behaviors in 500+ heterogeneous stock rats. Behavioral responses using the limited access model were not predictive of the development of addiction-like behaviors, highlighting the necessity of using the chronic extended access model to better understand and predict addiction-like behaviors. A wide variety of biological samples were collected longitudinally and are made available to the community through the Cocaine Biobank to facilitate identification of biomarkers of addiction-like behavior and facilitate reproducibility and replicability efforts.

Keywords: Psychostimulant, Outbred Rats, Substance-Related Disorders

Disclosure: Nothing to disclose.

P678. The Impact of the COVID-19 Pandemic on Alcohol Consumption and Related Outcomes Across the Spectrum of Alcohol Use and Alcohol Use Disorder

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Background: The diverse and pervasive impact of the COVID-19 pandemic continues to unfold globally on individuals, communities, health systems and the economy. Stress, social isolation, economic losses and uncertainty can significantly affect mental health and alcohol use; therefore, it is critical to prospectively assess the effect of the pandemic on alcohol use and associated behaviors and outcomes. With this goal, we identified a sample of participants previously enrolled in NIAAA clinical research studies and invited them to participate in a longitudinal survey study to assess the effects of the pandemic on alcohol use and consequences. Specifically, we aimed to examine pandemic-related changes in alcohol consumption and underlying determinants such as craving, negative life events, and alcohol use disorder risk.

Methods: Previous participants in NIAAA clinical studies across the spectrum of alcohol use were contacted by phone for study participation. Following consent, participants completed an initial survey to obtain pre-pandemic baseline and initial impact data. Following this, participants complete electronic or phone surveys at intervals ranging from weekly to every six months for 2 years. Assessments include the Alcohol Use Disorder Identification Test (AUDIT), and questionnaires assessing craving (Penn Alcohol Craving Scale), stress (Perceived Stress Scale), and negative life events (Life Events Questionnaire). Participants also complete a COVID-19 Scale, derived from CDC and WHO questionnaires, that assesses the impact of the pandemic on medical and mental health symptoms and behaviors as well as stressors (work, family, financial, etc.). Responses from the COVID-19 scale were used to obtain a COVID-stress score. Due to rolling enrollment, initial impact surveys were collected during different "waves" of the pandemic: Wave 1: March-July 2020, Wave 2: August-November 2020, Wave 3: December 2020 onward.

Results: Between June 2020 and March 2021, we enrolled 386 participants (180 *F*/206 M), into 3 groups: Individuals with AUD that had undergone treatment ($n = 116$), non-treatment seeking AUD ($n = 54$), and non-AUD controls ($n = 216$). Analysis of the initial impact data indicated a wide variation in pandemic-related changes in alcohol use, with 31% of participants reporting increases, 32% reporting decreases, and 37% reporting no change in AUDIT-Consumption scores. The magnitude of change in AUDIT scores was inversely related to the pre-pandemic AUDIT score. COVID-19 related stress scores showed significant racial differences with the Black/African-American group reporting higher COVID-related financial stress. Overall, greater COVID-related stress was associated with higher AUDIT scores. Additional analysis focused on differences between groups defined by pandemic-related change in AUDIT scores. The group that showed increases in AUDIT showed significantly higher pre-pandemic measures of perceived stress, negative life events and craving compared to the group that showed decreases in AUDIT and no change in AUDIT scores (all p values < 0.05). Predictors of increases and decreases in AUDIT scores were evaluated using separate general linear models, and included changes in negative life events, perceived stress and craving, covarying for pre-pandemic AUDIT scores, AUD diagnosis, race, sex and pandemic wave. Results indicated that increases in negative life events and craving were significantly associated with increases in AUDIT scores, while a decrease in negative life events was significantly associated with decreases in AUDIT score.

Conclusions: The findings of this study indicate great heterogeneity and bi-directional shifts in alcohol consumption associated with the pandemic. Changes in AUDIT scores were driven by pre-pandemic alcohol use, and associated with changes in negative life events and craving. Ongoing longitudinal data collection and analysis in this sample of individuals will expand on these initial changes in drinking patterns, craving and AUD risk and the role of COVID-related stress on these changes. Results will help identify potential targets for intervention to help individuals vulnerable to negative outcomes following the pandemic and other major public health disasters.

Keywords: Alcohol Use Disorder, the COVID-19 Pandemic, Perceived Stress, Craving

Disclosure: Nothing to disclose.

P679. Influence of Dopaminergic System on Blink Rates Assessed by Methylphenidate

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Background: Blink rate has been proposed as a biomarker of the dopamine system that could be valuable for detection and monitoring of relevant neuropsychiatric diseases (i.e., SUD, Parkinson's disease) 1,2. Findings on the relationship between blink rates and dopamine receptors (DRD1, DRD2) are not consistent3. D1 and D2 receptor direct agonists can independently increase blink rates in primates 4,5, but indirect agonists (e.g.., cocaine) could decrease blink rates6. Concomitant stimulation of D1 and D2/3 receptors by indirect agonists (e.g., cocaine or methylphenidate) could lead to variable effects on blink rates. Here we explored dopaminergic underpinning of blink rates by using a combination of eye tracking during resting state, as well as PET measures of DRD1 and DRD2 availability (following a methylphenidate (MP) challenge).

Methods: PET: [11C]raclopride and [11C]NNC112 scans were performed in 32 healthy subjects (20-50 yrs) on one of two scanners: a high-resolution research tomography (HRRT) scanner ($n = 16$; 7 female; Siemens AG; Germany) or a Biograph PET/CT scanner ($n = 16$; 5 female; Siemens AG; Germany). Participants were scanned once with [11C]NNC112 (15 mCI, 90 minutes of dynamic imaging) to assess DRD1 at baseline (i.e., no drug challenge) and twice with [11C]raclopride on separate days: once 1 h after administration of an oral placebo (PL) to assess baseline dopamine D2/3 receptor availability, and once 1 h after administration of 60 mg oral methylphenidate (MP) to assess DA changes (single blind; counterbalanced session order; 10 mCi, 60 minutes of dynamic imaging). The [11C]NNC112 scan was performed (10 AM) either before the [11C]raclopride scan (1PM) on the same day or on separate days. DRD1 and DRD2 binding potentials (BPnd) were calculated using simplified reference tissue model with Magia pipeline 7. Eye-tracking: Blink measures were collected during resting state (approximately 2 hours after the PET scan and 3 hours after PL or MP. We used ASL long-range LRO eye-tracker camera and ET7 software system (sample rate = 120hz). To detect eye-blinks, blink duration was set to a minimum of 0.1s and a maximum of 0.4s. Problematic eye-tracking sessions were eliminated, yielding 25 eye-tracking sessions (15 males, 10 females) from MP days, and 17 eye-tracking sessions (10 males, 7 females) from PL days; 16 subjects had both MP and PL eye tracking available. MRI: Structural scans were collected before fMRI to extract individual freesurfer brain segmentations, which were used to delineate putamen, caudate and ventral striatum as regions of interest (ROIs).

Results: Subjects blinked less and kept their eyes open more during MP than PL (Eye closures % during rest: PL = %21.2 (15.1), MP = %4.06 (5.3), paired-$t(15) = 5.1$, $p < .001$; Blink rates (blinks/s): PL = .56 (.23), MP = .42 (.29), paired-$t(15) = 2.48$, $p < .05$). DRD2 BPnd in putamen (not in caudate or VS) was positively associated with blink rates in the MP but not PL session ($n = 25$, $r = 0.69$, $p < .001$). Baseline DRD1 BPnd in caudate was positively associated with blink rates in the MP but not PL session ($n = 25$, $r = 0.53$, $p < .01$) and was negatively associated with MP-induced changes in blink rate (PL-MP) ($n = 16$, $r = -.69$, $p < .01$), which were also negatively correlated with DRD1 in putamen ($r = -.56$, $p < .05$) ROIs. D2R availability in striatum was significantly reduced by MP (reflecting DA increases) for the whole group ($n = 32$) (1-tailed t-tests, caudate $t = 3.31$, $p < .01$ and putamen $t = 7.27$, $p < .01$), MP-induced 'dopamine increases' were not correlated with MP-induced changes in blink rates.

Conclusions: Our findings are consistent with DA's modulatory role on blink rate but indicate distinct roles for DRD1 than DRD2 across striatal regions. Specifically, we showed that the effects of MP on blink rates were dependent on baseline DRD1 but not on baseline DRD2/3. Participants with low DRD1 in putamen and caudate had greater blink reductions with MP. Our findings also show that the associations between dopamine receptors and blink rates emerged when presumably D1R and D2/3R are simultaneously stimulated.

Keywords: Dopamine, DRD1, DRD2, Eye-Blinks, Methylphenidate

Disclosure: Nothing to disclose.

P680. Reports of Taste and Smell in AUD Participants During Different COVID-19 Pandemic Waves

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Background: Alcohol use disorder (AUD) is the most prevalent substance use disorder with 3.3 million deaths globally each year. Taste and smell are reportedly disturbed in individuals with AUD, and some studies have shown that individuals with severe AUD suffer from the inability to discriminate between taste and smell. The aim of this analysis was to investigate reports of chemosensory dysfunction, if any, in AUD within an ongoing longitudinal COVID-19 impact study. This was important because the COVID-19 pandemic had a major effect on multiple factors associated with AUD, including access to alcohol and changes in social and environmental variables (e.g., social isolation and access to health care).

Methods: Analyses were conducted on taste and smell measures from an ongoing longitudinal COVID-19 impact study initiated in June 2020. Because there were only nine COVID-positive participants, we decided to exclude the data of these participants to focus on the impact of alcohol consumption on taste and smell in the context of the COVID-19 pandemic. Of 307 participants with available diagnoses, there were 140 AUD (89 males/51 females, 46.4 ± 13.5 years) and 167 non-AUD controls (75 males/92 females, 43.4 ± 14.5 years). All participants were asked to complete a series of online surveys for 24 months. Taste and smell data were recorded using a VAS self-rating online questionnaire (0-100: higher score, a better sense of taste/smell). Notably, in this analysis, we considered three waves of the pandemic. This allowed us to examine different onset time points in the pandemic and the progression of symptoms over time. The three COVID waves were defined as (i) early pandemic (March 11 – July 31, 2020) (ii) mid pandemic (August 1 – November 22, 2020), and (iii) late pandemic (November 23, 2020, onward). We hypothesized that there would be a decrease in taste and smell scores related to alcohol drinking behaviors during the different COVID waves. Clinical trails # NCT04391816

Results: We analyzed the mean differences in smell and taste scores within the AUD and non-AUD groups during the three

COVID waves. Contrary to our hypothesis, AUD participants showed an increase in smell scores at week 4 from baseline ($p = 0.05$) during the early pandemic. While in non-AUD participants we observed a decrease in mid-pandemic mean smell scores at week 8 from baseline. Moreover, at baseline and week 4 we observed that AUD participants had significantly higher mean smell scores compared to non-AUD individuals ($p < 0.05$). However, this difference was only seen during the early COVID pandemic wave. This result shows increased smell perception in individuals with increased alcohol consumption. A significant decrease in the mean taste score of AUD individuals was seen at various time points from baseline during the different COVID waves: (a) the mid pandemic data revealed a decrease in the mean score at weeks 4, 8, and 12 ($p < 0.01$), (b) whereas the late pandemic data showed a decrease in mean taste score at week 12 from baseline score ($p < 0.01$).

Conclusions: Some of the earlier literature on smell perception alterations in individuals with excessive alcohol consumption report contradictory results. While some described an impairment in smell perception with increased intake, others have reported preserved olfactory abilities. The preliminary findings provide evidence of increased complications associated with COVID-19-induced signs and symptoms, including taste perception alterations in individuals with increased alcohol consumption. Interestingly, we observed significantly higher mean smell and taste scores at six months in the AUD compared to the non-AUD group. This was observed only during the late COVID wave. As approximately 80% of the AUD individuals were treatment seekers, higher smell, and taste scores at the end of the study period might reflect the effect of the treatment these participants were undergoing. Study limitations included self-report bias, reduced sample size due to attrition, and the lack of psychophysical measures to provide an objective assessment of taste and smell. As we showed in our meta-analysis (Hannum et al. 2020), objective measures are a more sensitive method to identify smell and taste loss because of infection with SARS-CoV-2. As part of our future directions, we plan to add objective chemosensory measures to this ongoing study when participants can return to the NIH. To better understand the observed smell scores changes, we plan to conduct an analysis on how the smoking status of individuals both in the AUD and the non-AUD group affects taste and smell scores. Moreover, we acknowledge that amid the different COVID waves there were unique social and environmental variables, such as people having limited access to alcohol and the risk of alcohol withdrawal syndrome. Therefore, we additionally plan to build upon our findings and examine other COVID-related factors including alcohol drinking patterns among AUD individuals.

Keywords: AUD, Taste, Smell, COVID-19

Disclosure: Nothing to disclose.

P681. Alcohol Use Disorder Compromises the Amygdalar Noradrenergic System

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Background: Alcohol use disorder (AUD) is a leading preventable cause of death worldwide. The central amygdala (CeA) functions as an integrative hub for the negative emotional responses associated with anxiety, stress and AUD. The noradrenaline (NA) system mediates stress responses, and its dysfunction is implicated in the craving and relapse risk of abstinent alcoholics.

Methods: Here we investigated whether alcohol (ethanol) dependence and withdrawal (abstinence) alter noradrenergic regulation of the amygdala in rodents. Male rats were housed under control conditions, subjected to chronic intermittent ethanol vapor exposure (CIE) to induce dependence, or withdrawn from CIE for two weeks, and ex vivo electrophysiology, in situ hybridization, biochemistry (catecholamine quantification by the HPLC system) and behavioral brain-site specific pharmacology studies were performed.

Results: We found that NA enhances basal CeA GABA release to 212.6 ± 24.0% of baseline in 9 out of 15 cells [$t(8) = 4.69$, $p < 0.01$ by one-sample *t*-test] via α1 adrenergic receptors in control rats, and that dependence recruits β adrenergic receptors to inhibit NA release. Despite a functional recovery of NA's effects on GABA signaling after 2 weeks of withdrawal, CeA cells expressing α1 and β1 receptor RNA are still altered. Additionally, withdrawal significantly decreased the concentration of NA in the CeA. We next used CeA microinfusions of prazosin or propranolol to determine the role of these receptors in alcohol consumption in non-dependent (moderate drinking) and dependent (excessive drinking) rats. We found that α1 receptors mediate the low to moderate levels of alcohol intake associated with non-dependent rats [$p < 0.05$ vs. vehicle by Sidak post hoc test following two-way ANOVA with non-dep vs. dep rats: $F(1,26) = 4.98$, $p < 0.05$; prazosin: $F(3,78) = 5.79$, $p < 0.01$; rats x doses interaction: $F(3,78) = 2.46$, $p > 0.05$], while β receptor activity drives excessive drinking in dependence [$p < 0.001$ vs. vehicle by Sidak post hoc test following two-way ANOVA with non-dep vs. dep: $F(1,26) = 12.80$, $p < 0.01$; propranolol: $F(3,78) = 6.86$, $p < 0.001$; rats x doses interaction: $F(3,78) = 2.65$, $p > 0.05$] .

Conclusions: Thus, CeA α1 and β adrenergic receptors are key neural substrates of AUD. Identification of these novel and complex mechanisms as significant drivers of alcohol drinking, particularly during the alcohol-dependent state, can guide promising ongoing medication development.

Keywords: Alcohol Dependence, Noradrenergic System, GABA, Central Amygdala

Disclosure: Nothing to disclose.

P682. Trait Negative Emotionality Predicts Subjective Drug Effects Across Drug Classes

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Background: The self-medication hypothesis suggests that individuals use psychoactive drugs to treat psychiatric symptoms including negative emotional states. However, it is not known whether individuals prone to such negative emotional states experience subjective drug effects differently, nor is it known to what extent this relationship may vary across drug classes. Here we investigate the relationship between trait negative emotionality and subjective effects of five different psychoactive drugs, including a psychedelic (LSD), amphetamines (d-amphetamine and MDMA), cannabinoid (THC), and opioid (buprenorphine).

Methods: Healthy adult volunteers completed the Multidimensional Personality Questionnaire (MPQ) and then completed at least two drug testing sessions during which they received placebo or one of the following: LSD (13ug), MDMA (1.5mg/kg), d-amphetamine (20mg), THC (15mg), or buprenorphine (0.2mg). At regular intervals throughout the sessions, subjects completed ratings of how much they felt a drug effect and whether they liked the effects. These ratings were examined in relation to the Negative Emotionality (NE) scale of the MPQ.

Results: Individuals with high trait NE gave higher ratings of "feel drug" under the influence of LSD ($N = 41$; $r = 0.37$, $p < 0.05$), buprenorphine ($N = 58$; $r = 0.35$, $p < 0.05$), and d-amphetamine ($N = 379$; $r = 0.11$, $p < 0.05$), but not THC. They did not, however show higher ratings of "like drug" during these sessions, and for d-amphetamine, individuals with greater trait NE gave significantly lower ratings of "like drug" ($r = -0.14$, $p < 0.01$).

Conclusions: Higher trait NE was related to greater sensitivity to experiencing the interoceptive, or subjective, effects of drugs. This increased sensitivity does not seem to be related to greater ratings of drug liking. It will be of interest to investigate to what extent sensitivity to experiencing drug effects are associated with risk for using or misusing psychoactive drugs.

Keywords: MDMA, LSD, Buprenorphine, THC, Personality

Disclosure: Nothing to disclose.

P683. Acute Withdrawal From Long Term Alcohol Consumptions Alters Spontaneous Home-Cage Behaviors and Paraventricular Nucleus of the Hypothalamus Corticotropin-Releasing Hormone Neurons in Mice

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Background: Alcohol use disorder (AUD) is a chronic relapsing condition that affects approximately 14.1 million Americans. Despite the prevalence of this disorder, we lack a thorough understanding of the underlying neurobiological mechanisms. One of the major mechanisms believed to contribute to the development of AUD is the dysregulation of central stress systems. The hypothalamic-pituitary-adrenal (HPA) endocrine axis is one of the body's primary stress response systems and is heavily implicated in the risk for and development of AUD. The HPA axis is triggered by the release of corticotropin releasing hormone (CRH) from CRH neurons in the paraventricular nucleus of the hypothalamus (PVNCRH). Dynamic changes in the HPA axis are linked to alcohol misuse, but alterations in PVNCRH neurons, which initiate the HPA axis, have not been rigorously examined following alcohol consumption. PVNCRH neurons are of particular interest, because in addition to initiating the HPA axis, they have been shown to mediate rapid stress-related behavioral responses independent of CRH release. Therefore, we tested the hypothesis that chronic alcohol consumption alters stress coping behaviors and PVNCRH neuron function.

Methods: Male C57BL/6J mice underwent a long-term, voluntary water or alcohol (20% w/v) drinking paradigm that consists of three 24-hour sessions per week for a total of six weeks. In acute, 24-hrs withdrawal, water and alcohol access mice were separated into a no stress or stress condition (2-hr restraint) to determine alcohol stress responsivity ($N = 13$-14/group). Immediately following stress, spontaneous home-cage behaviors were recorded for 15-mins. Mice were then placed in the open field test for 5-mins. To probe PVNCRH physiological function after chronic alcohol, a second experiment was conducted in male and female CRF-reporter mice that underwent the same long-term voluntary drinking paradigm. In acute withdrawal, ex-vivo electrophysiology experiments were conducted to measure synaptic transmission and excitability. To determine if home-cage spontaneous behaviors correlate with physiological function, a 15-min home cage recording was conducted before sacrificing the mice for ex-vivo recordings. Two-way ANOVAs were conducted for statistical analysis, and Tukey's corrected multiple comparison post-hoc tests were conducted when relevant.

Results: To date, we have found that during acute withdrawal, male mice demonstrate a series of spontaneous behaviors in the home-cage indicative of stress coping, such as increased rearing (no stress water vs alcohol groups, $p = 0.002$) and decreased digging (no stress water vs alcohol groups, $p = 0.021$). Notably, stress appears to impact water and alcohol mice similarly in the home cage (HC) and open field (OF), as both alcohol and water stress mice spent significantly more time grooming in both arenas (main effect of stress in home cage, $p < 0.0001$ and open field, $p = 0.02$) and less time rearing (main effect of stress HC, $p < 0.0001$ and OF, $p = 0.007$) and digging (main effect of stress HC, $p < 0.001$). Preliminary findings from experiment 2 indicate that during acute withdrawal, female mice exhibit heightened excitability of PVNCRH neurons compared to water control mice. Specifically, in response to increasing current injections, alcohol mice fired more action potentials (main effect of alcohol: $p < 0.0001$). We also found a trend towards male mice exhibiting similar alterations in PVNCRH neurons (main effect of alcohol: $p = 0.092$).

Conclusions: We find that a single acute stressor alters spontaneous behaviors in the home cage of both water and alcohol consuming mice. Interestingly, home cage behaviors are altered in naïve, non-stressed alcohol mice, in general being indicative of higher stress coping behaviors. This suggests that even 24 hours following the final alcohol drinking session, mice have a heightened basal stress state that can be further exacerbated by acute stressors. Additionally, PVNCRH neurons appear to be more excitable during this same acute withdrawal timepoint.

Keywords: Alcohol Withdrawal, Stress Coping, Paraventricular Nucleus of the Hypothalamus, Stress and Anxiety Behavior, Neural Circuit and Animal Behavior

Disclosure: Nothing to disclose.

P684. The Effects of Early Life Adversity on Risky Decision-Making and the Orbitofrontal Cortex Transcriptome in Male and Female Rats

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Background: Early life experiences can alter risk/resilience for disorders linked to changes in motivated behavior. Early stress that is not overwhelming can have an "inoculating" effect that promotes later resilience. Our lab uses the limited bedding and nesting (LBN) model of mild early life adversity. We found that this model reduces impulsivity and morphine self-administration in male but not female rats, suggestive of an "inoculation" effect against addiction-related behaviors in males. Here we extend this work to examine how early life experiences alter risky decision-making and explore the genetic alterations that can contribute to stress inoculation.

Methods: In LBN, pups from postnatal day 2–9 and their dams were exposed to a low resource environment and compared to rats raised in standard housing. Once these pups reached adulthood, their risky decision-making was compared to rats raised in standard housing conditions. Here we predicted that LBN would reduce male preference for risky choices in a probability discounting task. In probability discounting, rats must choose between two levers: one that delivers a small reward always (certain lever) vs. one that delivers a large reward sometimes (risky lever). Probabilities of earning a reward varied throughout the session and preference for the risky lever is only advantageous when the odds of winning the large reward are high (i.e., 100% or 50%). Given the role of the orbitofrontal cortex (OFC) in impulsive

and risky decision-making processes, we then evaluated whether LBN-induced changes in gene expression in this region by using RNA sequencing (RNA-seq). OFC tissue from adult rats (male control $n = 5$; female control $n = 5$; male LBN $n = 5$; female LBN $n = 5$) was collected and was sequenced on an Illumina HiSeq 4000. Fastqc version 0.11.8 was used to evaluate the quality of reads with adaptors and nonpaired reads were 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. RNA-seq analysis was performed, and 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 found that LBN exposure reduces risky decision-making on the probability discounting task in male and female rats [$F(4, 376) = 2.845$, $p = .024$]. High risk taking is associated with increased drug taking, but here we found LBN reduced risky choices on this task. Because this manipulation produces a resilient-like phenotype against addiction-related behaviors, we then were interested in examining possible molecular underpinnings that promote stress-induced resilience. RNA-seq on OFC tissue revealed LBN induced sex-specific changes in gene transcription. RRHO analysis revealed little overlap between both up-and down-regulated genes between males and females, but we also found a distinction between genes upregulated in males and downregulated in females due to LBN. We next narrowed down our analysis to genes showing a significant difference between control and LBN and found 161 differentially expressed genes (DEGs) in males and 138 DEGs in females. These gene changes were largely sex-specific with only 18 common genes altered by LBN. KEGG pathway analysis revealed "retrograde endocannabinoid signaling" was significantly enriched in males and not females (adjusted $p = .01$), which may drive behavioral changes induced by LBN in males. The endocannabinoid system is of interest because it has been implicated in impairing inhibitory control and promoting risk-taking behavior. Future studies aim to validate top gene targets, including one involved with the endocannabinoid system, with RNAScope.

Conclusions: Collectively, these results suggest that LBN reduces the expression of behaviors associated with increasing risk for substance use disorders. Although initially unexpected, these findings add to a growing body of literature supporting the stress inoculation hypothesis: that some early adversity promotes later resilience. Understanding mechanisms that promote resilience can lead to better treatments for disorders exacerbated by stress.

Keywords: Early Life Stress, Risk Taking Behaviors, Sex Differences, Orbitofrontal Cortex

Disclosure: Nothing to disclose.

P685. Moderators of Subjective Response to Alcohol in the Human Laboratory

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Background: Subjective response (SR) to alcohol represents a biobehavioral risk factor for heavy drinking and for developing alcohol use disorder (AUD). Identifying moderators of SR has been hindered by small sample sizes that are often used in alcohol administration studies. This study culled from multiple alcohol administration trials to test whether sex, family history of alcohol problems, and impulsivity (via delay discounting) predict SR to alcohol, comprised of four domains: stimulation, sedation, negative affect, and craving.

Methods: Male and female non-treatment-seeking heavy drinkers ($N = 250$) completed a battery of self-report scales and behavioral measures of alcohol use and problems, mood, and impulsivity. All participants completed an intravenous alcohol administration session wherein SR domains were measured at baseline, 20, 40, and 60mg%. Due to the nested data structure, a series of multilevel models tested whether potential moderators predicted SR during the alcohol challenge. The nested structure of the data was as follows: repeated SR measurements across the alcohol challenge (Level 1) nested within individuals (Level 2), who were nested within studies (Level 3).

Results: We found that male sex independently predicted higher alcohol-induced stimulation ($B = .820$, $SE = .32$, $p = .011$) and alcohol craving ($B = .295$, $SE = .10$, $p = .005$) after controlling for other moderators. Family history of alcohol problems independently predicted alcohol craving controlling for other moderators ($B = .995$, $SE = .26$, $p = .0001$). Delay discounting did not significantly predict any subjective response domain.

Conclusions: Through a large sample and advanced data analytic methods, this study extends the literature by suggesting important moderators of SR in heavy drinkers, namely male sex and family history of alcohol problems. These findings consolidate and extend a growing body of research on who is most likely to report the SR features that confer risk for AUD.

Keywords: Subjective Response, Alcohol, Sex Differences

Disclosure: Nothing to disclose.

P686. Role of CHRNA5 rs16969968 Polymorphism in Alcohol Consumption and Sensitivity in Non-Dependent Heavy and Light Drinkers

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Background: The nicotinic acetylcholine receptor gene variant CHRNA5 rs16969968 (G > A) has been strongly associated with nicotine use and dependence. A recent preclinical study has also implicated the rs16969968 polymorphism in alcohol consumption and alcohol use disorder (AUD). However, its role in alcohol consumption and sensitivity in humans is still unclear. In this study, we examined the effects of the rs16969968 polymorphism on alcohol consumption and sensitivity in heavy and light drinking groups of people without AUD.

Methods: The study included 176 healthy adult, non-AUD drinkers who were classified into heavy and light drinking groups based on Alcohol Use Disorders Identification Test (AUDIT-C) scores (light drinking: ≤5, heavy drinking: >5). Real-world alcohol consumption was assessed by AUDIT-C subscores and Timeline Followback (TLFB) measures. Human laboratory alcohol seeking, and consumption were evaluated using a free-access intravenous alcohol self-administration (IV-ASA) paradigm implemented in the Computer-Assisted Infusion System (CAIS). IV-ASA measures included the peak and average breath alcohol concentration (BrAC) at different time points (30, 60, 90, and 120 minutes) and total ethanol (in grams) infused during the session. Subjective responses were assessed serially during the IV-ASA session using the Drug Effects Questionnaire (DEQ), Alcohol Urge Questionnaire (AUQ) and Biphasic Alcohol Effects Questionnaire (BAES). In addition, Self-Rating of the Effects of Alcohol (SRE), Alcohol Sensitivity Questionnaire (ASQ), and Alcohol Effects Questionnaire (AEFQ) were used to assess alcohol response sensitivity and expectancies. Peripheral blood samples were collected from the participants for genotyping. Alcohol consumption, subjective

responses and related phenotypes were compared between heavy and light drinking groups and by rs16969968 genotype. The effect of the rs16969968 polymorphism was tested using a dominant model (GG and AA/AG).

Results: Initially, we compared the alcohol consumption and IV-ASA measures between heavy and light drinking groups, and as expected, AUDIT-C and total AUDIT scores as well as TLFB measures were significantly higher in heavy drinkers than light drinkers. BrAC levels and ethanol consumption during the IV-ASA session did not vary between drinking groups. However, in the GG genotype group, heavy drinkers reached significantly higher BrAC levels at 60, 90, and 120-min time points (all p values < 0.05) and administered more ethanol ($p = 0.045$) compared to light drinkers. In the AA/AG genotype group, there were no differences in IV-ASA measures between heavy and light drinkers.

Furthermore, heavy alcohol drinkers showed significantly higher scores for alcohol sensitivity measures, including all the measures of SRE (first five, recent, heaviest, and total), descending and ascending scores of ASQ, and social and physical pleasure expectancy scores of AEFQ. In the AA/AG genotype group, heavy drinkers showed higher SRE, ASQ, and social and physical pleasure expectancy scores of AEFQ than light drinkers. Additionally, sexual enhancement expectancies were higher in heavy drinkers than light drinkers. In the GG genotype group, heavy drinkers showed higher scores for recent, heaviest, and total scores of the SRE scale, ASQ measures and social and physical pleasure expectancy scores of AEFQ compared to light drinkers.

Conclusions: This study identified a significant effect of the CHRNA5 polymorphism (rs16969968) on increased alcohol consumption and related phenotypes in a human laboratory paradigm of alcohol consumption. A main effect of drinking pattern was observed for several subjective and sensitivity measures across CHRNA5 genotype groups. The AA/AG genotype group showed significantly higher sexual enhancement expectancies but no difference in alcohol consumption, potentially reflecting a protective effect. The GG genotype group showed significantly lower sensitivity and increased alcohol consumption in heavy drinkers.

Keywords: Alcohol, Sensitivity, CHRNA5

Disclosure: Nothing to disclose.

P687. Latent Structure of the Addictions Neuroclinical Assessment: Differences Between Individuals With and Without Alcohol Use Disorder

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Background: The Addictions Neuroclinical Assessment (ANA) is a clinical framework comprised of three neurofunctional domains that are thought to underlie substance use disorders: Executive Function (EF), Negative Emotionality (NE), and Incentive Salience (IS). The ANA battery consists of neurocognitive tasks and self-report questionnaires that provide a deep phenotypic characterization of individuals based on the three neurofunctional domains. The goal of ANA is to provide an improved understanding of the heterogeneity and etiology of substance use disorder, to develop personalized treatment for the individual, and identify potential treatment mechanisms. The present report focused on the latent structure underlying the ANA battery, and examined differences in these domains between individuals with and without alcohol use disorder (AUD).

Methods: The study comprised 298 individuals (41.3% female; mean age = 42.6 years; 68.5% with a current AUD diagnosis), representing a wide range of alcohol consumption, and who were enrolled in the NIAAA natural history protocol. Behavioral tasks and self-report questionnaires assessed the three ANA domains, EF, NE and IS. EF measures included response inhibition (Stop Signal Reaction Task), working memory (Digit Span – Backwards), inferential reasoning (Beads in a jar), task switching (Trail Making Task), mental rotation (Manikin), attention (Continuous Performance Task), metacognition (Metacognition Questionnaire), and interoception (Multidimensional Assessment of Interoceptive Awareness). NE measures were distress tolerance (Paced Visual Serial Addition Task), ostracism (Cyberball), motivation for rewards (Effort Expenditure for Rewards Task), anhedonia, (Snaith-Hamilton Pleasure Scale), resilience (Connor-Davidson Resilience Scale), social support (Inventory of Socially Supportive Behavior), affect (Positive and Negative Affect Scale), and alexithymia (Toronto Alexithymia Scale). IS was measured via approach-avoidance bias (Alcohol Approach Avoidance Task), implicit alcohol associations (Drinking Identity Implicit Association Task, and alcohol demand (Hypothetical Purchase Task). Ancillary measures were state anxiety and depression (Comprehensive Psychopathological Rating Scale), trait impulsivity (UPPS-*P* and Barratt Impulsivity Scale), and state craving (Penn Alcohol Craving Scale). Separate factor analyses were conducted within each domain to derive underlying factor structures. The interrelationships of ANA domains were modeled in a structural equation modeling framework. Group differences between individuals with and without AUD were identified by assessing structural invariance and comparing factor scores.

Results: Five factors emerged within the EF domain: Response Inhibition, Working Memory, Interoception, Rumination, and Impulsivity (CFI = 0.93, TLI = 0.92, RMSEA = 0.05). Three factors were identified in the NE domain: Negative Disposition, Positive Disposition, and Negative Actions (CFI = 0.95, TLI = 0.94, RMSEA = 0.07). Two factors were found in the IS domain: Alcohol Motivation and Alcohol Insensitivity (CFI = 0.98, TLI = 0.96, RMSEA = 0.06). We then combined the latent factors from each domain into a single model to examine cross-domain associations (CFI = 0.89, TLI = 0.87, RMSEA = 0.06). These cross-domain correlations varied from undetectable to strong (absolute r's = 0.01 – 0.86). Negative disposition and impulsivity displayed the strongest and most robust associations with other factors across domains. Compared to individuals without AUD, those with AUD displayed stronger associations between factors of the EF and IS domains, and between factors of the IS and NE domains. Individuals with AUD significantly differed from individuals without AUD on all factor scores, with the exception of response inhibition and positive disposition.

Conclusions: Factor analysis of ANA domains revealed substructures relevant to AUD. Across ANA domains, negative disposition and impulsivity showed the strongest associations to other factors, suggesting that they may play a key role in the etiology or progression of AUD. Those with AUD showed stronger associations between the EF and IS domains, and the IS and NE domains. These differences may reflect the pathology of AUD and are thereby potential targets for treatment interventions, which may lead to improvements in other dysfunctions central to AUD. Future work will focus on identifying the relevant neural networks associated with the domains, and categorizing the distinct phenotypic profiles of AUD based on the ANA domains.

Keywords: Addiction Phenotypes, Alcohol Use Disorder and Drug Addiction, Neurocognitive Functioning

Disclosure: Nothing to disclose.

P688. Cell-Type Specific Effects of Opioid Exposure in Human Brain

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Background: Opioid use disorder (OUD) remains a major public health problem as opioid dependency and overdose deaths continue to rise in the U.S. Although animal studies show substantial neurobiological abnormalities caused by long-term exposure to opioids, studies in human tissue are limited and the effects of opioids on individual brain cell types is unknown. We performed comprehensive multi-omics analysis of cell-type specific gene expression signatures in OUD and generated preliminary single nuclei gene expression data from postmortem brain to validate these signatures.

Methods: We performed whole tissue next-generation RNA sequencing (RNAseq) ($N = 27$ opioid users, 14 controls) and liquid chromatography mass spectrometry-based proteomics ($N = 20$ opioid users, 12 controls) in Brodmann area 9 (BA9), with subsequent differential expression analysis and pathway enrichment and gene ontology analysis of genes and proteins. Whole tissue gene expression was correlated with cell composition using cell type deconvolution. Further, we performed preliminary single-nuclei RNAseq (snRNAseq) in BA9 from a subset of subjects. Cell types of each sequenced nucleus were determined by cell-type specific markers. All analyses accounted for covariates including age, sex, PMI, pH, and RNA integrity.

Results: We identified 394 differentially expressed (DE) coding and long noncoding (lnc) RNAs as well as 213 DE proteins in BA9 of OUD subjects. The RNA and protein changes converged on pro-angiogenic gene networks and cytokine signaling pathways. We found cell-type specific effects in these networks with enrichment in astrocyte, endothelial, and microglia correlated genes. WGCNA identified hub genes involved in endothelial, astrocyte, microglial, and neuronal function. snRNAseq identified cell-type specific clusters that were used to validate gene expression findings obtained from whole tissue.

Conclusions: There is dysregulation of inflammatory and angiogenic gene networks in brains of opioid users, likely mediated by perturbation of endothelial, astrocyte, and microglial cells. Single nuclei RNA sequencing is feasible in our tissue samples and can be used to validate these signatures. To our knowledge, this is the first multi-omics study investigating perturbation of cell type specific networks in OUD. This work will help to further our understanding of the molecular effects of opioids in the brain.

Keywords: Opioid Abuse, Proteomics, Single-cell RNA Sequencing

Disclosure: Nothing to disclose.

P689. An Atlas of Transcriptionally Defined Cell Populations in the Rat Ventral Tegmental Area

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Background: The ventral tegmental area (VTA) is a complex brain region essential for reinforcement and reward learning, and whose dysregulation has been implicated in substance use disorders. While decades of research on VTA function have focused on the role of dopaminergic neurons, recent evidence has identified critical roles for VTA GABAergic and glutamatergic neurons in reward processing as well. Interestingly, molecular characterization has revealed that subsets of these neurons express genes involved in the transport, synthesis, and vesicular packaging of multiple neurotransmitters, providing evidence for the presence of co-release neurons. However, these studies have largely relied on low-throughput methods, and the molecular architecture of the VTA has not been comprehensively examined.

Methods: Here, we performed single nucleus RNA-sequencing (snRNA-seq) on 21,600 nuclei from male and female Sprague-Dawley rats to generate a transcriptional atlas of the rat VTA. Differential gene expression analyses and Gini coefficient calculations were leveraged to identify putative molecular markers for unique neuronal subclasses. Finally, to examine which VTA cell types exhibit preferential enrichment of GWAS risk-associated genes, we conducted gene set analysis with MAGMA (Multi-marker Analysis of GenoMic Annotation), a multiple regression model that allows for assessment of the contribution of multiple gene markers to a particular clinical phenotype.

Results: We identified 16 transcriptionally distinct cell types, including 7 dissociable neuronal populations. Further subclustering revealed several VTA populations harboring gene markers for more than one neurotransmitter system, including a cluster exhibiting high expression levels of genes involved in the synthesis and transport of GABA, glutamate, and dopamine. Between-cluster statistical comparisons identified novel gene markers for canonical and combinatorial dopamine neuron subclasses. Finally, MAGMA analysis identified pan-neuronal enrichment of gene SNPs implicated in Schizophrenia and smoking initiation, as well as enrichment of ADHD risk genes in in glutamatergic VTA neuron populations.

Conclusions: The use of snRNA-seq allowed for unbiased, comprehensive transcriptomic profiling of the VTA, and the resulting atlas is publicly available as a searchable online resource (www.day-lab.org/resources). Investigation of neuronal subpopulations confirmed the presence of combinatorial neurons, and identified novel marker genes for co-release neurons and classically-defined dopamine neurons. SNP-level gene set analyses revealed cell-type specific enrichment of GWAS risk-associated genes, highlighting the contribution of distinct cell populations to polygenic traits across several clinical disorders.

Keywords: Single-cell RNA Sequencing, Ventral Tegmental Area (VTA), Dopamine

Disclosure: Nothing to disclose.

P690. Impaired Cortical and Subcortical Responses During Infant-Oriented Face Mirroring in Postpartum Women With Opioid Use Disorder

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Background: Maternal sensitivity is critical to parenting behaviors and child outcomes, but it may be altered in mothers with the opioid use disorder (OUD). OUD incidence in pregnancy is sharply rising with >2.5% of pregnant women chronically using opioids and more than 100,000 postpartum women and their families suffering OUD every year. Despite "gold standard" buprenorphine treatment (BT) for withdrawal, mothers with OUD are at high risk for comorbid depression, polysubstance use and 30% relapse to illicit opioids, which can increase the risk of infant abuse or neglect and costly foster care utilization. BT mitigates the psychophysiological stress associated

with repeated cycles of maternal withdrawal, but little is known of how BT/OUD may affect maternal brain function, sensitive parenting behavior and child outcome. This is of concern because opioid-induced deficits have been established in preclinical rodent models. Furthermore, we know that human maternal behaviors are governed by the Maternal Behavior Neurocircuit (MBN) which is evolutionarily conserved among mammals to regulate adaptive maternal caring and aggressive behaviors. We have recently reported that human maternal brain responses in MBN and other cortical networks during own-child-oriented face mirroring in a functional magnetic resonance imaging (fMRI) task can reflect their capacity for maternal sensitivity. Here, we used this task to examine whether BT/OUD mothers' own-child-oriented face mirroring responses are impaired.

Methods: Of 22 mothers (aged 18-40) studied, 6 received BT for OUD and 16 were in a non-OUD control group (CG). They underwent a Child Face Mirroring Task (CFMT) in fMRI, at 4 months postpartum. In CFMT, the participants viewed their own child's pictures that were repeatedly presented in three different task conditions, OBSERVE, REACT, and JOIN, that were interleaved in a random order. In OBSERVE, the participants were instructed to coldly observe the child' picture without imitating the child's facial expressions or emotions. In REACT, the participants were instructed to respond to the child as they normally would. In JOIN, the participants were instructed to empathically imitate (mirroring) the child's facial expressions and emotions. Each task condition for their own child was presented in four blocks (16s/block). In each block, four pictures of the child were consecutively presented for 4 seconds per picture. Each of these four pictures exhibited one of four different emotional expressions, Joyful, Distressed, Ambiguous, and Neutral expressions, presented in a random order. fMRI data were analyzed using statistical parametric mapping software (SPM8).

Results: We examined the group differences in Join vs. Observe of the participants' own child. The supplemental motor area (SMA) showed significant activation during the Join vs. Observe of Own Child (MNI coordinate [0, 2, 58], 63 voxels, $Z = 3.53$). Furthermore, this region's response was impaired in BT/OUD mothers, as compared to CG (MNI coordinate [2, 8, 60], 230 voxels, $Z = 3.13$). Within MBN, the left pallidum had a similar deficit in BT/OUD mothers, as compared to CG (MNI coordinate [-16, -4, -6], 23 voxels, $Z = 4.21$).

Conclusions: We show preliminary effects of BT/OUD on neural responses during child-oriented mirroring. As compared to the comparison group, BT/OUD mothers showed impairments for own-child oriented face mirroring responses in brain areas that are critical to maternal sensitivity, i.e., the SMA that is important for face mirroring and the left pallidum important for maternal caregiving responses in the MBN. Further work is needed to connect these deficits in the neural responses to actual maternal sensitivity, parenting behavior, and child outcome. However, these preliminary results suggest potential mechanisms for mothers with BT/OUD. More allied research is needed on parenting interventions that can modulate the same MBN brain circuits affected by BT/OUD, reduces stress and augment maternal sensitivity to reduce transgenerational mental health risks.

Keywords: Opioid Use Disorder, Maternal Brain, Maternal Sensitivity, Emotional Empathy, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P691. Central Nervous System Monoamine Metabolite Response to Initial and Repeated Alcohol Exposure is Associated With Future Alcohol Intake in a Nonhuman Primate Model (Macaca Mulatta)

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Background: Studies show that initiation of high alcohol intake is a precursor to alcohol use disorders, and it is widely held that the central monoamine neurotransmitters modulate variation in alcohol intake, particularly early on. Nevertheless, most recent research has focused on elucidating variables that maintain addiction, such as the allostatic changes in the CNS that result from chronic alcohol intake as well as the opponent processes that reduce alcohol's rewarding effects and the role of negative reinforcement that drives craving and relapse. One recent paper notes that studies of the relationship between the central monoamines and alcohol intake are conspicuously absent from recent reviews, and when a search on Medline was performed, the authors of this study could not find a recent review paper that primarily focused on the relationship between the central monoamines and alcohol intake. Despite Cloninger's predictions concerning the role of monoamines in high intake, few studies have experimentally assessed the relationship between baseline, and alcohol-induced monoamine turnover and variation in alcohol intake in initial alcohol consumption in alcohol naïve subjects. Nor have studies assessed the role of change from baseline and subsequent exposures as predictors of variation in alcohol intake in initial alcohol consumption.

Methods: Using a nonhuman primate model, this study investigated baseline, alcohol-induced monoamine activity, and alcohol-induced monoamine change in activity following repeated exposure and their relationship to subsequent alcohol intake. Alcohol-naïve, adolescent rhesus macaques (Macaca mulatta, $N = 114$) were administered a standardized intravenous bolus of alcohol solution (16.8%, v/v) on two occasions, approximately one month apart. One month prior to and one hour following each of the alcohol infusions, cisternal CSF was obtained and assayed for monoamine metabolite concentrations. Approximately six-to-seven months later, subjects were allowed unfettered access to an aspartame-sweetened alcohol solution (8.4%, v/v) for one hour/day, 5 days/week, over five-to-seven weeks. Baseline, alcohol-induced, and monoamine change scores were used to assess the relationship between the monoamines and alcohol intake variation.

Results: Overall results from a power analyses show a statistical power of .90 for detecting medium to large effect sizes. The f2 values for the results ranged from .02-.19, indicating small to large effects. Results showed strong positive correlations between baseline and post-infusion CSF monoamine metabolite concentrations, indicating a trait-like response. Low baseline and post-infusion serotonin and dopamine metabolite concentrations and a smaller change in serotonin and dopamine metabolites from one infusion to the next, were associated with higher alcohol intake. Low baseline and post-infusion norepinephrine metabolite concentrations predicted high alcohol intake, but unlike the other monoamines, a greater change in norepinephrine metabolite concentrations from one infusion to the next was associated with higher alcohol intake.

Conclusions: There is a paucity of studies that measure central monoamine systems before and after alcohol intake, which limits the understanding of the role of monoamines in the acquisition of high alcohol intake and binge drinking as well as the acquisition of tolerance to alcohol's pharmacological effects. The findings of this study are important because they suggest that the preexisting, as well as the initial CNS monoamine response to alcohol, may be important factors in predicting and potentially moderating future alcohol intake, with the possibility that following alcohol exposure, subjects with low levels of serotonin and dopamine, and low alcohol-induced serotonin and dopamine change, consume more alcohol to compensate for their lower central monoamine functioning. These findings suggest that individual differences in

naturally-occurring and alcohol-induced monoamine activity, as well as the change between exposures, are interindividually stable, and likely important moderators of initial alcohol consumption and may play a role in the risk for subsequent excessive alcohol intake.

Keywords: Adolescent Alcohol Use, Rhesus Monkey, Norepinephrine, Dopamine, Serotonin

Disclosure: Nothing to disclose.

P692. Sex Differences in Alcohol Withdrawal Symptoms and Brain Structure in Alcohol Use Disorder

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Background: Alcohol Use Disorder (AUD) is a chronic relapsing disorder characterized by compulsive alcohol intake and the emergence of a negative emotional state and withdrawal symptoms when access to alcohol is interrupted. Women are at greater risk than men for adverse effects of alcohol, including alcohol-related diseases and alcohol- or alcohol withdrawal-induced brain damage. While a recent preclinical study found that female rats showed a stronger depressive state during alcohol abstinence than male rats, there is a need to study sex effects on withdrawal symptoms, liver function, depressive mood and sleep quality during abstinence in humans with AUD.

Methods: Here we investigated the effects of AUD and sex on sleep quality and depressive mood data, collected as part of a screening and natural history study in $n = 644$ participants with AUD (193 female) and $n = 509$ non-dependent healthy controls (264 female). A subgroup also completed brain MRI scans for brain volumetric evaluations ($n = 142$ AUD, $n = 180$ controls). In this cohort, $n = 455$ individuals with AUD (136 female) completed a 3-4 inpatient detoxification treatment program, in whom we tested sex differences on alcohol withdrawal (CIWA-Ar), need for benzodiazepines to alleviate withdrawal, liver function tests, sleep quality, and depressive mood over the course of treatment.

Results: We found that AUD participants showed worse sleep quality ($F1,1153 = 534.4$, $p < 0.0001$) and depressive mood ($F1,1153 = 543.4$, $p < 0.0001$) compared to healthy volunteers. Female AUD had worse sleep ($t642 = 2.8$, $p = 0.005$) and depressive mood ($t642 = 4.4$, $p < 0.0001$) than male AUD participants, despite equal weight-adjusted drinking quantities between them. Moreover, female AUD inpatients undergoing detoxification demonstrated stronger withdrawal symptoms ($F1,454 = 12.0$, $p = 0.0006$), higher benzodiazepine use ($F1,451 = 5.8$, $p = 0.016$), and impaired ALT ($F1,453 = 6.6$, $p = 0.011$) but not AST ($F1,454 = 0.6$, $p = 0.80$) liver function tests, despite equal weight-adjusted drinking quantities before alcohol abstinence. As expected, AUD showed smaller total GMV ($F1,322 = 19.9$, $p < 0.0001$) and larger lateral ventricles ($F1,322 = 15.0$, $p = 0.0001$) than controls (corrected for intracranial volume, age, smoking, and BMI). Within the AUD group, ALT and AST were negatively associated with GMV and ventricle size (all $p < 0.0001$), but withdrawal scores, sleep quality, or depressive mood were not associated with brain structural measures. Nevertheless, women with AUD showed larger GMV ($F1,142 = 50.5$, $p < 0.00001$) and smaller lateral ventricles ($F1,142 = 4.3$, $p = 0.040$) than AUD men (corrected for intracranial volume, age, smoking, and BMI); suggesting less sensitivity to the deleterious effects of alcohol on brain structure compared to men.

Conclusions: In sum, these findings suggest that alcohol has more severe effects on withdrawal, liver function, sleep quality and depressive mood, but not on brain structural measures, in women than in men. They also suggest that even without cirrhosis, liver dysfunction in AUD might contribute to brain structural changes.

Keywords: Alcohol, Alcohol Withdrawal, Sex Difference

Disclosure: Nothing to disclose.

P693. Attentional Functioning in Opioid, Cocaine, and Cannabis Use Disorders: A Head-To-Head Comparison

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Background: Disordered use of several drugs has been linked to decrements in executive function (EF) such as motoric or "rapid-response" impulsivity, especially stimulant use disorders. This decrement in self-control is thought to either reflect a core EF deficit in earlier neurodevelopment that promoted vulnerability to transition from recreational to compulsive substance use or may be a consequence of chronic neurotoxic effects of drugs. However, many metrics of motoric impulsiveness, such as raw commission errors, are at least partially dependent on core attentional capacity, which is seldom probed.

Methods: As a component of piloting the NIDA Phenotypic Assessment Battery (PhAB) as a potential standard assessment for clinical trials, men and women with each of opiate use disorder (OUD; $n = 85$), cannabis use disorder (CaUD, $n = 48$) and cocaine use disorder (CoUD; $n = 35$), as well as neurotypical controls ($n = 82$) completed the Attentional Network Task (ANT) version of the flanker task, as well as a stop-signal task (SST).

Results: Orienting capacity (leveraging spatial information on where the next target would appear to enable shorter reaction time (RT)) decreased with age, whereas the conflict (flanker) effect of incongruent arrows to increase RT worsened with age. Controlling for age, participants with OUD ($p < .05$) and CaUD ($p < .01$) each showed deficient alerting capacity compared to controls, wherein these groups did not leverage advance signaling (temporal cueing) of an imminent flanker target to enable shorter reaction times (RT) compared to controls. In addition, CaUD participants showed a trend ($p < .10$) toward an increased vulnerability to the conflict (flanker) effect to prolong RT compared to controls. Relatedly, when performing the SST, OUD and CaUD participants, but not CoUD participants showed slowed RT to target stimuli compared to controls ($p < .05$), but with no differences from controls in stop-signal reaction time itself in any SUD.

Conclusions: These data suggest that among SUD, CaUD and OUD may be uniquely characterized by attentional decrements in the absence of motoric impulsivity.

Keywords: Impulsivity, Attention, Cocaine and Opioid Use Disorders, Cannabis Use Disorder

Disclosure: Nothing to disclose.

P694. A Reduced Complexity Cross Between BALB/c Substrains Identifies Zhx2 as a Candidate Gene Underlying Brain Concentration of the Oxycodone Metabolite Oxymorphone and State-Dependent Oxycodone Reward

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Background: Understanding the pharmacogenetics of opioid pharmacokinetics is vital to therapeutic success, as genetic

mutations can drastically alter therapeutic efficacy and addiction liability of opioids. Oxycodone (OXY) is a semisynthetic opioid (active compound in Oxycontin®) and is metabolized in humans by CYP2D6 to a more highly potent mu opioid receptor agonist oxymorphone (OMOR) and by CYP3A4 to inactive noroxycodone (NOR). OMOR is then metabolized in humans by UGT2B7 to inactive oxymorphone-3-glucuronide and by CYP3A4/CYP2D6 to bioactive noroxymorphone. Because OMOR is a highly potent mu opioid receptor agonist and is both analgesic and addictive, understanding regulation of brain OMOR brain concentration ([OMOR]) is critical to understanding neurobehavioral effects of OXY. We observed robust differences in brain [OMOR] between BALB/cJ and BALB/cByJ substrains, with BALB/cJ (J) females showing higher brain [OMOR] at 30 min post-OXY (1.25 mg/kg, i.p.) compared to BALB/cByJ (ByJ) mice. Behaviorally, we also observed enhanced state-dependent OXY conditioned place preference (CPP) and locomotor stimulation in J females vs. ByJ mice, suggesting that increased brain [OMOR] could underlie the increase in OXY-induced behaviors. Because BALB/c substrains are nearly genetically identical with only 8K variants distinguishing them, an F2 reduced complexity cross could greatly facilitate the identification of causal genetic factor(s) underlying substrain variance in brain [OMOR] and behavior. The goal of this study was to use quantitative trait locus (QTL) mapping of brain [OMOR] brain and gene expression (eQTL) in BALB/c substrains to identify causal candidate genetic factors underlying increased brain [OMOR] and OXY behaviors.

Methods: We conducted quantitative trait locus (QTL) mapping of brain [OMOR] and behavior in 133 BALB/cJ x BALB/cByJ F2 reduced complexity cross (68F, 65M). BALB/c substrains and F2 mice were trained and assayed for state-dependent CPP over 9 days in a 2-chamber apparatus. Mice were assessed for initial preference on Day 1, trained for Days 2-5 (1.25 mk/kg OXY i.p. or volume matched saline), tested for drug-free CPP on Day 8, and state-dependent CPP on Day 9. For OXY/metabolite quantification, naïve parental substrains and F2 mice previously serving as controls for the CPP study were administered a single injection of OXY (1.25 mg/kg, i.p.) and whole brains were collected and flash frozen 30 min later. Samples were homogenized and brain [OXY/metabolite] were measured via combined liquid chromatography-electrospray ionization-tandem mass spectrometry. QTL mapping was conducted in R/qtl and striatal/hippocampal eQTL analysis in MatrixEQTL. Differential mRNA expression analysis of liver between BALB/c substrains was conducted with the scruff package. Whole brain proteomics was conducted in parental substrains using flash-frozen whole brains collected immediately after state-dependent CPP and LC-MS and MaxQuant for peptide identification and quantification.

Results: There was a greater state-dependent CPP ($F(1,156) = 18.8$, $p = 0.014$) and brain [OXY] ($t(14) = 2.55$, $p = 0.023$), [NOR] [$t(14) = -1.917$, $p = 0.076$], and [OMOR] [$t(14) = 2.06$, $p = 0.058$] in J females vs. ByJ mice. QTL analysis identified a single, genome-wide significant QTL on chromosome 15 underlying increased brain [OMOR] with the J allele (LOD = 6.53, $p = 0.001$; Bayes Interval: 32-94 Mb; 29% of the variance explained) that was driven by females. Both striatal and hippocampal cis-eQTL analysis identified Zhx2 as a highly significant (AdjP = 7.34E-09) and highly promising candidate gene located within the middle of the QTL interval (58 Mb) for brain [OMOR]. Zhx2 codes for a transcriptional repressor that is highly expressed in the brain and liver, and J mice harbor a 6.2 Kb mouse endogenous retrovirus (MERV) within intron 1 of Zhx2 that dramatically reduces Zhx2 transcript and ZHX2 protein levels, leading to female-specific dysregulation of pharmacokinetic genes (e.g., CYPs) within the liver. Alignment of whole genome sequence short-reads confirmed the presence of the Zhx2 MERV in J and the absence in ByJ. Whole brain mass spectroscopy proteomics in the BALB/c substrains corroborated decreased brain ZHX2 (AdjP = 2.8E-09), along with increased CYP2D11 protein (CYP2D6 homolog) in J mice (AdjP = 6.6E-03). Liver transcriptomics also corroborated decreased Zhx2 expression (AdjP = 1.54E-10) in J mice, along with a plethora of differentially expressed pharmacokinetic genes, including genes coding for CYP and UGT enzymes that could underlie the quantitative trait mechanism linking the Zhx2 MERV to increased brain [OMOR] and OXY-induced behaviors.

Conclusions: Systems genetic analysis of brain OXY [metabolite] and behavior in a reduced complexity cross between BALB/c substrains combined with a multi-pronged -omics approach identified the private Zhx2 MERV in the J substrain as a highly promising candidate quantitative trait variant underlying increased brain [OMOR] and OXY-induced state-dependent reward in J versus ByJ. We hypothesize that decreased Zhx2 expression leads to altered liver expression of one or more key pharmacokinetic enzymes that regulate liver and brain [OMOR] (e.g., CYPs and/or UGTs). Future studies will use CRISPR/Cas9 gene editing to validate the Zhx2 MERV as the quantitative trait variant and determine the quantitative trait mechanism linking the Zhx2 MERV with brain [OMOR] and OXY behaviors.

Keywords: Pharmacogenetics, Oxycodone, Opioid Use Disorder, GWAS, Drug Metabolism

Disclosure: Nothing to disclose.

P695. Polygenic Contributions to Risk Taking as Measured by the Balloon Analogue Risk Task

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Background: Risky decision-making, a core feature of several psychiatric disorders, has a complex but largely unknown genetic architecture.

Methods: We examined risk taking in two independent samples of primarily healthy adults. One sample ($n = 1138$) included psychiatric patients (53 schizophrenia, 42 bipolar disorder, 47 ADHD); the other ($n = 911$) excluded participants reporting recent treatment or medication for various psychiatric disorders but did not screen for ADHD. Mean adjusted pumps, the index of risk taking, was recorded during performance of the Balloon Analogue Risk Task. DNA was obtained to perform GWAS analyses in both samples. Polygenic risk scores (PRS) were derived in each dataset and then tested against the other sample. In addition, a PRS was constructed in the combined sample, using genome-wide MEGA-analysis, in order to test genetic correlation between BART performance and risk-taking self-report in the UK Biobank sample and psychiatric phenotypes characterized by impulsivity and risk-taking (ADHD, Bipolar Disorder, Alcohol Use Disorder, Cannabis Use Disorder) in the Psychiatric Genomics Consortium.

Results: The PRS for BART performance derived in the larger discovery dataset predicted task performance in the replication sample ($r = 0.13$, $p = 0.000012$, pFDR = 0.000052), as did the reciprocal analysis ($r = 0.09$, $p = 0.0083$, pFDR = 0.04) using the smaller dataset for discovery. Exclusion of subjects with psychiatric diagnoses produced significant results as well. A single genome-wide significant association emerged from the MEGA-GWAS, implicating a gene with strong face validity, IGSF21. IGF21 is known to function at inhibitory brain synapses. Common single nucleotide polymorphisms captured 27% of the variance in task performance. A PRS for Cannabis Use Disorder ($p = 0.00047$, pFDR = 0.0053) predicted risky decision-making in our sample. The other disorders tested and self-reported risk taking were not significantly associated with BART performance.

Conclusions: The findings indicate heritability of risky decision-making with shared vulnerability to Cannabis Use Disorder.

Keywords: Polygenetic Risk Score, Risk-Taking, Cannabis Use Disorder, Balloon Analogue Risk Task

Disclosure: Myriad Genetics: Advisory Board (Self, Tourette Association of America, Advisory Board, Self

P696. Consequences of Childhood Trauma and Substance Use Disorder Histories on Endocannabinoid Function in Adulthood

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Background: Exposure to trauma in childhood or adolescence is associated with an increased susceptibility to the development of a substance use disorder (SUD) in adulthood. One potential contributing factor is disruption of the endocannabinoid (eCB) system, a neuromodulatory system involved in stress and emotion processing. The eCB system undergoes extensive reorganization during adolescence and perturbations of this process, such as exposure to trauma, can last into adulthood. Thus, childhood trauma may impact eCB system function, influencing stress and emotion processing and potentially contributing to SUD development later in life.

Methods: Using a quasi-prospective approach, we have recently shown that individuals with documented childhood trauma are more likely to develop an SUD, even when controlling for potential sources of bias, such as recall bias or familial confounding (Capusan et al. 2021 Molecular Psychiatry). To explore the potential contribution of eCB function in this relationship, we recruited individuals ($N = 100$) with or without documented histories of childhood trauma and/or a substance use disorder. All participants provided blood samples at baseline and following exposure to a laboratory stressor for analysis of endocannabinoids and cortisol. Emotion processing was assessed using facial electromyography before and have stress exposure.

Results: Individuals exposed to trauma (trauma only) had higher baseline levels of the eCB ligand anandamide (AEA; $p = 0.003$), which remained elevated even after stress exposure ($p = 0.005$). This effect was absent in trauma-exposed individuals who went on to develop an SUD. There were no differences in cortisol reactivity to stress ($p = 0.60$), though the SUD groups showed blunted autonomic stress reactivity ($p = 0.031$). The trauma only group had lower self-reported ratings of depression than both the SUD only and trauma + SUD groups ($p = 0.01$).

Conclusions: For individuals exposed to childhood trauma, elevated AEA may serve as a protective factor, mitigating the negative consequences of stress exposure. This data supports the notion that pharmacologically enhancing AEA may serve as a novel therapeutic target for the treatment of trauma-related disorders, in support of ongoing clinical trials targeting this mechanism.

Keywords: Endocannabinoid, Stress and Trauma, Substance Use Disorders

Disclosure: Nothing to disclose.

P697. Input Specific Enhancements in NAc Excitatory Transmission Following Junk-Food Diet Consumption and Mechanisms of CP-AMPAR Insertion

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Background: Activity in the nucleus accumbens (NAc) contributes to food and drug 'cravings' in response to cues paired with these reinforcers. These cue-triggered urges contribute to drug addiction and obesity. Blockade of calcium-permeable AMPA receptors (CP-AMPAR) in the NAc prevents the expression of cue-triggered food-seeking and the 'incubation' of cocaine craving. In addition, NAc CP-AMPAR expression and transmissions are increased following withdrawal from extended access cocaine self-administration, and after consumption of a sugary, fatty junk-food diet. These effects persist for over one month after cessation of cocaine use or junk-food consumption. However, what initiates and maintains the long-term up-regulation of NAc CP-AMPARs is poorly understood. In addition, the degree to which junk-food diet promotes their synaptic incorporation in specific inputs to the NAc or cell types is unknown.

Methods: Male rats (10-12 per group) were given free access to junk-food or chow, and whole-cell patch clamp recordings were made using electrical stimulation or optogenetic stimulation of either medial prefrontal cortex (mPFC) or basolateral amygdala (BLA) inputs to the NAc (pAAV-CamKII-Chronos-GFP). Recordings from D1- and D2-type MSNs in the NAc were made from D1-Cre+ and A2a-Cre+ rats. Cre+ cells were visualized by viral transduction of tdTomato (pAAV-FLEX-tdTomato; Addgene 28306-AAV1). The contribution of CP-AMPARs to AMPAR transmission was determined using the CP-AMPAR selective antagonist naspm (200 μM). Pharmacological or DREADD-mediated blockade of excitatory transmission was used to test the hypothesis that reductions in glutamate transmission promote CP-AMPAR transmission. Data are analyzed using two-way and one-way ANOVAs followed by Sidak's post-tests or unpaired *t*-tests as appropriate.

Results: We found that junk-food increases CP-AMPAR transmission in mPFC-to-NAc but not BLA-to-NAc inputs after brief exposure (10 days; two-tailed unpaired *t*-test based on a priori predictions: $t11 = 2.4$, $p < 0.05$). Initial results suggest that increases in CP-AMPAR transmission are found in D2-type MSNs (studies of D1-MSNs are ongoing). In addition, blockade of excitatory transmission facilitated the recruitment of CP-AMPARs within the NAc.

Conclusions: Both mPFC and BLA influence cue-triggered food-seeking via interactions with the NAc, but mPFC is more tightly linked to behavioral flexibility and goal-directed behavior while BLA is linked to affective motivation. Thus, preferential effects of junk-food on mPFC-inputs suggest potential reductions in behavioral flexibility. In addition, high-fat diet al.ters perineuronal nets and spine density in the mPFC, suggesting that changes in NAc transmission may be downstream of these effects. Finally, initial results suggest that CP-AMPAR increases may result from a reduction in glutamate activity. This effect was not dependent upon prior junk-food diet exposure.

Keywords: Incentive Motivation, Glutamatergic Synapses, Addiction

Disclosure: Boehringer-Ingelheim: Consultant (Self)

P698. Transcriptional Profiling of the Nucleus of the Solitary Tract After Alcohol Dependence and Stress

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Background: Stress exposure contributes to the development of drug and alcohol addiction. Animal models show that stress exacerbates escalations in alcohol consumption in alcohol-dependent animals. The nucleus of the solitary tract (NTS) is a critical brainstem region for integrating and relaying peripheral signals to regulate stress responses.

Methods: To examine the molecular adaptions within this brain region that may contribute to stress-induced alcohol drinking, we

exposed animals to chronic intermittent bouts of ethanol vapor (CIE), forced swim stress (FSS), or both (CIE + FSS) and then transcriptionally profiled the NTS at three different timepoint after the last vapor exposure (0-h, 72-h, and 186-h).

Results: Using the three different timepoints, we were able to identify transient, persistent, and compensatory gene expression patterns related to each of the behavioral treatments. We identified interferon signaling as a critical gene network correlated with alcohol consumption levels. Using a likelihood ratio test, we identified genes that were differentially expressed across time and between groups. Clustering analysis of these genes to identify unique expression patterns identified a subset of genes that fail to normalize in the CIE + FSS group, but not the others. These genes were enriched for cell-to-cell interaction and cellular movement pointing to long-term structural and functional changes in this brain region caused be the unique interaction of alcohol dependence and stress. Specific genes of interest identified in this group include Aqp4, Il16, Reln, Grm4, Gabrd, and Gabra6. We also compared gene expression changes in the NTS to the PFC and found a significant overlap of differentially expressed genes between the two brain regions. Overlapping genes in the CIE + FSS group were enriched for type I interferon signaling.

Conclusions: Overall, these results summarize the transcriptomic changes across time in the NTS that may be critical to the development of stress-induced increases in alcohol consumption and alcohol dependence.

Keywords: Brainstem, Nucleus Tractus Solitarii, Acute Stress, Alcohol Dependence, Transcriptome

Disclosure: Nothing to disclose.

P699. Dopaminergic Modulation of Brain Functional Connectivity Using Simultaneous PET/Pharmacological MRI

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Background: The speed at which drugs enter the brain affects their rewarding effects, the faster the entry the stronger the reward, which is why intravenous (iv) injection and smoking are the most addictive routes of drug administration. However, the rate of DA increases and brain connectivity changes associated with the speed of drug entry into the human brain have not been investigated. To address this neglect, we compared DA changes and resting-state functional connectivity (FC) patterns associated with iv versus oral administration of methylphenidate (MP) using a double-blind, counterbalanced, placebo-controlled study in healthy controls tested on three different days. We hypothesized that the speed and the amount of DA release in the striatum would be higher for iv- than for oral-MP, and that the speed of DA increase would be associated with FC changes and subjective reward from MP.

Methods: Fifteen healthy participants (36.7 ± 9.5 years old; 7 females) underwent simultaneous PET/fMRI in the resting state for 90 minutes to assess dynamic DA increase, FC and reward (feeling of 'high') at 1-min temporal resolution using MP as challenge in 3 randomly ordered sessions. In each session participants were given an oral pill (60mg-MP or placebo) 30 minutes prior the bolus injection (11C-raclopride; 16 mCi) and an i.v. injection (0.25mg/kg-MP or placebo) 30 minutes after the bolus. Dynamic DA increase was estimated from time-varying differences in standardized uptake value ratio (SUVr) to cerebellum between placebo and MP conditions (oral/iv). The fMRI timeseries (3mm isotropic, TR = 3 seconds, 1800 time points) were realigned, distortion corrected, and spatially normalized to MNI space using the image preprocessing pipelines of the human connectome project and underwent standard motion regression and 0.01-0.10Hz band-pass filtering. Cardiac, respiratory, and head motion artifacts were removed. Whole-brain dynamic FC was estimate for each fMRI session using a sliding window approach in an edgewise fashion (long-range FC matrices) and in a voxelwise fashion (short-range lFCD). Within-subjects ANOVA was used to test for main effects of DA increase and MP condition (iv vs oral), as well as for DA-by-MP interaction effects on edgewise and voxelwise statistical analyses, while controlling for multiple comparisons with a stringent familywise error approach (p-FWE < 0.05).

Results: The 'high' ratings maxima were stronger for oral- and iv-MP than for placebo ($p < 0.001$). The increase in striatal DA with time was smoother and weaker for oral- than iv-MP ($p < 0.03$, within-subjects ANOVA, one-tailed), in which DA sharply increased after iv-MP, paralleling the participants' ratings of 'high' in response to MP. Dynamic DA increase was more sensitive than the traditional static measure of DA release to detect differences between drug conditions. Perceived reward ('high' ratings maxima) and DA increase in putamen at the time of iv-injection were correlated during oral- ($R = 0.7$; $p = 0.004$) but not during iv-MP. The speed of DA changes in putamen during the first 20 minutes after iv injection was higher for iv- than for oral-MP ($p < 0.01$, two tailed t-test). The speed of DA changes in other striatal regions was not significant. Similarly, the decrease in average FC degree from baseline across subjects after iv injection was faster and stronger for iv- than for oral-MP ($p < 2E-16$), paralleling the observed faster DA increase after iv-MP. A MP-sensitive network showing significant negative correlation in 125 edges between FC strength and DA-release in putamen emerged from the network analysis of the long-range matrices. Predominantly, iv-MP reduced subcortical-cortical (53 edges) and motor (49 edges), visual (25 edges), and saliency (22 edges) network connectivity across subjects compared to placebo (p-FWE < 0.05; Bonferroni corrections for 35778 edges). Though FC decreases were larger for iv- than for oral-MP, the association between DA increases and mean MP-network connectivity was stronger for oral- than iv-MP ($p < 2E-08$), such that for a given DA increase the reduction in FC was larger for oral- than iv-MP, suggesting a greater contribution from additional neurotransmitters for iv-MP (i.e., noradrenergic stimulation). The speed of FC changes during the first 20 min after iv injection did not differ for oral- and iv-MP. Time-varying increases in striatal DA were also associated with time-varying lFCD decreases in the default mode network (DMN), motor, parietal, temporal and occipital cortices and with lFCD increases in orbitofrontal cortex and cerebellum for iv-MP but less so for oral-MP (p-FWE < 0.05).

Conclusions: Using simultaneous PET-MRI, we demonstrate for the first time in the human brain that DA-release is faster for iv-MP than for oral-MP. Striatal DA increase was concomitant with increases in self-report of "high" and with decreases in FC of the subcortical and motor networks, which are modulated by DA. The decrease in FC with MP is likely to reflect predominant DA stimulation of D2 receptors, which are inhibitory but are also likely to be influenced by MP's noradrenergic effects. Overall, findings support our working hypotheses that DA increase would be faster for iv- than for oral-MP and that dynamic changes in striatal DA are associated with dynamic changes in FC.

Keywords: Reward, Dopamine (D2, D3) Receptors, Resting State Functional Connectivity, Simultaneous PET-MR, Methylphenidate

Disclosure: Nothing to disclose.

P700. Perceived Stress During the COVID-19 Pandemic: Effects of Childhood Trauma and Associations With Pandemic Alcohol Use

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Background: The negative impact of the COVID-19 pandemic on stress and mental health both locally and globally has been well publicized in the media, while more scientific data on the specific effects of pandemic-related stressors (e.g., social isolation, economic insecurity, health concerns) are still emerging. Perceived stress, or the degree to which life events are appraised as stressful by an individual, is an important measure to consider in the context of the pandemic as it encompasses emotional and coping responses to life's challenges. Individual differences in stress perception and coping are well documented in the literature and are influenced by a variety of factors, including childhood trauma, personality, and history of AUD. The current study had three objectives: 1) to investigate changes in perceived stress levels due to the pandemic, assessing the role of pandemic wave and alcohol use disorder (AUD) status; 2) to evaluate whether childhood trauma exposure is associated with increased perceived stress during the pandemic; 3) to investigate the association of perceived stress with alcohol use during the pandemic, and the moderating of role of AUD status on this association.

Methods: From June 2020 to March 2021, a sample of participants previously enrolled in NIAAA clinical research studies were contacted by phone and invited to participate in a longitudinal survey study to assess the effect of the pandemic on alcohol use and related outcomes. Following consent, participants completed a survey to obtain pre-pandemic baseline ("In the month before the pandemic [February 2020]….") and initial impact data ("In the last month…."). Due to rolling enrollment, initial impact data were collected during different "waves" of the pandemic across participants: Wave 1: 3/11/2020-7/31/2020; Wave 2: 8/1/2020-11/22/2020; Wave 3: 11/23/2020 onward. The survey included, among other assessments, the Perceived Stress Scale (PSS) and the Alcohol Use Disorder Identification Test (AUDIT). Screening data from when participants initially enrolled in NIAAA clinical research studies, including the PSS, the Childhood Trauma Questionnaire (CTQ) and the NEO Five Factor Personality Inventory, were also included in the current analysis. Participants in the current study ($n = 390$; 183 females, 207 males) represented a wide spectrum of alcohol use, from non-drinkers to those with severe AUD (219 non-AUD, 171 AUD). Perceived stress scores were compared between pre-pandemic and pandemic timepoints using linear mixed models. Associations between childhood trauma (measured by the five CTQ subscales of emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect), pre-pandemic and pandemic perceived stress levels, and alcohol use during the pandemic were analyzed using path analysis. A multigroup path model was utilized to evaluate the moderating effects of AUD status on these associations.

Results: Overall, perceived stress levels were significantly elevated during the pandemic compared to pre-pandemic ($p < 0.0001$). There was no interaction effect with pandemic wave, indicating that perceived stress levels were comparable across the three waves. There was a significant interaction between pandemic timepoint and AUD status ($p = 0.03$), such that perceived stress levels significantly increased in non-AUD participants but not in AUD participants; however, stress levels were higher in the AUD vs. non-AUD participants at both timepoints. These effects remained significant when controlling for perceived stress level, CTQ score, and the NEO neuroticism factor measured at initial screening. Path analysis indicated that emotional abuse, sexual abuse, and emotional neglect were all positively associated with perceived stress levels during the pandemic. Sexual abuse had both a direct effect on perceived stress, and an indirect effect that was mediated by neuroticism and pre-pandemic stress levels; effects of emotional abuse and neglect were all indirect. Alcohol use during the pandemic (AUDIT-C score) was associated with both pandemic perceived stress and pre-pandemic alcohol use, with the latter exhibiting a stronger effect. Analysis of a multigroup path model showed that the direct effect of sexual abuse on pandemic perceived stress was only observed in AUD participants, and that there was a stronger association between pandemic stress and alcohol use in AUD participants.

Conclusions: Perceived stress levels were elevated during the pandemic among participants across all wave timepoints in which they were initially evaluated, although our results suggest this increase was only true for individuals without AUD. Exposure to childhood trauma was both directly and indirectly associated with increased perceived stress during the pandemic through neuroticism and pre-pandemic stress levels. These findings add to existing evidence that early life stress exposure may sensitize individuals to heightened stress responses later in life. Lastly, alcohol use during the pandemic, while primarily influenced by pre-pandemic consumption levels, was also associated with pandemic perceived stress, particularly in those with AUD. A better understanding of the trajectory and impact of the COVID-19 pandemic on alcohol and related outcomes may help develop interventions that benefit individuals vulnerable to pandemic-related negative outcomes.

Keywords: COVID-19, Early Life Stress, Perceived Stress, Alcohol

Disclosure: Nothing to disclose.

P701. Identification of Individuals With Resistant, Mild, Moderate, and Severe Opioid Addiction-Like Behaviors in 400 + Heterogeneous Stock Rats

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Background: A key question for opioid addiction research remains why casual drug consumption escalates to problematic use associated with high motivation and compulsivity typical of substance use disorder in some individuals but not others. A better characterization of individual differences in the propensity to develop addiction-like behaviors could help identify new pharmacological targets, biomarkers, gene variants, and facilitate medication development. However, most studies have used small sample sizes ($N < 8$-20), limited access self-administration paradigms, or used animal models with limited individual differences. Such limitations reduce the translational relevance of the results and limit the reproducibility and replicability of these studies. To address this issue, we characterized addiction-like behaviors using an advanced model of extended access to oxycodone self-administration in large cohorts of heterogeneous stock (HS) rats ($N = XXX$), a unique outbred strain of rats with large individual differences.

Methods: HS rats were allowed to self-administer oxycodone ~11 weeks under various duration (2-12h/day and were also

screened for oxycodone-induced analgesia (tail flick), compulsive oxycodone use using progressive-ratio (PR) responding, and withdrawal-induced hyperalgesia/allodynia-like behavior (von Frey test). Every animal was also tested for their sensitivity to methadone (0.5 mg/kg), naltrexone (3 mg/kg), and buprenorphine (0.5 mg/kg). Large cohorts ($n = 46$-60) were used to minimize cohort-specific effects, and the level of responding was normalized within cohorts. *T*-tests and ANOVA were performed to determine significant effects. With this many animals, small effect sizes (cohen $d = 0.2$) can be detected with high power (1-b = 0.95, a = 0.05).

Results: Behavioral characterization showed large inter-individual differences in addiction-like behaviors, with small intra-individual differences. Final oxycodone intake showed a positively skewed distribution. Small sex differences were observed, with females having a higher responding under fixed ratio (FR) than males ($p < 0.01$), but not under progressive ratio ($p < 0.001$). Both male and female rats exhibited opioid-induced analgesia and withdrawal-induced hyperalgesia/allodynia. There were considerably larger differences between individuals than between sex, with individuals showing resistance, middle, moderate, and severe addiction-like behaviors. For instance, ~25% of animals showed a resistant phenotype with a low escalation of oxycodone intake, low opioid-induced analgesia, a lack of tolerance to the analgesic effect of oxycodone, and low withdrawal-induced hyperalgesia/allodynia (all $p < 0.01$). We found that 65 % of rats decreased their motivation to take oxycodone with at least one treatment ($p < 0.05$). There were substantial individual differences in response to each treatment, with individuals being sensitive to one, two, or all three medications. Buprenorphine and naltrexone were associated with the highest number of responsive animals, and around 8% of individuals were responsive to all three medications.

Conclusions: This is, to our knowledge, the most extensive intravenous oxycodone self-administration study in rats. We identified individuals with resistant, mild, moderate, and severe opioid addiction-like behaviors in 400+ heterogeneous stock rats. Low sensitivity to the analgesic effect of oxycodone and lack of tolerance was a strong predictor of the resistance to the development of addiction-like behaviors, while withdrawal-induced hyperalgesia was only observed in animals with moderate to severe addiction-like behaviors. Pharmacological testing demonstrates individual differences in response to medications to treat opioid use disorder in a genetically diverse population of rats. A wide variety of biological samples were collected longitudinally and are made available to the community through the Oxycodone Biobank to facilitate the identification of biomarkers of addiction-like behavior and facilitate reproducibility and replicability efforts.

Keywords: Addiction, Opioids, Self-Administration, MAT, Pain

Disclosure: Nothing to disclose.

P702. Adolescent Voluntary Alcohol Drinking Impairs Response Inhibition and Alters Cortical-Striatal Dynamics in Adult Male and Female Rats

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Background: Background: Development of alcohol use disorder (AUD) is strongly associated with initiation of drinking during adolescence. A better understanding of the impact of alcohol exposure on the adolescent brain in the context of motivated behavior is fundamental for understanding the etiology and pathophysiology of AUD. Deficits in response inhibition are a critical feature of AUD and are a clinically relevant endophenotype of the disorder. However, little is known about the functional and mechanistic consequences of adolescent alcohol drinking on response inhibition and related cognitive measures in adulthood.

Methods: We used a moderate voluntary adolescent drinking model combined with a recently developed Cued Response Inhibition Task (CRIT) to assess response inhibition, stimulus-response relationships, attentional processes and learning in male and female adolescent or adult rats. This method was combined with single unit and local field potential activity in two brain regions implicated in response inhibition, the orbitofrontal cortex (OFC) and dorsal striatum (DS). Dependent variables for the behavioral analyses included number of correct and premature pokes, number of pokes and trough entries during the inter-trial interval, latency to make a premature poke and ratio of correct over incorrect responses (response inhibition ratio). We performed analysis of variance (ANOVA) for all dependent variables with factors sex, reward and age. Unit firing rate was assessed during the following epochs of interest: correct responses, premature responses, reward delivery and inhibitory tone presentation. All firing rate data was analyzed using ANOVA testing. To determine how individual neurons in a network influence the firing rate of other neurons within the same network, dependent on behavior or event, we computed spike correlation and assessed group differences with ANOVA testing. To investigate neural synchrony between two brain regions, the phase locking index γ was computed and group differences were next assessed using ANOVA testing.

Results: We find adult rats who voluntarily drank ethanol during adolescence made more (irrelevant) nose pokes during the inter-trial-interval, were quicker to make premature responses, and exhibited reduced response inhibition, relative to sucrose exposed controls. Our analysis of recording data revealed that ethanol exposed rats have an enhanced response to reward in the OFC and an augmented response in the DS during premature responding.

Conclusions: Because dysfunction of cortico-striatal circuits is a critical feature of AUD, these translational results will enhance our mechanistic understanding of brain changes that occur in these circuits as a consequence of adolescent ethanol exposure.

Keywords: Adolescent Alcohol Use, Electrophysiology, Orbito-frontal Cortex (OFC), Dorsal Striatum, Response Inhibition

Disclosure: Nothing to disclose.

P703. Parsing Genetically Influenced Risk Pathways: Genetic Loci Impact Problematic Alcohol Use via Externalizing and Specific Risk

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Background: Characterizing whether genetic variants for psychiatric outcomes operate via specific versus general pathways provides more informative measures of genetic risk, and, potentially, allows us to design more targeted prevention and interventions. We employ multivariate methods to tease apart variants associated with problematic alcohol use through either general or specific pathways and compare results to standard univariate genetic analysis of problematic alcohol use.

Methods: We compared results from a univariate genome wide association study (GWAS) of problematic alcohol use to those from a previous multivariate GWAS of externalizing phenotypes. We identified genetic variants associated with problematic alcohol use

through a broad liability to externalizing, and those that remain after removing shared variance with externalizing. We compared these results across SNP overlap, bioannotations, genetic correlations, and polygenic scores. We included GWAS summary statistics from existing GWAS, and two US based hold out samples: The National Longitudinal Study of Adolescent to Adult Health (Add Health) and the Collaborative Study on the Genetics of Alcoholism (COGA).Outcomes included problematic alcohol use (ALCP-O), shared risk for externalizing (EXT), and problematic alcohol use-specific risk (ALCP-S) for the GWASs; a preregistered list of 99 available phenotypes for genetic correlations; and substance use, substance use disorder criteria, and alcohol misuse in the polygenic score analyses.

Results: The analysis differentiated SNPs operating through common versus specific risk pathways. While ALCP-O was associated with multiple phenotypes, ALCP-S was predominantly associated with alcohol use and other forms of psychopathology. Polygenic scores for ALCP-O were associated with a variety of other forms of substance use and substance use disorders, polygenic scores for ALCP-S were only associated with alcohol phenotypes. Polygenic scores for both ALCP-S and EXT show differential patterns of associations with alcohol misuse across development.

Conclusions: Focusing on the differential impacts of shared and specific risk can better characterize pathways of risk for alcohol use disorders. Multivariate methods can be a useful tool for studying many psychiatric conditions. Parsing risk pathways will become increasingly relevant as genetic information is incorporated into clinical practice for psychiatric outcomes.

Keywords: Alcohol and Substance Use Disorders, Psychiatric Comorbidity, Multivariate Analysis

Disclosure: Nothing to disclose.

P704. Sex- and Dose-Dependent Differences in Preference for Ethanol in Preadolescent and Adolescent Rats

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Background: Ethanol is the drug of choice among adolescents in the United States. Adolescence is a pivotal period in brain development, making young people specifically at risk for a variety of different consequences as a result of ethanol use. Previous literature using the conditioned place preference (CPP) paradigm, a validated animal model of drug reward, has revealed the difficulty of reliably demonstrating ethanol preference in adolescent rats, potentially due to sex and dose-related differences. Therefore, we hypothesized that male and female adolescent rats would exhibit dose-dependent differences in ethanol preference.

Methods: Rats underwent a 10-day CPP procedure to assess ethanol preference during preadolescence (experiment 1) and adolescence (experiments 2 and 3). On day 1, preconditioning, an initial preference for a two-chamber CPP apparatus was assessed during 15 min (experiments 1 and 2) or 20 min (experiment 3) sessions. On days 2-9, conditioning, rats were conditioned with saline in their initially preferred chamber or ethanol in their initially non-preferred chamber on alternating days for 15 min. On day 10, postconditioning, the preference for the ethanol-paired chamber was assessed using identical procedures to the first day of the experiment. In experiments 1 and 2, rats were randomly assigned to receive an ethanol injection (0.0, 0.5, 1.0, or 2.0 g/kg, IP, $n = 7$-11) before being placed in the ethanol-paired chamber. In experiment 3, rats were randomly assigned to receive an ethanol injection (0.0, 0.0156, 0.0313, 0.0625, 0.125, 0.5, or 2.0 g/kg, IP, $n = 8$-11) before being placed in the ethanol-paired chamber. A preference score was computed by taking the time spent in the ethanol paired side during postconditioning minus the time spent in the ethanol paired side during preconditioning. CPP was defined as having a significantly higher preference score compared to the saline control group (between-group comparisons using ANOVA and post hoc Tukey where appropriate) and/or a significant increase in the time spent in the ethanol-paired side between preconditioning and postconditioning tests (within-group assessments using planned comparisons).

Results: In experiment 1, both male and female rats demonstrated ethanol-induced CPP when administered the highest dose of ethanol (2.0 g/kg), evident as a significant shift in preference towards the ethanol paired side in males ($p < 0.05$) and a significantly higher preference score compared to saline controls in females ($p < 0.05$). In experiment 2, sex differences emerged as female rats continued to prefer a high dose of ethanol (2.0 g/kg) and male rats demonstrated a modest preference for the ethanol-paired chamber with the lowest ethanol dose (0.5 g/kg). In experiment 3, male rats demonstrated a robust preference for ethanol when administered low ethanol doses (0.0625, 0.125 g/kg), evident as a significantly higher preference score compared to saline controls ($p < 0.05$).

Conclusions: Overall, sex differences did not emerge in preadolescence because rats exhibited a similar preference for ethanol, regardless of sex. However, during adolescence, a shift in preference was seen in male rats that demonstrated ethanol-induced CPP at low doses of ethanol. In conclusion, ethanol-induced CPP can be reliably demonstrated in adolescent rats, which can aid in a better understanding of the underlying brain mechanisms involved in adolescent ethanol use.

Keywords: Alcohol Use Disorder and Drug Addiction, Adolescence, Alcohol, Reward, Developmental

Disclosure: Nothing to disclose.

P705. Genome-Wide Association Study of Pavlovian Conditioning Phenotypes Reveals Loci Associated With the Reinforcing Effects of Psychostimulants and Nicotine

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Background: Addiction vulnerability is influenced by genetic and environmental factors, and is also related to non-drug traits including the response food-associated stimuli. Using over 3200 Heterogenous Stock (HS) rats tested at the University at Buffalo and the University of Michigan, we performed a genome-wide association study (GWAS) to identify genomic regions influencing the response to food-associated stimuli in a Pavlovian conditioning paradigm.

Methods: In this paradigm, a conditioned stimulus (an illuminated lever) predicted the delivery of a food pellet into a food magazine (25 trials per day for 5 days). Lever contacts (sign-tracking) and magazine contacts (goal-tracking) and the last two days were the major measures subjected to GWAS analysis, although other measures such response bias, latency, probability, and PavCA Index were also obtained. We identified several loci associated with these measures.

Results: One of the most robust loci associated with sign-tracking was identified on chromosome 1, which contained a cluster of trace amine-associated receptor (Taar) genes. Previous genetic and pharmacological investigations of TAAR1 indicate that this receptor is involved the reinforcing effects of

methamphetamine, cocaine, and nicotine. To test whether TAAR1 is involved in the development and expression of PavCA phenotypes, we administered the TAAR1 agonist RO5262297 to a subset of HS rats tested in this paradigm. RO5262297 reduced both the development and expression of sign-tracking, but not goal-tracking, and sign- and goal-trackers were also differentially sensitive to the hypothermic effects this drug.

Conclusions: These studies provide pharmacological evidence for Taar1 as a candidate gene underlying the sign-tracking phenotype, and further suggest that sign-tracking and drug reinforcement are similarly influenced by individual differences in Taar1 activity.

Keywords: Pavlovian Conditioning, Self-Administration, Sign-Tracking, Trace Amines-Associated Receptor 1, Methamphetamine

Disclosure: Nothing to disclose.

P706. Exposure to Nicotine Using a Model of Electronic Vapor Inhalation Increases Risky Choice and β2 Nicotinic Receptor Subunit Gene Expression in Rats

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Background: Targeted advertising, addition of palatable flavors, and misconceptions about safety has led to a dramatic increase in the recreational use of e-cigarettes over the last 10 years. Higher concentration of nicotine, increased rate of use, and unique additives and metabolites drive distinct pharmacological properties for nicotine when delivered through e-cigarettes and emphasize a need for research on the effects of nicotine vapor on the brain and behavior. Pre-clinical research investigating the effects of nicotine have primarily used nicotine delivery via injections or subcutaneous pumps; however, more recent rodent studies have begun to investigate the neurochemical and behavioral consequences of nicotine by administering it through vapor inhalation. The goals of this study were to 1) validate an emerging model of electronic nicotine vapor inhalation in our laboratory by assessing nicotine dependence following cessation of repeated nicotine vapor exposure in rats and to 2) investigate the effects of nicotine vapor exposure on cost-benefit decision making and acetylcholine receptor (AChR) subunit gene expression in striatal and cortical regions of the rat brain.

Methods: For Experiment 1, fifty-six adult male Sprague Dawley rats were exposed to either 0, 12, or 24 mg/mL nicotine vapor for 9 days. Cotinine levels were assessed on day 6, physical withdrawal signs following mecamylamine precipitated withdrawal on day 7, and anxiety-like behavior following mecamylamine precipitated withdrawal on day 9 of exposure. A separate group of twelve rats had stimulating electrodes implanted into the medial forebrain bundle and were trained in intracranial self-stimulation (ICSS). The effects of spontaneous nicotine vapor withdrawal on ICSS thresholds were then assessed. For Experiment 2 twenty-four adult male Sprague Dawley rats were trained in the probability discounting task until stable. Following training rats were exposed to either 0 or 24 mg/mL nicotine vapor for 10 consecutive days, with testing in the discounting task occurring immediately after daily exposures. The brains of the animals were collected and rt-PCR was used to assess AChR α4 and β2 subunit gene expression in the ventral striatum and prefrontal cortex.

Results: Increases in cotinine levels, physical withdrawal signs, anxiety-like behavior, and ICSS thresholds were seen in male rats exposed to 24 mg/mL nicotine vapor, relative to 0 mg/mL controls. Furthermore, relative to 0 mg/mL controls, rats exposed to 24 mg/mL nicotine vapor showed immediate, short-term increases in risky choice, as well as increases in the expression of genes coding for the AChR β2 subunit, dopamine d1 receptor, and dopamine d2 receptor in the medial prefrontal cortex.

Conclusions: Observed increases in ICSS thresholds following cessation of repeated nicotine vapor exposure expand on existing literature suggesting that e-cigarette use may lead to nicotine dependence and withdrawal symptoms similar to that seen with traditional cigarette smokers. Findings also provide data showing nicotine vapor-induced changes in cortical AChR subunit gene expression, that may underlie the changes in cost-benefit decision making seen in smokers. The proposed work helps establish a much needed pre-clinical rodent model of human e-cigarette use and identifies new possible mechanisms driving nicotine's effects on decision making. Additional information on the effects of nicotine vapor exposure on the brain and behavior will be necessary for the development of effective government regulations and educational campaigns dedicated to reducing recreational e-cigarette use.

Keywords: Nicotine Vapor, Model Systems, Nicotine Dependence, Decision Making, a4b2 Receptors

Disclosure: Nothing to disclose.

P707. Neural and Hormonal Factors Underlying Sensitivity to Alcohol Effects on Inhibitory Control in Heavy Drinking Women

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Background: The sex gap in alcohol consumption is closing rapidly due to alarming increases among women. As such, it is important to determine potential women-specific factors underlying risk for Alcohol Use Disorder (AUD). Data from our lab and others suggest that poor inhibitory control, both in sober and intoxicated individuals, is a stronger risk factor for women than for men. We previously examined neurobiological factors underlying poor inhibitory control in heavy drinking women and found less brain activity during response inhibition in the early follicular phase of the menstrual cycle, when sex hormones (estradiol and progesterone) are low. Here we extend these findings to examine neural and hormonal factors underlying sensitivity to alcohol effects on brain activity during inhibition in heavy drinking women.

Methods: Female heavy drinkers performed the stop signal task to assess inhibitory control while undergoing fMRI on four sessions. Women were tested while receiving intravenous alcohol (60mg%) and saline infusions in both the early follicular phase of their menstrual cycle (low estradiol and progesterone) and in the mid-luteal phase (moderate estradiol and high progesterone). Women also reported subjective feelings of stimulation and sedation at baseline and during the infusions. Blood samples were taken to assess serum levels of estradiol and progesterone before and after infusions at all sessions.

Results: Data collection is currently ongoing, and to date 7 women have completed the study. Preliminary analyses confirmed low hormone levels in the early follicular phase (mean estradiol across alcohol and saline sessions = 28.5 ng/mL; mean progesterone = 0.44 ng/mL), and moderate to high levels in the mid-luteal phase (mean estradiol = 93.6 ng/mL; mean progesterone = 13.2 ng/mL). Alcohol effects on neural correlates of inhibition differed according to menstrual cycle phase. Specifically, alcohol decreased brain activity during inhibition relative to saline in right frontal regions, including the right supplementary motor area, inferior frontal gyrus, insula, and middle frontal gyrus during the early follicular phase, whereas alcohol increased brain activity in these regions in the mid-luteal phase. Additionally, alcohol

increased subjective stimulation in the early follicular phase, whereas alcohol increased sedation in the mid-luteal phase.

Conclusions: These data suggest that fluctuating levels of estradiol and progesterone influence sensitivity to the disinhibiting and subjective effects of alcohol in heavy-drinking women. Greater disinhibition and stimulation following alcohol are both associated with increased risk for heavy drinking. Thus, 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. Identification of such vulnerable periods for problematic alcohol consumption, as well as related brain mechanisms, could have important implications for prevention and treatment of AUD in women.

Keywords: Women's Health, Alcohol, Inhibitory Control

Disclosure: Nothing to disclose.

P708. Epigenetic Priming Drives Aberrant Gene Expression in Cocaine Relapse

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Background: Substance use disorders represent an enormous public health crisis and are among the most intractable illnesses in our society. An ongoing focus of research into the molecular pathology of addiction are mechanisms by which neuronal gene regulation is altered in a central brain region of reward, the nucleus accumbens (NAc). Stable changes in chromatin are proposed to underlie the maladaptive transcriptional states in this brain region, which persist despite long-term drug withdrawal. However, there is no direct link between drug-induced epigenetic marks and aberrant gene expression programs that drive relapse. 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 immediate 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: Our studies investigate an emerging view of epigenetic adaptation that may promote substance use disorders, providing new insight into gene priming as a key mechanism whereby drugs of abuse alter brain function and behavior. Specifically, our data indicate that cocaine-induced chromatin remodeling involving the histone variant H2A.Z underlies the persistent effects on circuit-specific gene regulation in the NAc. Together, these findings support epigenetic priming as a critical mechanism and promising clinical target that drugs of abuse engage in modifying brain function and behavior in lasting ways.

Keywords: Epigenetics, Chromatin Modifications, Gene Priming, Histone Variants

Disclosure: EpiVario, Inc.: Founder (Self)

P709. Oral and Intravenous Methylphenidate Produce Opposing Patterns of Brain Activity Despite Comparable Levels of Dopamine Release: A Simultaneous PET-fMRI Study

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Background: The faster an addictive drug enters the brain, the greater its rewarding effects. Hence drugs when taken by routes of administration that results in fast brain delivery, e.g., intravenously (IV) are more rewarding than when taken orally, which leads to slow drug delivery into the brain. Yet it is unclear how human brain function changes as a function of the speed of a drug's brain delivery, which is relevant to the mechanisms underlying their rewarding effects. Here we used simultaneous PET-fMRI to examine brain activity and dopamine signaling to IV (0.25 mg/kg) versus oral (60 mg) methylphenidate (MP) for doses that achieve comparable dopamine transporter blockade in the brain, in a double-blind, counterbalanced, placebo-controlled study in healthy adults. We hypothesized that oral and IV MP would evoke decreases and increases in striatal activation, respectively, because slow dopamine release would be more likely to stimulate high-affinity inhibitory D2 receptors, whereas fast dopamine release would be sufficient to temporarily stimulate the lower-affinity excitatory D1 receptors.

Methods: Fifteen healthy participants (36.7 ± 9.5 years old; 7 females) underwent simultaneous 11C-raclopride (16 mCi) PET/fMRI in the resting state in a 3T Siemens Biograph mMR scanner for 90 minutes to assess dopamine striatal changes, brain activation, and euphoria (feeling of 'high') in response to MP over 3 different sessions (placebo, oral MP, or IV MP). Dynamic PET images were reconstructed using the 3D OSEM algorithm and corrected for scatter and attenuation using a fully convolutional neural network. Striatal dopamine changes were estimated from differences in standardized uptake value ratio (SUVr) to cerebellum between placebo and MP conditions (oral/IV). The fMRI timeseries (3mm isotropic, TR = 3 seconds, 1800 time points) were realigned, distortion corrected, and spatially normalized to MNI space using the image preprocessing pipelines of the Human Connectome Project, and underwent standard motion regression and 0.01-0.10Hz band-pass filtering. To model brain activation responses to oral and IV MP, we fit gamma variate functions to the BOLD timeseries in a whole-brain, voxelwise general linear

modeling analysis. Paired *t*-tests were used to test for main effects of treatment (oral or IV MP vs placebo) on striatal dopamine changes and brain activity while controlling for multiple comparisons (pFWE < 0.05). We also used a within-subjects repeated-measures ANOVA to test for treatment-by-time interactions in 'high' ratings.

Results: IV MP elicited a stronger 'high' than oral MP with a faster rise and fall, replicating prior work ($p < .05$). In line with our hypothesis, IV MP elicited a pattern of relatively fast (~5 min duration) increased activation in striatum, with a bilateral cluster centered on the head of caudate, whereas oral MP elicited a pattern of slow, plateauing reduction in activation in ventral striatum, with additional clusters in ventromedial/ventrolateral prefrontal cortex and amygdala/hippocampus.

Conclusions: We found that 'fast' versus 'slow' MP administration elicited positive and negative patterns of striatal activation, respectively, which paralleled the different temporal patterns of subjective euphoria ('high') to IV versus oral MP. Thus, the speed rather than the overall magnitude of dopamine increases may be driving different patterns of brain activation that contribute to a drug's rewarding effects. We speculate the divergent patterns of striatal signaling may reflect predominantly excitatory D1 signaling to IV MP versus inhibitory D2 signaling to oral MP.

Keywords: Dopamine, Drug Abuse, Simultaneous PET-MR

Disclosure: Nothing to disclose.

P710. Top Down Regulation of Dopamine Activity Affects Cognitive Flexibility in a Sex-Specific Manner When Behaviors are Well-Learned

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Background: Cognitive flexibility is the act of adjusting behavior in response to changing reward contingencies. Deficits in cognitive flexibility are pervasive across psychiatric disorders, and represent core, predictive features in disorders such as depression, bipolar disorder, addiction, and schizophrenia. For example, the inability to cease drug taking despite negative consequences may stem from a loss of flexible control over behavior and cognitive flexibility deficit severity in adolescence predicts mood symptom onset and severity in depression and bipolar disorder. The circuitry that regulates cognitive flexibility has been well-studied, highlighting the necessity of dopamine (DA) release and receptor activation in cortex and ventral or dorsomedial striatum. These studies, however, typically measure flexibility 1-2 sessions after the initial discrimination was learned, while few have examined pathways by which flexibility could be reinstated after overtraining. This gap in knowledge is important because rewarded behaviors that are repeated eventually become outcome-insensitive, stereotyped habits, which coincides with a shift in related DA release patterns from ventral to dorsolateral striatum. This suggests that the circuitry that regulates cognitive flexibility following learning may be different than reinstating flexibility once a behavior becomes well-learned, particularly in regards to the regulation of DA. Previous studies have hinted at a role for the medial septum (MS), and its ability to increase DA population activity in the ventral tegmental area (VTA) and decrease DA population activity in the substantia nigra (SNc), in cognitive flexibility. However, the extent of this role, and how it may be affected by level of training, is unclear.

Methods: To investigate this question, I trained male and female rats to discriminate between sides of a touch screen apparatus for 100 trials per day for 1, 10, or 15 days. The day after training concluded, we activated the MS (systemic CNO) or prelimbic cortex to MS pathway (intra-prelimbic CNO) using DREADDs. Thirty minutes later, we measured their performance on a set-shifting task where they were required to switch from the side rule they had learned to a visual discrimination rule (e.g. follow a particular image side-to-side rather than choosing only left or right). The number of trials and errors committed en route to switching to the new rule (quantified as 10 correct-in-a-row) were recorded. A separate set of male and female rats were trained for 15 days and then I pharmacologically prevented MS-mediated effects in VTA, SNc, or both just after systemic injection of CNO, but prior to test testing. This was done by bilaterally infusing scopolamine, which we previously showed selectively prevents the MS-mediated increase in VTA DA population activity, bicuculline, which selectively prevents the MS-mediated decrease in SNc DA population activity, or both into the ventral hippocampus. Thirty minutes after CNO injection and ten minutes after ventral hippocampus infusions rats performed the same set-shifting task described above.

Results: Male and female rats who performed the set-shifting task following 1 day of discrimination training showed similar results and had no effect of MS activation (sexes combined, trials: $P = 0.71$, errors: $P = 0.57$). Following 10 days of training, however, activation of the MS or the prelimbic to MS pathway significantly improved set-shifting performance in female rats, reducing the number of trials ($P = 0.0005$) and errors ($P = 0.001$) committed en route to reaching criterion of 10 correct trials-in-a-row. This effect was not seen in male rats ($P = 0.18$ and 0.11, respectively). Following 15 days of training, MS activation improved set-shifting performance in both male and female rats to a similar degree, again reducing the number of trials (sexes combined, $P = 0.041$) and errors (sexes combined, $P = 0.018$). These effects were mediated by the MS's regulation of VTA and SNc DA activity, as prevention of the MS-mediated decrease in SNc DA population activity and increase in VTA DA population activity eliminated the MS activation-induced improvement in set-shifting in both sexes. Prevention of the decrease in SNc DA population activity or increase in VTA DA population activity alone reduced the MS activation-induced improvement in set-shifting, but did not eliminate it. The difference in MS activation efficacy in male and female rats may be explained by learning rate during training. Female rats reached an average of >92% correct performance during discrimination training in fewer days (training day 4) than males (training day 6), and more days at this performance criteria tended to predict worse performance in controls and better performance following MS activation.

Conclusions: These data suggest that MS activation may be more effective at aiding flexibility as training increases. They also suggest that the MS, via its bidirectional regulation of VTA and SNc DA activity, may be a critical relay from prelimbic cortex to midbrain by which flexibility is reinstated once behaviors are well-learned.

Keywords: Cognitive Flexibility, Dopamine, Medial Septum, Sex Differences, Top-Down Control

Disclosure: Nothing to disclose.

P711. Characterization of a Prkcd-Cre Knock-in Rat Model

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Background: The central amygdala (CEA), a nucleus predominantly composed of GABAergic inhibitory neurons, plays an important role in alcohol addiction-related behaviors, including alcohol preference, withdrawal- or dependence-induced escalation of self-administration, and choice of alcohol over natural rewards. We recently found PKCδ-mediated signaling in this

structure as a potential mechanism behind individual differences in vulnerability for compulsive alcohol taking. In order to define the functional role of CEA- Prkcd microcircuits and their output connections in alcohol related behaviors we developed and validated the novel transgenic Prkcd-Cre Knock-in rat model.

Methods: Prkcd-IRES-Cre Knock-in rat line was developed by genOway applying CRISPR nuclease technology in Wistar genetic background. Presence of the knock-in construct in the offspring was confirmed by PCR and DNA sequencing and further breeding with Wistar wild type rats confirmed germline transmission. To confirm specific Cre and PKCδ neuronal expression and further validate CeA PKCδ + projection input and output targets we used Cre-dependent adeno-associated viral tracing combined with immunohistochemical labelling. The anterograde AAV5-hSyn-DIO-mCherry and the retrograde viral tracer AAVretro-hSyn-DIO-EGFP were injected bilaterally (0.5µL, 0.25 µl/min) into the CeA of Prkcd-Cre rats ($N = 5$/group). We next examined the electrophysiological properties of PKCδ + neurons in acute amygdala slices. Finally, female and male Prkcδ-Cre rats and their wild type counterparts ($N = 10$-17/group) were evaluated for alcohol self-administration and a battery of control behaviors.

Results: In the CeA, Cre expression was limited to PKCδ + neurons, and detected in approximately 62% of all PKCδ + neurons.The insertion of the Cre-cassette into the Prkcd locus did not affect the general expression patterns of PKCδ in the CeA. PKCδ + expression was approximately 3.5-fold higher in the CeL compared to the central medial amygdala (CeM) in concordance with expression patterns of PKCδ in the CeA in mice. The most prominent CeL- PKCδ + output projection targets included the CeM, the orbitofrontal and insular cortex, the bed nucleus of the stria terminalis (BNST), hypothalamus, the ventral pallidum, and the stria terminalis. Additionally, we found direct CeL-PKCδ + projections onto the dopaminergic midbrain nuclei and brainstem projections specifically in the ventrolateral columns of the periaqueductal gray area, which has been mainly attributed to the CeL SOM + neuronal subpopulation. We confirmed retrograde labelling in the insular cortex, BNST, basolateral amygdala, multiple nuclei of the periamygdaloid cortex and the rostromedial tegmental nucleus. However, we did not find input signaling from the nucleus accumbens, previously described as a main input region towards CeL PKCδ + neurons in mice.

Electrophysiological recordings showed similar passive electrophysiological properties in CeL-PKCδ + and PKCδ- neurons. Optogenetic stimulation (5 ms) evoked inhibitory postsynaptic currents in all CeL neurons that were completely abolished by pretreatment with picrotoxin (100 uM), indicating their GABAergic nature. Moreover, we found that optogenetic activation of CeL PKCδ + neurons inhibited firing activity of PKCδ- neurons, and this effect was abolished by pretreatment with picrotoxin (100 uM).

Prkcd-Cre rats self-administered similar levels of alcohol reinforcers when compared to Wistars and there was no effect of the genotype in either sex $F(1,81) = 1.1$; $p = $ NS). Unsupervised clustering identified two subpopulations that responded differentially to punished alcohol self-administration in both male and female Prkcd-Cre rats, in agreement with our recent findings of a bimodal distribution in vulnerability for compulsive alcohol taking. Prkcd-Cre rats did not display deficiencies when tested in a range of control behaviors including locomotor activity, anxiety, and memory.

Conclusions: Our findings demonstrate that Prkcd-Cre Knock-in rats represent a valid genetic tool to investigate the properties and the function of PKCδ + neurons as well as cell-type specific manipulations of PKCδ + neuronal circuitry in a range of behavioral models in rats.

Keywords: Central Amygdala, PKCdelta, Transgenic Rats, Alcohol and Substance Use Disorders

Disclosure: Nothing to disclose.

P712. Chronic Methamphetamine Produces Hypofrontal States and Cognitive Deficits by Increasing Perineuronal Nets in the Prefrontal Cortex

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Background: Hypofrontality refers to a pathological state of reduced frontal cortex function that is observed in numerous neuropsychiatric disorders, including substance use disorder. In abstinent, methamphetamine (METH)-dependent individuals, the hypofrontality is associated with deficits in working memory and attention, and increases in impulsive behavior, which reduces quality of life and enhances relapse vulnerability. However, the mechanisms underlying METH-induced hypofrontality remain elusive. In the medial prefrontal cortex (mPFC), perineuronal nets (PNNs) comprise extracellular matrix (ECM) glycoproteins that surround predominantly parvalbumin-positive fast spiking interneurons (PV + FSIs). PNNs are involved in synaptic plasticity in response to experience, and PNN-related genes are dynamically regulated by neuronal activity.

Methods: Two-month-old Long Evans rats received daily injections (i.p.) of saline or METH for 14 days (1 mg/kg on days 1 and 14; 5 mg/kg on days 2-13), followed by 7 days of forced abstinence in the home cage. In a separate study, LE rats were allowed to self-administer METH (0.02 mg/infusion) using intravenous operant responding at fixed ratio 1 for 15 days until stable taking was established. PNNs are labeled using WFA staining, together with anti-parvalbumin immunohistochemistry. Using acute, ex vivo slices, whole-cell patch clamp recordings were made from pyramidal neurons in prelimbic cortex deep layers (V/VI). Evoked inhibitory postsynaptic currents (eIPSCs) were pharmacologically isolated by adding CNQX and APV. To digest PNNs, chondroitinase ABC (chABC) was injected bilaterally into the mPFC. Temporal order memory (TOM) was assessed by standard design including exploration of 2 identical objects for 5 min (phase 1), followed 30 minutes later by exploration of a different pair of identical objects (phase 2), and 30 minutes later, the rat is allowed to explore an object from each set for 5 mins (test) and a preference ratio is calculated.

Results: We found that either repeated, non-contingent METH administration or contingent METH self-administration induced in the mPFC a significant increase in the percentage of PNN-surrounded PV + FSIs, but without altering the density of PV + FSIs. This increase in mPFC PNNs correlated with an increase in PV + FSIs intrinsic excitability and an increase in the amplitude of both evoked and spontaneous IPSCs onto deep-layer pyramidal neurons in mPFC. In addition, chronic METH exposure elicited deficits in the mPFC-dependent temporal order memory (TOM) task, but this deficit was rescued by enzymatic digestion of mPFC PNNs in chronic METH-exposed rats. Similarly, mPFC PNN digestion by chABC normalized the amplitude of evoked IPSCs recorded from mPFC deep layers pyramidal neurons in METH rats.

Conclusions: Together our data suggest that chronic contingent or non-contingent METH exposure increases the percentage of PNN-surrounded PV + FSIs, which promotes hypofrontal states and cognitive deficits through increased PV + FSI excitability and inhibitory synaptic transmission. Moreover, enzymatic digestion of mPFC PNNs can reverse METH-induced changes in mPFC activity and behavioral deficits, suggesting a potential therapeutic avenue for treating cognitive symptoms in individuals suffering from methamphetamine use disorder and other disorders with psychopathology linked to hypofrontal states.

Keywords: Perineuronal Nets, Hypofrontality, Working Memory, Parvalbumin Interneurons, Methamphetamine

Disclosure: Nothing to disclose.

P713. Nucleus Accumbens Neurons Receiving the Densest Prelimbic Input are Required for Cocaine Seeking and are Embraced by Astrocytes During Relapse

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Background: Prelimbic cortical projections to the nucleus accumbens core are critical for cue-induced cocaine seeking, but the relevance of the neurons most innervated by this projection and the neuroadaptations contributing to relapse within these cells remain unknown.

Methods: Male Sprague-Dawley rats underwent cocaine or sucrose self-administration, extinction and cue-induced reinstatement. Pathway-specific chemogenetics, patch-clamp electrophysiology, in vivo electrochemistry, and high-resolution confocal microscopy were used to characterize the subset of nucleus accumbens core neurons that receive dense prelimbic cortical input and to elucidate the role they play in cue-induced sucrose and cue-induced cocaine seeking.

Results: Pathway-specific chemogenetic inhibition of prelimbic cortical projections to the nucleus accumbens core suppressed cue-induced cocaine relapse and normalized real-time cue-evoked increases in accumbens glutamate release to levels observed in sucrose seeking animals. Furthermore, chemogenetic inhibition of anterogradely targeted nucleus accumbens core neurons receiving the densest prelimbic cortical input suppressed cocaine, but not sucrose seeking. Interestingly an even smaller subset of these neurons were specifically activated by cocaine versus sucrose-paired cues. We find that accumbens neurons receiving dense prelimbic input also undergo morphological plasticity during the peak of cocaine seeking in the form of dendritic spine expansion and increased ensheathment by astroglial processes, specifically at large dendritic spine heads.

Conclusions: We identified and characterized a unique subpopulation of nucleus accumbens neurons that receive dense prelimbic cortical input. The functional specificity of this subpopulation is underscored by their ability to mediate cue-induced cocaine relapse, but not sucrose seeking. This subset of cells represents a novel target for addiction therapeutics revealed by anterograde targeting to interrogate functional circuits imbedded within a known network.

Keywords: Cocaine, Transsynaptic, Astrocyte, Prelimbic Cortex, Nucleus Accumbens Core

Disclosure: Nothing to disclose.

P714. Dissecting the Genetics of Intravenous Cocaine Self-Administration in a Genetically Diverse Mouse Population

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Background: Cocaine intravenous self-administration (IVSA) in laboratory animals models the volitional initiation and progression of drug use in humans. This behavior 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 laboratory animals to rigorously and rapidly identify relevant genetic influences. Large and genetically diverse mouse populations, including the Hybrid Mouse Diversity Panel (HMDP) and the BXD recombinant inbred panel (a subset population of the HMDP), have been developed for these types of forward genetic approaches. These populations enable high-resolution genome-wide association studies, as well as the discovery of genetic correlations.

Methods: All subjects ($n = 86$ genetically unique inbred strains, $n = 8$-12 mice per strain) were surgically catheterized, providing access to the jugular vein. Following recovery from the surgery, half of the mice received 10 daily sessions of cocaine IVSA, while the other half received access to a saline infusate (as a control). IVSA data for BXD recombinant inbred strains ($n = 52$ strains) were utilized for quantitative trait locus (QTL) mapping and prioritization of positional candidate genes using publicly available RNA expression data obtained from addiction-relevant brain regions (striatum, nucleus accumbens and ventral tegmental area) (software and data provided by genenetwork.org). Candidates were evaluated for cis-expression QTL (eQTL) and significant, strain-level correlations to cocaine IVSA behavior.

Results: We found cocaine IVSA to be substantially heritable in this population, with strain-level intake ranging from near zero to >25 mg/kg/session. Though saline IVSA was also found to be heritable, a very modest genetic correlation between cocaine and saline IVSA indicates that operant responding for the cocaine reinforcer was influenced by a substantial proportion of unique genetic variants. QTL mapping in the BXD strains revealed a suggestive QTL ($p = 0.063$) on chromosome 1 for cocaine, but not saline, intake early in acquisition. Analysis of positional candidates prioritized 3 genes (Pappa2, Nme7 and Kifap3) that demonstrate both cis-eQTL and significant genetic correlation to cocaine intake. Expression Phewas analysis (systems-genetics.org) across the whole genenetwork.org BXD phenome database revealed that Kifap3 expression is also genetically correlated to alcohol conditioned taste aversion to saccharin, indicating this gene may regulate response to both cocaine and alcohol.

Conclusions: These data indicate that the HMDP and BXD populations are suitable for forward genetic approaches for the analysis of cocaine IVSA. Furthermore, we have identified a genetic locus potentially associated with cocaine self-administration, as well as novel candidate genes. This project is ongoing and includes whole-transcriptome RNA sequencing in key brain regions for differential expression (cocaine relative to saline IVSA) phenotypes across all strains in the study. Integration of the genetic association data with expression profiling should aid in identifying candidate genes that regulate risk for cocaine use.

Keywords: Cocaine Self-Administration, Behavioral Genetics, QTL, Substance Use Disorder

Disclosure: Nothing to disclose.

P715. Dietary Polyphenols Inhibit Low Dose Morphine Conditioned Place Preference in Mice: Implications for an Epigenetic Mechanism

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Background: Opioid use disorder (OUD) is a neuropsychiatric condition which results in significant morbidity and mortality, as well as social, economic, and personal costs to patients and their families. In 2020 alone, approximately 70,000 lives were lost to opioid overdose in the US. Currently, available pharmacotherapies for OUD are ineffective or intolerable for many patients. As such,

development of novel interventions particularly those focusing on strategies to promote overall health and resilience to problematic opioid use are of immense clinical and societal interest. In recent years, botanically derived dietary polyphenols such as resveratrol have been shown to be effective at promoting behavioral resilience and adaptive neuroplasticity in models of neuropsychiatric disease. In this study we investigated the potential for dietary polyphenols to reduce the formation of addiction-like behaviors and neurobiological changes in a mouse model of OUD.

Methods: In order to investigate the influence of dietary polyphenols on the behavioral and molecular response to morphine, male C57BL6/J mice aged 7 weeks old were treated with either the Bioactive Dietary Polyphenol Preparation (BDPP) comprised of grape seed polyphenol extract (1g/l), Concord grape juice (1 part juice: 3 parts water), and resveratrol (1g/l) or drinking water containing matched concentrations of sucrose (152g/l) for 2 weeks, followed by assessment of behavioral response to morphine using a number of paradigms. Effects of polyphenols on development of locomotor sensitization was assessed with daily injections of 5 or 15mg/kg morphine. The conditioned place preference (CPP) model of drug seeking was used to assess preference for a morphine paired chamber at a range of doses (2.5 - 15mg/kg given subcutaneously). To assess the effects of BDPP on morphine preference following withdrawal, an additional cohort received five daily injections of 5mg/kg morphine two weeks prior to CPP training. As polyphenols are known enhancers of Sirtuin-1 (Sirt-1) histone deacetylase activity, an additional cohort received microinjections of the Sirt-1 inhibitor EX527 into the nucleus accumbens (NAc) daily during CPP training. For molecular analyses, mice were sacrificed 24 hours after CPP testing and the nucleus NAc was dissected for qPCR analysis and RNA sequencing and cecal samples collected for 16s sequencing. An additional cohort of mice were sacrificed 1 hour after an acute injection of 5mg/kg morphine, and the NAc dissected for analysis of acute gene expression changes.

Results: In the CPP paradigm we found that at lower doses of 2.5 mg/kg and 5mg/kg morphine, control animals developed robust place preference ($p < 0.05$ and $p < 0.001$ respectively), but BDPP treated animals did not develop a significant preference at either dose (two-way ANOVA followed by Sidak's post-hoc, $n = 8$-32 per group). Similarly, polyphenol treated mice did not form preference for 5mg/kg morphine even when pretreated with a week of morphine followed by two weeks of withdrawal prior to CPP training, while this regimen led to formation of robust preference in control mice. At the highest dose of 15 mg/kg of morphine, BDPP treated animals developed preference ($p < 0.001$), but control mice did not ($n = 24$ per treatment group). Moreover, data from the locomotor sensitization paradigm revealed a significant reduction in ambulation following 15 mg/kg of morphine in animals pretreated with BDPP compared to control animals on day 1 and day 3 of morphine treatment (two-way ANOVA followed by Tukey's post-hoc $p < 0.0001$ and $p = 0.05$ respectively). Taken together, these data suggest that polyphenol treatment leads to a shift in the behavioral dose-response curve for morphine. Gene expression data from the NAc following CPP showed robust effects of polyphenol treatment on expression of the p65 subunit of the nuclear factor-κB (NF-κB) transcription factor, as well as multiple changes in gene networks related to epigenetic regulation. qPCR data from animals sacrificed 1 hour after an acute dose of 5mg/kg morphine revealed a significant main effect of BDPP treatment on immediate early genes including EGR4 as well as epigenetic editors including Sirt1 and HDAC1 levels (two-way ANOVA followed by Sidak's post-hoc $p < 0.05$, $N = 6$-8). To assess a possible mechanistic role for Sirt-1 activity in mediating these effects, control and BDPP treated mice received microinjections of the Sirt-1 specific inhibitor EX527, directly into the NAc during CPP conditioning for 5mg/kg morphine ($n = 5/4$ per group). Treatment with EX527 did not alter the polyphenol effect on reducing CPP, suggesting that behavioral effects of polyphenols are not mediated via Sirt-1 activity in the NAc.

Conclusions: Taken together these data suggest that dietary polyphenol pretreatment robustly reduces formation of CPP at lower doses of morphine even after a period of withdrawal. At the highest dose of morphine, BDPP appears to have the opposite effect of enhancing CPP, perhaps by increasing morphine associated reward memory consolidation. Molecular analysis thus far reveals BDPP treatment influences expression of multiple transcription factors and epigenetic editors in the NAc. Inhibition of Sirt-1, which is well established to be activated by resveratrol, did not alter the behavioral effects of polyphenol treatment. Taken together, these studies lay the foundation for dietary polyphenols to reduce the formation of addiction-like behaviors in mice, potentially via epigenetic or transcriptional mechanisms.

Keywords: Opioid Addiction, Resveratrol, Epigenetics, Resilience, Microbiota-Gut-Brain Axis

Disclosure: Nothing to disclose.

P716. Comparison of Brain Nicotine Uptake From Electronic Cigarettes and Traditional Combustible Cigarettes

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Background: Recently, there has been enormous growth in the popularity of electronic cigarettes (E-cigs). While E-cigs are likely less harmful than traditional combustible cigarettes (C-cigs), a major concern is that long-term use of these products can lead to the development and maintenance of nicotine dependence. Similar to other drugs of abuse, brain accumulation rate and magnitude of nicotine are critical for its acute reinforcing effects. Despite recent findings that plasma concentrations of nicotine after E-cig use can be comparable to that of cigarette smoking, the capability of E-cigs to produce cigarette-like rapid brain nicotine delivery remains understudied.

Methods: Brain uptake of nicotine was directly assessed in 16 adult dual users (10 females; Mean ± SD, 36 ± 11 y) of E-cigs and C-cigs using 11C-nicotine and positron emission tomography (PET). Race was self-reported (White/African American/others: 56%/6%/38%). PET imaging was performed with a Discovery MI DR PET/CT system (GE Healthcare). The Institutional Review Board of the Duke University Health System and the Institutional Review Board of the Wake Forest University Health Sciences approved this study and all subjects provided written informed consent. Each participant went in a randomized order through two PET scanning sessions during which the head was scanned after he/she inhaled a single puff of vapor (55 mL over 4 sec) or smoke (35 mL over 2 sec) containing 11C-nicotine. Each standardized puff of vapor was produced from 15 µL V2 Red e-liquid (1.2% nicotine, 20/80 VG/PG) mixed with 11C-nicotine via a V2 EX Blanks refillable cartomizer, coupled with a programmable air syringe pump. The smoke was generated from a shortened Capri Magenta cigarette (R.J. Reynolds, USA) through a customized smoke delivery device after 11C-nicotine was applied. The subject's head was scanned over 15 min in a sequence of 249 frames of 1 - 10 sec each. Afterwards, a full-body scan was conducted to measure total absorbed dose of 11C-nicotine (TAD), which was used to normalize the 11C-nicotine uptake values between subjects and between conditions. The brain nicotine concentration was expressed as the % TAD per kg brain tissue. Final data were analyzed using paired *t*-test for each of four kinetics parameters

and Holm–Bonferroni correction for multiple comparisons was applied.

Results: After inhalation of a single puff of E-cig vapor or a single puff of C-cig smoke: 1) The mean maximal nicotine concentration (Cmax), normalized to total administered 11C-nicotine dose (TAD), from E-cig was 23% lower than from C-cig (Mean ± SE, 3.7 ± 0.2% TAD vs 4.7 ± 0.3% TAD); 2) The mean area under the time activity curve from 0 to 15 min was 22% lower for E-cig compared to C-cig (48.5 ± 2.7% TAD*min vs 62.1 ± 3,1% TAD*min); 3) The mean time to reach the maximal nicotine concentration (Tmax) for E-cig was approximately twice as long as that for C-cig (9.0 ± 0.8 min vs 4.4 ± 0.5 min); and 4) The mean time to reach one-half of the maximal nicotine concentration (T1/2) for E-cig was approximately two and a half times as long as that for C-cig (1.04 ± 0.13 min vs 0.38 ± 0.06 min). All four of these *P* values are lower than 0.0005 after correction for 4 comparisons. This lower brain nicotine accumulation following E-cig use as compared with C-cig smoking is due at least in part to the lower arterial blood nicotine concentration resulting from E-cigs, which in turn likely results from less nicotine reaching the alveoli where rapid nicotine absorption occurs. Indeed, our preliminary whole-body imaging results indicated greater nicotine retention in the upper respiratory tract from inhalation of E-cig vapor versus C-cig smoke. A possible reason for greater upper airway deposition is the higher pH levels typical of E-cig liquids relative to C-cig smoke (pH 7-9 for E-cigs and 5-6 for C-cigs). Alkaline pH enhances evaporation of nicotine base from droplets thereby enhancing its retention in the upper respiratory tract. Upper airway deposition of nicotine is predicted to be reduced by using E-cig liquids having low pH levels.

Conclusions: These results suggest that E-cigs can deliver nicotine rapidly to the brain, although less so than C-cigs under the present conditions. Therefore, E-cigs may lead to the development and maintenance of nicotine dependence, but they are also promising substitutes for combustible cigarettes and thereby may promote smoking cessation and harm reduction.

Keywords: Nicotine Addiction, Electronic Cigarette (e-cigarette), PET Imaging, Pharmacokinetics

Disclosure: Nothing to disclose.

P717. Morphological Plasticity of Nucleus Accumbens Astrocytes Differentially Impacts Synapses From D1- And D2-MSNs

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Background: Cues predicting heroin delivery induce heroin seeking by initiating synaptic glutamate release in the nucleus accumbens core (NAcore). The intensity of heroin seeking is negatively modulated by cue-induced increases in synaptic proximity of astrocytes and increased surface expression of the glutamate transporter GLT-1. We sought to determine whether cue-induced increases in astrocyte synaptic proximity and surface GLT-1 were selective for D1 or D2 receptor-expressing medium spiny neurons (D1- or D2-MSNs) in the NAcore, and how increased synaptic adjacency of astroglial processes impacted synaptic function.

Methods: Rats were trained to self-administer heroin or sucrose before undergoing extinction and cued reinstatement of seeking. Using confocal microscopy, we quantified astrocyte association with synapses from D1- or D2-MSNs. Next, we used an ezrin-targeted morpholino oligonucleotide to knock down astrocyte insulation in the NAcore of transgenic mice, and used whole cell patch-clamp electrophysiology to examine the impact of astrocyte insulation on D1- and D2-MSN synapses.

Results: Extinction from heroin, but not sucrose self-administration, downregulated GLT-1 and induced retraction of astroglial processes from NAcore synapses. Heroin cues increased synaptic proximity of astroglial processes and surface expression of GLT-1 in parallel with heroin seeking in separate populations of NAcore astrocytes. Astroglial retraction increased release probability onto D1-MSNs in the NAcore, and reduced spontaneous excitatory post-synaptic currents in D2-MSNs.

Conclusions: Our data demonstrate that increased synaptic insulation by NAcore astroglia does not reduce heroin seeking through GLT-1-dependent mechanisms. Instead, synaptic insulation by astroglia differentially impacts D1- and D2-MSNs to suppress relapse behavior.

Keywords: Astrocytes, Reinstatement, Heroin Self-Administration, GLT-1

Disclosure: Nothing to disclose.

P718. Daily Methocinnamox Treatment Attenuates Fentanyl Self-Administration in Rhesus Monkeys

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Background: Opioid abuse remains a serious public health challenge despite the availability of effective medications. Methocinnamox (MCAM), a long-acting mu opioid receptor antagonist, attenuates the positive-reinforcing and ventilatory-depressant effects opioids such as heroin and fentanyl in rhesus monkeys, suggesting it could be an effective treatment for opioid abuse and overdose. In previous studies, MCAM (≥ 0.32 mg/kg) given acutely or intermittently (i.e., once every 12 days) decreased self-administration of fentanyl. This study evaluated effects of daily injections of smaller doses of MCAM on fentanyl self-administration.

Methods: Five rhesus monkeys (2 females and 3 males) lever-pressed for i.v. infusions of fentanyl (0.032-320 µg/kg/infusion) under a fixed-ratio 30 schedule. MCAM was injected s.c. one hour prior to each session. The daily dose of MCAM increased in half-log unit steps from 0.0032 to 0.1 mg/kg/day across conditions, and the fentanyl dose-effect curve was redetermined with each treatment dose of MCAM. Effects of MCAM were quantified by dividing the ED50 of fentanyl (ascending limb of the dose-effect curve) during each daily MCAM treatment by the ED50 of fentanyl before daily MCAM treatment (i.e., potency ratio).

Results: Before MCAM treatment, the number of fentanyl infusions increased and then decreased with increasing dose; mean (95% confidence interval) ED50 was 0.13 (0.07-0.22) µg/kg/infusion. Daily MCAM treatment dose-dependently shifted the fentanyl dose-effect curve rightward, with mean (95% CI) potency ratios of 1.9 (1.2-2.6), 3.7 (2.5-4.8), 14.6 (10.1-19.1), and 49.0 (44.3-53.6) for treatment doses of 0.0032, 0.01, 0.032, and 0.1 mg/kg/day MCAM, respectively.

Conclusions: MCAM attenuated fentanyl self-administration and dose-dependently shifted the fentanyl dose-effect curve rightward nearly 50 fold with the largest daily treatment dose of 0.1 mg/kg. These results, taken together with previous studies demonstrating long-lasting and selective attenuation of opioid self-administration, support the view that MCAM could be a safe and effective treatment for opioid use disorder.

Keywords: Fentanyl, Self-administration, Methocinnamox, Repeated Treatment, Rhesus Monkeys

Disclosure: Nothing to disclose.

P719. Individuals with Substance Use Disorder Show Consistently Reduced Learning Rates in Response to Negative Outcomes Over a 1-Year Period

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Background: Computational modelling is a promising approach to parse behavioral processes and dysfunctions in individuals with substance use disorders (SUDs), but it is unclear how much these processes change during the recovery period.

Methods: We assessed 1-year follow-up data on a sample of treatment-seeking individuals with one or more SUDs (alcohol, cannabis, sedatives, stimulants, hallucinogens, and/or opioids; $N = 83$) that were previously assessed at baseline within a prior computational modelling study. Relative to healthy controls (HCs; $N = 48$), these participants were found at baseline to show altered learning rates and less precise action selection while completing an explore-exploit decision-making task. Here we replicate these analyses when these individuals returned and re-performed the task 1 year later to assess the stability of these baseline differences. We also examine whether baseline modelling measures can predict symptoms at follow-up.

Results: Bayesian analyses indicate that: (a) group differences in learning rates were stable over time (posterior probability = 1); (b) relationships between model parameters at baseline and follow-up were all significant and ranged from small to moderate ($.25 < ICCs < .54$); and (c) learning rates and/or information-seeking values at baseline were associated with substance use severity at 1-year follow-up in stimulant and opioid users ($.36 < rs < .43$, $.002 < ps < .02$).

Conclusions: These findings support the notion that processing dysfunctions involving learning to arbitrate between exploration and exploitation are stable throughout the recovery period. At the same time, individual computational differences at baseline had some predictive value for changes in substance use severity. Taken together, this computational approach may measure trait dysfunctions that could have predictive utility for substance use severity.

Keywords: Substance Abuse Disorders, Computational Modeling, Active Inference, Learning Rate, Explore-Exploit Dilemma

Disclosure: Nothing to disclose.

P720. Voluntary Alcohol Drinking During Adolescence Alters Prefrontal Cortex Neural Signals of Pavlovian Conditioned Approach in Adult Rats

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Background: Drug taking behavior typically starts during adolescence, a time during which the brain – and in particular, the prefrontal cortex – continues to develop. Given this critical developmental time window, however, the impact of regular drug consumption at this time can alter developmental trajectories, creating persistent changes in adult brain function in these individuals.

Methods: To understand this process more thoroughly, male ($n = 16$) and female ($n = 16$) adolescent rats (PND28-42) were given two weeks (14d) of daily access of 4% v/v alcohol in a substrate of jello (Alcohol) or the same substrate without alcohol but additional sucrose added to calorically match the alcohol (Controls). Rats then matured to adulthood where they were assessed on a Pavlovian Conditioned Approach (PCA) paradigm in a novel context. In this task, one compound set of Pavlovian cues (e.g., left panel light / left lever extend / high tone) was presented for 10s and co-terminated with delivery of a sucrose pellet (CS +). A different stimulus (right panel light / right lever extend / low tone) had no programmed consequences (CS-); CS + and CS- stimuli were counterbalanced. Individual differences in conditioned approach during PCA is typically seen by rats who go to the food cup during cues in anticipation of reward (goal trackers; GT), compared to those who spend the cue period interaction with the cue stimuli such as making unnecessary lever presses (sign trackers; ST).

Results: Overall, adolescent alcohol exposure appeared to increase the probability and intensity of the ST phenotype during early learning (days 1-10 of PCA), and in particular showing increases in lever pressing during cues compared to Controls. Electrodes placed in the prelimbic (PL) and infralimbic (IL) cortex during these PCA behaviors demonstrated clear distinctions between ST and GT phenotypes, particularly in Controls, and often with greater phasic activity to stimuli seen in Alcohol animals than controls. At the same time, local field potentials collected in the same locations were decreased in both ST and GT Alcohol animals relative to Controls at CS + onset, particularly in the low and high gamma frequencies. However, this alcohol-related decrease was only seen in STers and not GTers later in the cue and during reward receipt.

Conclusions: These data collectively suggest that voluntary alcohol drinking during development can produce lasting changes in PFC function, even in drug-abstinent adults. These changes, in turn, may set the stage for more pathological engagement with drugs of abuse through cue-elicited craving, approach and relapse.

Keywords: Electrophysiology, Drug Abuse, Adolescent Alcohol

Disclosure: Nothing to disclose.

P721. Fos-Expressing Neuronal Ensembles in Rat Infralimbic Cortex Encode Initial and Maintained Oxycodone Seeking in Rats

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Background: Neuronal ensembles within the infralimbic cortex (IL) as well as their projections to the nucleus accumbens (NAc) have been shown to mediate opiate seeking in well-trained rats. However, it is unclear if this circuitry is recruited during initial oxycodone self-administration. Here, we tested the necessity of IL neuronal ensembles in initial and maintained oxycodone-seeking behavior using the Daun02 inactivation procedure.

Methods: We trained male and female transgenic Fos-LacZ rats ($n = 12$-13) to self-administer oxycodone for 3hr daily sessions until rats met acquisition criteria (>30 active lever presses, >75% responding on active lever). We then infused Daun02 to selectively inactivate IL Fos expressing ensembles associated with initial oxycodone self-administration. We then tested the rats' oxycodone-seeking behavior 2 days later. We then repeated the experiment using a longer training period to determine the role of IL neuronal ensembles in oxycodone seeking after prolonged training. Here, we trained male and female transgenic Fos-LacZ rats ($n = 9$-10) to self-administer oxycodone in 3hr daily sessions under an increasing schedule of reinforcement for 9 days. After 1 week of oxycodone abstinence, we put animals through a 30 min induction test to reactivate neuronal ensembles associated with recall of oxycodone self-administration and infused Daun02 into the IL. We measured the rats' oxycodone-seeking behavior 2 days later.

Results: We found that inactivation of IL neuronal ensembles reduced oxycodone-seeking after initial oxycodone self-administration on test day ($t23 = 2.5$, $p = 0.02$). Next, we found that Daun02 attenuated oxycodone seeking behavior in well-trained rats ($t17 = 2.6$, $p = 0.02$). In both experiments, Daun02 infusions decreased Fos-expression after the test, indicating ablation of Fos-expressing neuronal ensembles by Daun02.

Conclusions: These results suggest that IL neuronal ensembles are formed during initial learning of oxycodone self-administration and are required for initial and maintained oxycodone-seeking behavior.

Keywords: Oxycodone, Self-Administration, Reinforcement Learning

Disclosure: Nothing to disclose.

P722. Changes in Stress Reactivity and Stress-Related Behaviors Following Stress-Induced Escalation of Cocaine Intake

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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 is likely due to long-lasting neuroplastic changes that also increases susceptibility to reinstatement of drug-seeking behavior. This may be explained by changes in stress reactivity as increased stress reactivity is associated with the severity of relapse in humans. We hypothesize that repeated stress at the time of SA increases susceptibility to stress-induced reinstatement and produces long-lasting changes in stress-related behaviors and stress reactivity.

Methods: Male SD rats were trained to SA cocaine (0.5 mg/kg/inf) on a FR 4 schedule in 4 X 30 min SA sessions separated by 5-min drug-free periods. Some rats received intermittent electric footshock stress in the SA chamber during the 5 min drug-free period over 14 days. Rats were then tested for increased susceptibility to footshock-induced and yohimbine-induced (0, 1.25, 2.5 mg/kg, i.p.) reinstatement. Additional groups of rats were tested for changes in stress reactivity and stress-related behaviors following stress-induced escalation of cocaine intake. These rats were trained to SA cocaine as described above and additional saline SA groups, with or without footshock stress, were also run. All animals underwent an extinction period followed by yohimbine-induced reinstatement tests. Subsequently, rats were tested for changes in anxiety-related behaviors (elevated plus maze, open field, and marble burying), social interaction, and cognition (object location memory) on different days. After the last behavioral task, rats were then subjected to a 30-min restraint stress and blood was sampled from the tail at 0, 15, 30, 60, and 90 min after the onset of stress to measure changes in plasma corticosterone. Rats were euthanized following the 90 min time point and brains were collected to examine stress-induced cFos activation in stress- and reward-related brain regions.

Results: Electric footshock stress administered daily at the time of self-administration induced an emergent escalation of cocaine intake over 14 days that persists in the absence of stress ($n = 13$-15/group, $p < .05$). Stress-escalated rats also demonstrate increased susceptibility to later reinstatement. Rats with a history of stress at the time of SA show augmented reinstatement to a priming injection of cocaine (2.5, 5, 10 mg.kg, i.p.; $n = 11$-14/group, $p < .05$), re-introduction of the footshock stress during the 5-min drug-free period ($n = 9$-12/group), and to an injection of the alpha-2 adrenergic receptor antagonist yohimbine (0, 1.25, 2.5 mg/kg, i.p.; $n = 14$-17/group, $p < .05$). There were a variety of changes in anxiety-related behavior, cognition, and social interaction and HPA axis response to restraint stress that were dependent upon the rat's drug and/or stress history ($n = 11$-12/group). Analysis of cFos expression in stress- and reward-related brain regions is currently in progress.

Conclusions: Chronic stress induces a glucocorticoid-dependent escalation of cocaine intake that is the result of persistent neuroadaptations. These long-lasting neuroadaptations result in increased susceptibility to reinstatement of drug-seeking behavior. These stress-induced neuroplastic changes influence the behavioral, hormonal, and neural responses to stress and the changes in stress reactivity may be one factor in the increased reinstatement behavior observed in stress-escalated rats. Understanding the unique mechanisms by which stress can drive drug use has implications for identifying and treating sub-populations of patients with SUD in whom stress is a contributing factor.

Keywords: Stress Reactivity, Cocaine Self-Administration and Reinstatement, c-Fos

Disclosure: Nothing to disclose.

P723. Synthetic ZFP189 Transcription Factors Delivered to Mouse Nucleus Accumbens Drive Cocaine, but Not Morphine, Induced Behaviors and Transcription

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Background: Understanding the molecular drivers of the stages of drug addiction may allow for the design of pharmacotherapies that block or reverse key events of the progression of drug addiction. Zfp189 is a CREB-regulated gene which itself encodes a nucleus accumbens (NAc) neuronal transcription factor that has been demonstrated to regulate brain transcriptional networks in neuropsychiatric disorders. In rodents, NAc Zfp189 gene expression increases following exposure to psychostimulants, like cocaine. To further examine the downstream relationship between ZFP189 and physiological response to different classes of drugs of abuse, we designed synthetic ZFP189 variants to exert opposite forms of gene regulation at presently unknown in vivo ZFP189 target genes. These ZFP189 variants were delivered to mouse NAc and subsequent behaviors and transcriptional response following exposure to psychostimulants (cocaine) or opiates (morphine) were characterized.

Methods: Three ZFP189 variants were synthesized: ZFP189WT which is identical to the endogenously expressed ZFP189 and contains a transcriptionally repressive N-terminal Krüppel associated box (KRAB) domain and a C-terminal Cys2-His2 DNA-binding domain; ZFP189VPR in which the endogenous KRAB domain is replaced with the robust transcriptional activator VP64-p65-Rta (VPR) yet retains the endogenous ZFP189 DNA-binding domain; and ZFP189DN in which any transcriptional regulatory domain is entirely removed. Each variant was sub-cloned into identical (p1005) expression vectors and gene-targeted regulation was characterized in combination with a ZFP189 response element (RE) luciferase assay in Neuro2a cells ($n = 3$ in technical triplicate). Expression vectors were then packaged in herpes simplex virus (HSV) to enable brain-region targeted expression.

Both male and female C57Bl/6J mice, aged 8-10 weeks, were utilized in these studies. HSV-ZFP189WT, -ZFP189VPR, or -ZFP189DN were delivered by stereotaxic surgery bi-laterally to NAc (10°; +1.6 AP; ± 1.5 ML; -4.4 DV, 1uL). For locomotor sensitization, mice were administered saline, cocaine (10 mg/kg), or morphine (10 mg/kg) IP for seven sequential days and locomotion was quantified. Sample sizes are 10-15 mice per group and statistical analyses were performed with one- or two-way ANOVAs, including a post-test comparing the test groups (HSV-ZFP189WT, -ZFP189VPR) versus the control group (HSV-ZFP189DN). Following the test on day seven, we extracted RNA from virally infected NAc tissues, performed ribosomal RNA depletion, prepared libraries, performed RNAseq in the following configuration: 2x150 paired-end reads on an Illumina sequencing platform (HiSeq 2500) at a depth of >30M reads per sample ($n = 5$ mice for each condition). Differentially expressed genes (DEGs) were generated using DESeq2 in comparing either test group (HSV-ZFP189WT, -ZFP189VPR) to the control group (HSV-ZFP189DN) with Wald adjusted p-value < 0.05 and absolute log2 fold change > 1 DEGs called as significant.

Results: By co-transfecting the ZFP189 RE luciferase plasmid and iterations of our ZFP189 variants, we are able to observe, relative to GFP control: ZFP189VPR induces robust targeted gene activation ($P < 0.0001$), ZFP189WT induces gene-targeted repression ($P < 0.05$), and ZFP189DN exerts no transcriptional regulatory control in this assay ($P > 0.5$). In virally delivering each of these ZFP189 variants to mouse NAc, we observe that HSV-ZFP189VPR significantly potentiates the rodent's psychomotor response to daily 10 mg/kg IP cocaine injections ($P < 0.0001$). When comparing the total daily distance moved across viral groups, it can be seen that HSV-ZFP189WT and HSV-ZFP189VPR induce opposite locomotor behavioral responses to cocaine ($P < 0.05$). By repeating this locomotor sensitization assay with either saline or morphine IP injections, no significant differences for any of the viral treatments emerged ($P > 0.05$ for all comparisons). In the corresponding RNA-sequencing data from these NAc of these mice, 147 DEGs were identified in cocaine treated mice, whereas zero DEGs were identified in saline or morphine treated mice, indicating that some feature of cocaine exposure rendered ZFP189VPR capable of regulating target genes.

Conclusions: These experiments indicate that the transcriptional regulation exerted by ZFP189 within the NAc specifically contributes to the progression of cocaine abuse yet does not contribute to basal behaviors or morphine abuse. The synthetic ZFP189VPR was capable of singularly potentiating cocaine-induce locomotor sensitization and transcription. The NAc cell-types in which this is occurring, the degree to which this represents a cocaine-specific or general psychostimulant phenomenon, and the mechanism through which cocaine enables ZFP189VPR to regulate transcription are all areas of future investigation. This work is evidence that in vivo neurotranscriptional control allows researchers to untangle mechanisms governing drug-specific disorders.

Keywords: Cocaine and Opioid Use Disorders, Artificial Transcription Factors, RNA Sequencing

Disclosure: Nothing to disclose.

P724. Prelimbic Cortex Encoding of Reward-Predictive Cues Following Outcome Devaluation

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Background: Animals must modify their behavior based on updated expected outcomes to effectively navigate changing environments. Rats that have undergone a history of cocaine are impaired in their ability to update expected outcomes to suppress behavior towards reward predictive cue in a Pavlovian outcome devaluation task. We have recently shown in naïve animals that the growth of prelimbic cortex (PrL) neural encoding across learning (from Day 1 and Day 10) predicts this ability to suppress approach behavior towards a reward predictive cue following outcome devaluation (West et al. 2021). Thus, greater enhancement of neural activity during learning may strengthen specific cue outcome associations to allow for rats to be able to adjust behavior in response to expected outcomes. In contrast, the rats that lost phasic responsiveness in the PrL during learning exhibited an inability to stop engaging with the cues in spite of devaluation suggesting that inflexibility is linked to a loss of PrL engagement during learning. In support, PrL neural activity during learning is dampened in cocaine-exposed rats which are impaired in their ability to suppress responding to the cue after outcome devaluation and PrL optogenetic inhibition during learning recapitulates this behavioral phenotype (West et al. 2021). How the PrL encodes updated outcome value "online" to the cues that predict expected outcomes (i.e., post-devaluation testing under extinction) and to cues that predict the actual updated outcomes (i.e., post-devaluation testing with outcomes delivered) is unknown.

Methods: To determine if PrL neural signaling tracks the ability to shift behavior, we recorded PrL neural activity to reward predictive cues after were trained on a pavlovian task (10 sessions) and following outcome devaluation. Briefly, male Long-Evans rats ($n = 18$) were presented with 2 cues as conditioned stimuli (CS +; predicting a sugar or food pellet) and 2 cues that did not predict a reinforcer (CS-). After 10 sessions, sugar pellets were devalued by pairing them with LiCl (0.3 M, i.p.). Food pellets were paired with a saline injection remaining nondevalued. This was repeated once. Rats were then tested under extinction to assess their ability to avoid the CS + associated with the devalued outcome. Next, the session was repeated, but with both nondevalued and devalued outcomes delivered (relearning). We examined the % of time spent in the food cup during the cues that predicted nondevalued and devalued outcome and calculated a "Devaluation Index" for each rat (ND-D/ND + D) to correlate to neurophysiological data for both test sessions.

Results: In both post-devaluation test sessions, rats spent less time in the food cup during the CS + associated with the devalued outcome (13.2 % +/- 1.6, extinction, 14.9 +/- 1.7%, relearning) compared to the CS + that predicted the nondevalued outcome (19.7 +/- 1.9%, extinction, 20.3 +/- 2.5%, relearning). PrL recordings revealed distinct neuronal populations that were excited or inhibited during the cues (classified as "phasic"). The % of PrL phasic neurons to the cue that predicted the devalued (D) outcome was significantly lower than to the cue that predicted the nondevalued (ND) outcome in the extinction test (D: 19.0 +/- 3.9% vs ND: 32.6 +/- 5.5%). In addition, the % of PrL phasic neurons to the cue that predicted the devalued (but not nondevalued) outcome during the extinction test negatively correlated with the ability to suppress behavior post-outcome devaluation ($R2 = 0.33$, $p < 0.05$ and $R2 = 0.03$; behavior measured as a Devaluation Index). PrL phasic responsiveness during the relearning test, however, was not different to cues that predicted the devalued and nondevalued outcomes (D: 32.0 +/- 6.2% vs ND: 35.6 +/- 6.6%). In addition, there was no correlation between the % of phasic neurons to the cue that predicted the devalued or nondevalued outcome during the relearning test ($R2 = 0.01$ and 0.02, respectively, behavior measured as a Devaluation Index).

Conclusions: Increased engagement of PrL neurons across learning is correlated to the ability to later avoid a cue associated with a devalued outcome (West et al. 2021) during the test session (extinction). Here, it is decreased PrL phasic responsiveness to the cue that predicted the devalued outcome that correlates with the

ability to avoid cues based on expected outcome value. Perhaps the increased strengthening of cue-outcome associations during learning allows for the differential and dynamic encoding of the PrL neural activity to the distinct cues that predict the nondevalued and devalued outcome during testing. When rats were presented with the actual outcome during the relearning test session, PrL neurons re-establish engagement to the cue predicting both nondevalued and devalued outcome (despite most rats avoiding the cue that predicts the devalued outcome and the outcome itself). Future studies will examine how these PrL neural processes are disrupted in male and female rats with a history of cocaine.

Keywords: Reward Devaluation, Prelimbic Cortex, Neurophysiology

Disclosure: Nothing to disclose.

P725. An Operant Paradigm That Discriminates Between Appetitive and Consummatory Behaviors Reveals Distinct Behavioral Phenotypes in Commonly Used Strains of Outbred Rats

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Background: Operant self-administration is often used to study alcohol drinking in a pre-clinical setting. Frequently, studies using operant paradigms infer the amount of alcohol consumed from the number of reinforcers delivered following completion of an appetitive response (e.g., lever press). However, recent work in mice suggests that appetitive responses are not reliably predictive of consumption. Whether this is also true in rats is unknown.

Methods: To address this gap, we trained adult male Sprague-Dawley ($n = 16$), Wistar ($n = 32$), and Long-Evans ($n = 32$) rats to lever press for ethanol using a paradigm that precisely measures appetitive and consummatory behaviors. Rats were habituated to ethanol using a standard two-bottle choice intermittent-access home cage drinking procedure. Rats were then trained on a fixed ratio 3 schedule where three presses on the active lever resulted in presentation of a cue light and 15s access to a lickometer-equipped sipper tube containing 20% ethanol.

Results: Three distinct operant phenotypes emerged during training with differing prevalence across rat strains. A significant number of Sprague-Dawley (81.3%) and Wistar (50.0%), but not Long-Evans (6.3%) rats maintained appetitive responding but failed to consume ethanol (Responders). In contrast, appetitive responding was predictive of consumption (Drinkers) in most Long-Evans (50.0%) and some Wistar (28.1%), but not Sprague-Dawley (6.3%) rats. No significant strain differences in ethanol intake or appetitive responding were observed within the two phenotypes. Importantly, appetitive responding was significantly positively correlated with intake in Drinkers ($r = 0.79$, $p < 0.001$), but not in Responders ($r = 0.28$, $p = 0.14$). A retrospective analysis of the relationship between home cage and operant drinking revealed significantly higher 24h ethanol intake in future Drinkers vs. future Responders ($p < 0.001$). Withdrawal from chronic ethanol exposure using the Lieber-DeCarli liquid diet procedure resulted in a significant increase in appetitive responding in both Drinkers ($p < 0.001$) and Responders ($p < 0.05$). A similar escalation in ethanol consumption was only observed in Drinkers ($p < 0.001$) and not Responders.

Conclusions: Together, these results uncover important strain differences in the propensity to operantly self-administer ethanol and support previous findings in mice cautioning the use of appetitive measures to infer consumption.

Keywords: Animal Models, Operant Behavior, Alcohol Consumption

Disclosure: Nothing to disclose.

P726. Differential Association Between Genetic Variation at the Glucagon-Like Peptide-1 (GLP-1) Receptor and Brain Functional Connectivity in Individuals With Low Versus High Severity of Alcohol Use

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Background: Growing evidence suggests that the glucagon-like peptide-1 (GLP-1) system is involved in mechanisms underlying alcohol seeking and consumption. Accordingly, the GLP-1 receptor (GLP-1R) has begun to be studied as a potential pharmacotherapeutic target for alcohol use disorder (AUD). We hypothesized that genetic variation at the GLP-1R, specifically two missense single nucleotide polymorphisms (SNPs) that lead to amino acid substitutions in the GLP-1R (rs6923761, glycine to serine at position 168; rs1042044, phenylalanine to leucine at position 260) will differentially correlate with brain functional connectivity in individuals with low versus high severity of alcohol use.

Methods: Potential participants were screened at the National Institute on Alcohol Abuse and Alcoholism (NIAAA) clinical program and eligible individuals were enrolled into a neuroimaging protocol ($N = 181$). As part of the screening, AUD identification test (AUDIT) was performed, according to which participants were categorized into high-risk alcohol use (total AUDIT score ≥ 8, $n = 96$, 53%) and low-risk alcohol use (total AUDIT score < 8, $n = 85$, 47%). Genomic DNA was extracted from whole blood and genotyped using the genome-wide Illumina OmniExpress BeadChip array. Following a dominant model, participants were categorized into two genotype groups according to the presence of the mutant/risk allele for each of the two GLP-1R SNPs of interest. For rs6923761, the two groups were A-allele carriers (AA + AG, $n = 65$) and non-A-allele carriers (GG, $n = 116$), and for rs1042044, A-allele carriers (AA + AC, $n = 114$) and non-A-allele carriers (CC, $n = 67$). Brain images were acquired by a Siemens 3T Skyra MRI machine. The resting-state functional MRI (rs-fMRI) lasted 10 minutes during which participants were instructed to stay awake while lying on their back in the dark with their eyes open and no additional stimuli. Following preprocessing, independent component analysis was used to analyze the rs-fMRI data. Of the 75 initial components, 24 were removed, and the remaining 51 were included in the statistical models. Multivariate analyses of covariance (MANCOVA) were run separately for each of the two GLP-1R SNPs. The models included GLP-1R genotype group (A-allele carrier versus non-A-allele carrier), AUDIT group (low-AUDIT versus high-AUDIT), and their interaction (GLP-1R genotype group × AUDIT group) as independent variables, and within- and between-network connectivity as dependent variables. Age, sex, years of education, body mass index, smoking status, and ancestry informative markers scores were also included as covariates.

Results: For rs6923761, three ICs (21, 35, 47) showed significant genotype × AUDIT interaction effects on within-network connectivity. IC35 and IC47 were mapped onto the anterior salience network and IC21 was mapped onto the visuospatial network. Post-hoc analyses showed that in the group carrying the variant allele (AA + AG), high AUDIT, compared to low AUDIT, was associated with stronger within-network connectivity, but weaker or no association was found in the protected group (GG). For rs1042044, four ICs (8, 45, 46, 54) showed significant genotype × AUDIT interaction effects on within-network connectivity. IC8, IC45, and IC54 were mapped onto the dorsal default mode

network and IC46 was mapped onto the basal ganglia network. Post-hoc analyses showed that in the group carrying the variant allele (AA + AC), high AUDIT, compared to low AUDIT, was associated with stronger within-network connectivity, but an opposite association was found in the protected group (GG). No significant genotype × AUDIT interaction effects on between-network connectivity were found.

Conclusions: Genetic variation at the GLP-1R was differentially associated with brain functional connectivity in individuals with low versus high severity of alcohol use. Specifically, the presence of the variant alleles was associated with stronger within-network connectivity in those with high versus low severity of alcohol use. Significant findings in the salience and default mode networks are particularly relevant, given their established role in the neurobiology of addictive behaviors.

Keywords: Alcohol, GLP-1 Receptor, Imaging-Genetics, fMRI Resting State

Disclosure: Nothing to disclose.

P727. Interactions Between Gabapentinoids and Heroin

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Background: Recent epidemiological studies suggest increasing misuse of gabapentinoids in people with opioid use disorder, and that co-use of gabapentinoids (e.g., pregabalin) 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. This study examined the effects of pregabalin on the rate-decreasing and ventilatory depressive effects of heroin, and on naloxone reversal of heroin-induced ventilatory depression in rats.

Methods: Eight male Sprague Dawley rats were trained to respond for sucrose pellets under a fixed ratio 10 schedule of reinforcement across four, 15-minute cycles. Each cycle consisted of a 10-minute blackout period and a 5-minute response period during which rats could earn up to 5 food pellets. Rats received an intraperitoneal (i.p.) injection of saline prior to the first cycle, followed by sham injections, or injections of saline or heroin prior to the subsequent cycles. Dose-effect curves were determined within-session using a cumulative dosing procedure where increasing doses of heroin were given i.p. across cycles 2-4, resulting in cumulative doses of 0.1, 0.32, and 1.0 mg/kg, respectively. Before some sessions, pregabalin (0.1-32 mg/kg; i.p.) was administered 15 minutes prior to the start of the first cycle. The rate of lever pressing in each cycle was compared across pretreatment condition. In another, preliminary study three male Sprague Dawley rats were given pregabalin (1-10 mg/kg) or saline intravenously (i.v.) prior to increasing doses of heroin with ventilation monitored by whole-body plethysmography. Sessions consisted of a 30-minute baseline period followed by a test period in which a cumulative dosing procedure was used, where rats received infusions of heroin or saline at minutes 0, 3, and 6 during the test period of each session; each infusion of heroin resulted in a cumulative dose of 0.178, 0.56, and 1.78 mg/kg (i.v.), respectively. Naloxone (0.01 mg/kg) or saline was administered i.v. 5 minutes following the last infusion of heroin. The primary outcome of this experiment was minute ventilation–the volume of air ventilated per minute and the product of tidal volume and respiratory rate. All procedures were approved by the UT Health Science Center at San Antonio Institutional Animal Care and Use Committee.

Results: Heroin dose-dependently reduced the rate of lever pressing, with the 1 mg/kg dose completely suppressing responding. The nature of the interaction between pregabalin and heroin varied among subjects, with the heroin dose-effect curve shifted leftward approximately 3-fold in 5 of 8 rats pretreated with pregabalin (i.e., responding was completely suppressed at 0.32 mg/kg heroin). The potency of pregabalin to exert this effect varied across these 5 animals, with the effective dose ranging from 0.32-3.2 mg/kg. Heroin dose-dependently reduced minute ventilation with a dose of 0.56 mg/kg (i.v.) reducing minute ventilation to 78% of baseline. Following i.v. pretreatment with 1 or 10 mg/kg of pregabalin this effect was enhanced, reducing minute ventilation to 53% and 38% of baseline, respectively. When administered 5 minutes after the largest dose of heroin naloxone (0.01 mg/kg; i.v.) restored minute ventilation from 33% to 101% of baseline 1 minute following administration. This dose of naloxone was much less effective at reversing the ventilatory depressant effects of heroin in rats pretreated with 10 mg/kg pregabalin, restoring minute ventilation to only 62% of baseline.

Conclusions: Pregabalin enhanced the rate-decreasing and ventilatory depressive effects of heroin, and markedly reduced the effectiveness of a single dose of naloxone to restore baseline levels of ventilation following a large dose of heroin. These findings have significant clinical implications and might help explain recent trends in opioid overdoses involving gabapentinoids. Future studies will determine whether this interaction is shared with gabapentin, the other gabapentinoid approved for use in humans, and characterize interactions between pregabalin and other mu opioid receptor agonists.

Keywords: Pregabalin, Opioid, Respiration

Disclosure: Nothing to disclose.

P728. Examining Factors That Predict Drug Use: Coping Style is a Stronger Contributor to Drug Use Than the Family Micro-Environment or Community Macro-Environment

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Background: Family history of drug and alcohol use is linked to risk factors for drug use later in life, including earlier first alcohol and drug use, higher impulsivity, impaired executive function, depression, and other mental health conditions. Neighborhood and community factors such as safety concerns, poverty, crime, income and other environmental disadvantages are also associated with drug use. In addition, individual factors such as coping style, emotional regulation, stress, and traumatic life events likely play a role. Dysfunctional coping in particular is linked to increased substance use, co-occurring psychiatric conditions, and adverse health outcomes. Here we examined how the family micro-environment, the community macro-environment, and individual traits, in particular coping, influence drug use in a community sample.

Methods: Approximately 500 participants in Baltimore and surrounding areas were recruited from 2016-2018. Assessments of the family micro-environment included the Children of Alcoholics Screening Test and Family Drug and Alcohol Use questions on the Addiction Severity Index (ASI). Community macro-environment measures included subjective assessments using the Perceived Neighborhood Scale (PNS) and neighbor conflict (ASI), as well as objective assessments using the Centers for Disease Control Social

Vulnerability Index (SVI) and tax code data. Drug use was assessed with the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and/or ASI. Additional assessments included the Life Events Checklist (LEC), Perceived Stress Scale (PSS), Difficulties in Emotion Regulation Scale (DERS), Coping Orientation to Problems Experienced, and age at first drug use (PhenX Toolkit). Covariates included gender, age, race, ethnicity, marital status, education level, number of children, and years living in a neighborhood. Data were examined in three ways: 1) analyses of variance (ANOVAs) to identify group differences in family, community, and other traits among participants with lifetime drug use of opioids/stimulants, lifetime use of other drugs (marijuana, alcohol), and no reported lifetime drug use; 2) stepwise regression modeling to identify contributors to any lifetime drug use, lifetime opioid use, lifetime stimulant use, or other lifetime drug use; and 3) stepwise regression modeling to identify contributors to any past 30-day drug use, past 30-day opioid use, past 30-day stimulant use; or past 30-day other drug use.

Results: Overall, 154 participants reported no lifetime drug use, 139 reported opioid/stimulant use, and 207 reported other drug use. Compared to no drug use, participants with any lifetime use reported significantly greater family conflict and drug/alcohol use, more adverse community environments (PNS, SVI), earlier age at first use, higher (worse) scores on the PSS, LEC, DERS, and poorer coping strategies ($3.8 < F < 74.5$; $0.001 < p < 0.03$). Compared to marijuana/alcohol use, participants with lifetime opioid/stimulant use reported more adverse community environments, earlier age at first drug use, higher scores on the PSS and LEC, and poorer coping strategies ($0.001 < p < 0.04$). While lifetime opioid use was predicted by low satisfaction with neighbors, socioeconomic vulnerability, difficulty engaging goal-directed persistence, and coping (positive reinterpretation, substance use; $-0.12<\beta < 0.31$; $0.001 < p < 0.03$), lifetime stimulant use was predicted primarily by coping (positive reinterpretation, substance use; $-0.13<\beta < 0.23$; $0.001 < p < 0.03$). Other lifetime drug use was predicted by family alcohol use, family and neighbor conflict, higher life stress and trauma, and coping (substance use, humor; $0.11<\beta < 0.25$; $0.001 < p < 0.05$). Similar findings occurred with past 30-day drug use: social vulnerability, goal-directed persistence, and coping predicted opioid use ($-0.11<\beta < 0.34$; $0.001 < p < 0.05$), while neighbor conflict, lower tax code values, and coping predicted stimulant use ($-0.17<\beta < 0.29$; $0.001 < p < 0.04$). Other past 30-day use was predicted by family drug use, family and neighbor conflict, lower tax code values, lack of emotional awareness and coping ($-0.1<\beta < 0.36$; $0.001 < p < 0.04$).

Conclusions: Among the measures collected here, the family micro-environment and community macro-environment were weaker predictors of drug use than individual factors, especially coping style. Although individual factors generally contributed more to drug use than either family history or community, the particular predictors varied by drug type. While prior family and community experiences cannot be changed, intervention efforts aimed at teaching more effective coping may help to reduce or prevent future drug use.

Keywords: Substance Use Disorder, Opioids, Stimulants, Coping

Disclosure: Nothing to disclose.

P729. Non-Invasive Neuromodulation With Focused Ultrasound Alters Target Specific Brain Activity

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Background: Development of non-invasive treatments for brain disorders is at the forefront of neuroscience endeavors. Novel techniques for neuromodulation have been developed to alter targeted cellular function under both transient and stable control. Widely used technologies such as chemogenetics and optogenetics posit control of designer/engineered receptors and channels with otherwise inert exogenous drugs or stimulation (i.e., light). However, these techniques require surgical delivery of viral vectors and/or implantation of obtrusive hardware. Focused ultrasound (FUS) has emerged as a neuromodulation technology that has the potential to bypass the invasive procedures outlined above. However, unlike chemogenetics and optogenetics, it is unknown to what extent local FUS stimulation in a specific brain region can be used to modulate whole-brain activity as well as activity of discrete, molecularly targeted cell types at the specific site of stimulation. The aim of these experiments was to assess the effect of specific FUS stimulation parameters at the CA1 region of the hippocampus in awake, freely moving mice on i) in vivo calcium activity of discrete cell types within this region and ii) whole-brain metabolic activity as assessed via positron emission tomography (PET) and the [18F]fluorodeoxyglucose (FDG) radioligand.

Methods: For fiber photometry experiments, mice were transduced using adeno-associated viral vectors with two different genetically encoded calcium indicators in the CA1 region of the hippocampus. Parvalbumin-positive interneurons in CA1 were targeted with jRGECO1a and CaMKII-positive pyramidal neurons were targeted with GCaMP6s. A fiber optic implant was cemented on the skull targeting the hippocampus region to record from cells during ultrasound stimulation. FUS was delivered transcranially and effective parameters were optimized by manipulating both pressure and frequency of the stimulation. For PET experiments, mice were implanted with a peg on top of the skull with coordinates aimed over the CA1 region. The peg was used to hold the transducer in place to deliver FUS. Mice were injected with FDG and stimulated with a 900Hz stimulation 10 times over 30 minutes in an open field where mice were free to move. Following simultaneous FUS stimulation and FDG uptake, mice were anesthetized and scanned for 20 minutes with PET. FDG uptake was assessed via voxel-wise methods to determine unbiased changes in metabolic activity between the non- and stimulated hemispheres.

Results: Acoustic pressure fields were examined for the transducer operating attached to an explanted skull using a scanning hydrophone. To optimize pressure needed for effective stimulation of the targeted area, various pressures (0.26, 0.43, 0.56, and 0.76 Mpa) were tested and calcium signaling was recorded with jRGECO1a and GCaMPP6S. The higher pressures (0.56 and 0.76 Mpa) elicited the most significant increase in calcium signaling in both parvalbumin and pyramidal neurons. Frequency stimulation of 900Hz produced an initial brief increase in calcium signaling but quickly caused a prolonged depression of calcium signaling that persisted >30sec. The results found in the fiber photometry experiment were further similarly confirmed with PET. Following 900Hz stimulation in awake freely moving mice, FDG uptake analysis showed a significant decrease in the region proximal to the site of stimulation within the hemisphere that was stimulated versus the non-stimulated contralateral side. Threshold mapping found that the FDG signal was most depressed at the targeted CA1 region of the hippocampus.

Conclusions: Here we show that focused ultrasound can alter in vivo cellular activity in a manner that can facilitate further research into the field of neuromodulation. The invasive techniques used in these experiments (i.e., viral transduction, fiber implant) were done to test the feasibility of this novel FUS technique. As confirmed in the PET experiments where only minimally to non-invasive techniques were used, FUS produced reliable decreases in cellular activity at the targeted site. This specificity is crucial for elucidating neurocircuitry with a high potential for clinical applications to treat human brain diseases.

Future studies will examine the long-term effects of chronic stimulation as well as potential for manipulating drug related behaviors. Additionally, FUS may be used as a potential method to deliver systemic compounds to specific brain regions without the need for neurosurgery. Such approaches would also be amenable to be assessed via PET for efficacy both acutely and longitudinally.

Sponsored by Dr. Michael Lewis

Keywords: Positron Emission Tomography (PET), Focused Ultrasound, Non-Invasive Neuromodulation

Disclosure: Nothing to disclose.

P730. Oxycodone Physical Dependence Promotes Gene Adaptations in the Brain Reward Pathway Under Neuropathic and Pain-Free States

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Background: The development of physical dependence and addiction disorders due to misuse of opioid analgesics is a major concern with current pain therapeutics. Chronic pain, a debilitating disorder characterized by a range of sensory and neuropsychiatric symptoms, i.e., anxiety, depression, sleep disturbance, and increase stress, is linked with Opioid Use Disorder (OUD). Very few treatment strategies target both the physical and emotional symptoms associated with chronic pain, and most available treatments show limited efficacy and tolerability and produce severe side effects. Approximately 20-25 percent of chronic pain patients are treated with an opiate despite the lack of effectiveness and adverse outcomes associated with this treatment strategy. Moreover, the increased rates of prescription opioids for the treatment of chronic pain lead to a dramatic increase in the number of patients that become dependent on opioids and transition to addiction. Recent studies from our group revealed that chronic neuropathic pain promotes several gene adaptations in reward-related brain regions (Descalzi et al. Sci. Signaling, 2017). Here, we are using Next Generation RNA sequencing and pathway analysis to gain insight into gene expression adaptations triggered by persistent exposure to oxycodone in the presence and absence of chronic neuropathic pain.

Methods: To assess opiate misuse under conditions of chronic pain and pain-free states, we subjected two-month-old C57BL/6 mice to Spared Nerve Injury (SNI) model of neuropathic pain or sham surgery. Mice were assessed for sensory hypersensitivity (mechanical allodynia and thermal hyperalgesia) associated with long-term neuropathic pain bi-weekly for 9 weeks, followed by two weeks of daily s.cu oxycodone (30mg/kg) or saline injections. Following the two weeks of chronic Oxycodone exposure, animals underwent an extended period of spontaneous drug withdrawal. At that point, we monitored sensory hypersensitivity and emotional behaviors to understand the effects of oxycodone withdrawal in the context of chronic pain and pain-free states. To this extent, we performed several behavioral assays such as Novelty social recognition assay, Novel Suppress Feeding (NSF), marble burying, Light-dark assay, and locomotor activity throughout the 3-week drug withdrawal period to assess emotional behaviors associated with drug withdrawal. We next sort to understand the molecular mechanisms underlying oxycodone withdrawal, we did a separated cohort of mice in our oxycodone misuse paradigm, and 21 days post-drug cessation, we dissected medial prefrontal cortex (mPFC), Nucleus Accumbens (NAc), and Ventral Tegmental Area (VTA) and performed RNA sequencing on whole brain tissue. Using pathway analysis, we identified Histone deacetylase 1 (HDAC1) as a top upstream regulator in the NAc and mPFC. Using a novel inhibitor for HDAC1/2, RCY1305, we treated mice with RCY1305 (3mg/kg i.p daily) during oxycodone administration and drug withdrawal for 5- weeks. We then assessed the effect of HDAC1/2 inhibition on oxycodone-induced behavioral changes, i.e., sensory hypersensitivity and emotional and motivational deficits.

Results: Oxycodone administration induced thermal hyperalgesia after five days of daily injection in chronic pain mice but not pain-free mice. During early drug withdrawal, four days post drug cessation, both pain and pain-free developed significant thermal hyperalgesia compared with saline controls (ANOVA, F (3,248) =57.6). Oxycodone treatment alleviates symptoms of mechanical allodynia in SNI mice and produces significant mechanical allodynia in Sham mice during early drug withdrawal (d7) (ANOVA, (3,279) = 748.5). Next, we assessed the emotional symptoms affected during drug withdrawal states. In the social interaction test, all groups show a preference for the social target except for SNI mice undergoing oxycodone withdrawal, which displayed significant social deficits (ANOVA, F (3,58) =1.418). Furthermore, all groups except for our Sham saline controls display significant deficits in recognizing a novel social target (ANOVA, F (3,58) =0.5616). To assess anxiety-like behaviors, we perform the light-dark box and Marble burying assay. SNI mice undergoing oxycodone withdrawal spent significantly less time exploring the light side of the box. In the Marble burying assay Sham- Oxycodone mice buried a significant percent of marbles compared to SNI- oxycodone and their controls. Using RNA Sequencing, we monitored changes in gene expression in the medial prefrontal cortex, the nucleus accumbens, and the ventral tegmental area. Although oxycodone treatment promotes mostly unique transcriptome profiles across brain regions, we observed similar downstream effectors and transcription factors affected in pain states compared with non-pain states. Our pathway analysis revealed that Histone deacetylase 1 (HDAC1), an epigenetic modifier with a prominent role in striatal plasticity, is a top upstream regulator with opioid withdrawal in both the NAc and the mPFC.

Conclusions: Our findings suggest that chronic pain states exacerbate the behavioral and transcriptomic signatures of oxycodone withdrawal. Overall, our studies highlight intracellular pathways, thereby providing novel possibilities for treating oxycodone dependence under neuropathic and pain-free states.

Keywords: Chronic Pain, Opioid Dependence, Pharmacotherapy, Animal Model, Withdrawal, Transcriptomics, Negative Affect, Mesolimbic Reward Circuitry

Disclosure: Nothing to disclose.

P731. Role of Dynorphin/κ-Opioid Receptor on Fentanyl Vapor Self-Administration in Mice

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Background: The United States and many other countries are facing a deadly opioid overdose epidemic. Two-thirds of the drug overdose deaths in the United States in 2018 involved an opioid, and 66% were linked to synthetic opioids such as fentanyl. Currently, treatment options for opioid use disorder are substitution therapy (e.g., methadone and buprenorphine) or the nonselective opioid receptor antagonist naltrexone. We have previously found that the long-lasting κ-opioid receptor (KOR) antagonists 5'GNTI and norBNI reversed the hyperalgesia observed during heroin acute withdrawal in rats of both sexes and that norBNI blocks heroin self-administration escalation in male rats. Recently, short-acting κ-opioid receptor antagonists have gained interest as potential therapeutics for anhedonia and

depression, but little has been done with these compounds in addiction models.

The aim of this preliminary work was to investigate whether KOR antagonism and pro-dynorphin deletion decrease fentanyl self-administration.

Methods: First, we trained 64 C57BL/6J mice (32 males and 32 females) and pro-dynorphin knockout (KO) and wild-type (WT) mice (16 female KO, 18 female WT, 19 male KO and 20 male WT) to self-administer vaporized fentanyl (5 mg/mL, 60 W, 1.5 sec) and then split them between short access (ShA, 1-h sessions; non-dependent) and long access groups (LgA, 6-h sessions; dependent). We tested the hypothesis that (1) the short-acting KOR antagonist aticaprant and the canonical KOR antagonist norBNI would decrease fentanyl vapor self-administration in dependent but not nondependent C57BL/6J mice; (2) the deletion of the gene encoding pro dynorphin (pDyn KO) would prevent fentanyl self-administration escalation. All animal studies were approved by the local ACUC and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All data will be presented as mean + SEM and analyzed by RM one or two-way ANOVA followed by Dunnett's post hoc test.

Results: LgA mice escalated their fentanyl self-administration, whereas ShA mice did not. Using a within-subjects Latin square design, we tested aticaprant (0, 0.3, 1, 3, 10, and 30 mg/kg, PO, 75 min before the session) on fentanyl self-administration. Since we did not detect any sex differences, males and females were analyzed together to increase statistical power. Aticaprant failed to reduce fentanyl self-administration in the dependent groups ($F5, 75 = 1.781$, $P > 0.05$), whereas the highest dose produced a slight increase in fentanyl self-administration in nondependent mice ($F5, 74 = 4.362$, $P = 0.07$). The two cohorts of mice that went through escalation and behavioral testing then were allowed a 3-week abstinence phase and then received norBNI (10 mg/kg, IP, $N = 8$ male, and 8 female) or saline (10 mL/kg, IP, $N = 8$ male, and 8 female) before a 9-session re-escalation phase. Males and females were analyzed together since no sex differences were detected. A single treatment with norBNI before re-escalation, significantly reduced average fentanyl intake in dependent mice but not on non-dependent mice ($F1, 60 = 4.998$, $P = 0.03$).

Mice lacking the pro-dynorphin gene (pDyn KO) were tested using the same self-administration protocol. Dependent mice escalated their fentanyl self-administration ($F9, 287 = 6.262$, $P < 0.001$) regardless of the group, contrary to our hypothesis female pDyn KO mice showed a trend to escalate their intake faster and to take more fentanyl than their WT counterparts.

Conclusions: These preliminary data suggest that sustained antagonism of κ-opioid receptors may be required to reduce fentanyl self-administration. The effect of chronic treatment with short-acting κ-opioid receptor antagonists on opioid self-administration remains to be tested. Deletion of the pro-dynorphin gene may have sex-dependent effects that merit further study.

Keywords: Prodynorphin, Kappa Opioid Receptor Antagonist, Opioid Use Disorder

Disclosure: Nothing to disclose.

P732. Pharmacological Profiling of Non-Fentanyl Synthetic Opioids That are Appearing in Clandestine Drug Markets

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Background: Illicitly manufactured fentanyl is largely responsible for the current opioid overdose crisis, but a number of non-fentanyl synthetic opioids have emerged on non-medical (i.e., clandestine) drug markets worldwide. Little information is available about the pharmacology and toxicology of novel synthetic opioids when they first appear on the street. U-47700, brorphine, and isotonitazene are examples of non-fentanyl synthetic opioids that are associated with opioid overdose fatalities in human users.

Methods: Here, we profile the pharmacology of several non-fentanyl synthetic opioids - including U-47700, brorphine, and isotonitazene - using in vitro and in vivo methods. In vitro receptor bindings assays were carried out in rat brain membranes to examine the ability of test drugs to displace [3H]DAMGO, [3H] DADLE, or [3H]U-69593 from mu-, delta-, or kappa-opioid receptors, respectively. In vivo experiments were carried in male Sprague-Dawley rats to evaluate the effects of subcutaneously (s.c.) administered test drugs on antinociception, catalepsy, and body temperature. All experiments involving animals were approved by the Animal Care and Use Committee of the NIDA Intramural Research Program.

Results: Findings from [3H]DAMGO binding assays showed that Ki values for U-47700 (12.0 nM), brorphine (37.7 nM), and isotonitazene (14.9 nM) at the mu-opioid receptor are weaker than that of morphine (4.0 nM) ($N = 3$ experiments for each dose response curve). Nevertheless, all test compounds were selective for the mu-opioid receptor over delta and kappa sites. When administered to male rats in vivo, all compounds induced dose-dependent opioid-like effects, including antinociception, catalepsy, and hypothermia ($N = 8$ rats for each drug dose). Findings from the hot plate test revealed that ED50 values for U-47700 (0.404 mg/kg, s.c.), brorphine (0.086 mg/kg, s.c.), and isotonitazene (0.007 mg/kg, s.c.) are much more potent when compared to morphine (4.163 mg/kg s.c.). Isotonitazene was even more potent than fentanyl (0.021 mg/kg, s.c.) as an antinociceptive agent.

Conclusions: Our results demonstrate that non-fentanyl synthetic opioids can be much more potent than morphine in vivo, suggesting a serious risk of overdose for unsuspecting users. Importantly, the in vitro mu-opioid binding affinities for synthetic opioids may not predict in vivo potency. Comprehensive in vitro and in vivo investigations are needed to determine the pharmacology and toxicology of novel synthetic opioids that are emerging in clandestine drug markets.

Keywords: Opioid Abuse, Analgesia, Catalepsy, Mu-Opioid Receptors

Disclosure: Nothing to disclose.

P734. Assessing the Effect of Neuroimmune Modulation on Subjective Response to Alcohol in the Natural Environment

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Background: Development of novel and more efficacious treatments for alcohol use disorder (AUD) is a high research priority. Identifying treatment mechanisms of action is an important step toward improving treatment efficacy, which can be enhanced by using more reliable and cost-effective assessment methods, such as daily diary reports. A growing body of research connects the immune system with the development and maintenance of AUD. As such, considerable interest exists for novel treatments that can restore healthy levels of inflammation and immune signaling to reduce drinking and promote recovery. Phosphodiesterase (PDE) inhibitors have been tested extensively in preclinical animal models of AUD, as certain PDEs are expressed in brain regions indicated in the reinforcing effects of alcohol. Ibudilast, which is a neuroimmune modulator and selective PDE

inhibitor, reduced drinking and relapse in several animal models of AUD. Further, our laboratory has tested ibudilast in two clinical samples of AUD, where ibudilast treatment, compared with placebo, decreased neural alcohol cue-reactivity and reduced rates of heavy drinking. In addition, while ibudilast did not significantly alter subjective response to intravenous alcohol administration across all subjects, it attenuated the stimulant effects of alcohol among individuals with high depression symptomatology, which warrants further exploration. Thus, ibudilast is a promising AUD pharmacotherapy but its mechanisms of action remain largely unknown in human clinical samples. The current study sought to test the effect of neuroimmune modulation by ibudilast on subjective response to alcohol in the naturalistic environment in a two-week randomized trial, which enrolled participants with AUD.

Methods: This study serves as a secondary analysis of a two-week clinical study (NCT03489850) of ibudilast for negative mood improvement and drinking reduction that enrolled non-treatment seeking males and females with an AUD. Fifty-two eligible participants were randomized to ibudilast or matched placebo. Participants completed three in-person visits and morning electronic daily diary assessments to report on their drinking, craving, and mood from the previous day. When participants reported previous day alcohol consumption, they were asked to report on mood and craving both before and during drinking, as well as stimulation and sedation during drinking. Participants who completed at least one daily diary report following randomization ($n = 50$: ibudilast = 23, placebo = 27) were included in these analyses. Multilevel mixed models with random intercepts and relevant covariates (e.g., sex, baseline drinking, depression) tested the effect of medication condition (ibudilast vs. placebo) on craving, urge, stimulation, and sedation. Moreover, multilevel mixed models with random intercepts and cross-level interaction tested whether ibudilast moderated the effect of subjective response on same-day and next-day alcohol consumption.

Results: While participants on ibudilast reported lower average levels of stimulation and sedation, this between-subjects medication effect was not significant (p's > .05). However, analyses revealed a significant cross-level medication x stimulation ($p = .046$) and medication x sedation ($p = .049$) interaction on same-day but not next-day alcohol intake. Using simple slopes to probe these interactions, individuals on ibudilast reported a stronger relationship between stimulation ($p < .001$) and sedation ($p = .001$) on same-day drinking than those on placebo (p's > .250). Overall, participants across medication conditions reported alcohol-induced increases in urge and craving during the trial. Moreover, results revealed a significant medication x time (before vs. during drinking) interaction for alcohol craving, such that participants on ibudilast reported significantly smaller same-day alcohol-induced increases in craving ($p = .180$) than those on placebo ($p < .0001$). This same effect was not detected for urge to drink. However, ibudilast significantly moderated the effect of average change in urge (during - before drinking urge) on alcohol use, such that for ibudilast ($p = .022$) but not placebo ($p = .772$), a significant positive relationship between average urge to drink and number of drinks was detected.

Conclusions: This investigation furthers our understanding on mechanisms of action for neuroimmune modulators in clinical samples of AUD by utilizing ecologically valid reports. While ibudilast did not robustly alter stimulation and sedation in response to alcohol in the natural environment, it appeared to enhance participant perception of the relationship between subjective response and same-day alcohol consumption. In line with previous reports from our laboratory, ibudilast reduced craving, such that it attenuated alcohol-induced increases in craving. These findings should be considered in the context of ibudilast's immunomodulatory and biological actions. Future research should extend these findings to treatment-seeking and higher severity samples of AUD and explore sex differences.

Keywords: Alcohol Use Disorder - Treatment, Subjective Response, Neuroimmune

Disclosure: Nothing to disclose.

P735. Oxycodone Behaviors in Cacna1h Knockout Mice

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Background: The opioid use disorder (OUD) epidemic constitutes an expanding public health crisis, with opioid related deaths peaking in 2020. The majority of OUD related deaths are attributed to prescription opioids, with the semi-synthetic opioid oxycodone (OXY) being one of the most prescribed opioids and contributing substantially toward OUD-related deaths. However, current OUD therapeutics are limited, and relapse rates remain high, illustrating a critical need for understanding factors underlying OUD risk, which could lead to more effective OUD therapeutics. There is a heritable basis for OUD risk, opioid use, and opioid behavioral and physiological responses, so determining the genetic basis of OUD-related traits could aid in therapeutic developments through identifying novel genes, mechanisms, and downstream biological processes regulating drug responses. Human genome-wide association studies (GWAS) have so far identified few quantitative trait genes for OUD related phenotypes. Quantitative trait locus mapping in rodent models can overcome limitations of human GWAS, efficiently elucidating genetic causes of addiction-relevant model phenotypes. To identify genetic variants of OUD-relevant behaviors in mice, we surveyed 29 strains of mice for strain-specific differences in behavioral responses to OXY to identify strain-specific OUD addiction model behaviors and underlying genetic factors which we then served to validate through in vivo genetic manipulations.

Methods: All strains of mice were tested through our multistage addiction assessment protocol (MSAAP). Mice first underwent conditioned place preference (CPP) for 1.25mg/kg OXY to assess strain differences in OXY locomotion, OXY environmental preference, and OXY state-dependent reward learning. Mice subsequently were injected daily with 40mg/kg OXY for 1 week then assessed for 4 mg/kg OXY analgesic tolerance using a hotplate antinociceptive test. Mice were injected again with daily 40mg/kg OXY for 1 week then tested for acute OXY withdrawal using the elevated plus maze (EPM) and light-dark conflict tests. Data were analyzed using 2-way (Genotype, Treatment) and 3-way (Genotype, Treatment, Sex) ANOVAs, with subsequent post-hoc comparisons performed with Holms-Sidak corrections for significant main effects or interactions. Haplotype association mapping was performed on tissue samples to identify genetic regions associated with differential OXY phenotypes.

Sample Sizes (Genotype, Treatment, Sex):
Wild-type, Saline, Male: 14
Wild-type, Saline, Female: 13
Wild-type, OXY, Male: 11
Wild-type, OXY, Female: 10
KO, Saline, Male: 8
KO, Saline, Female: 10
KO, OXY, Male: 10
KO, OXY, Female: 10

Results: Using CPP, we found robust strain differences in OXY-induced locomotion, place preference, and state-dependent learning, indicative of causal genetics for these phenotypes. We additionally observed strong heritability for OXY-induced locomotion ($h2 = 0.72$). Subsequent haplotype association mapping

identified a region on chromosome 17 associated with OXY-induced locomotion containing the candidate gene Cacna1h, encoding a voltage-gated calcium channel subunit ($p = 2.9e-8$). We subsequently tested Cacna1h knockout (KO) mice on a mixed 129/C57BL/6N background backcrossed to C57BL/6N to the N2 generation (and then subsequently intercrossed) in OXY CPP, in addition to hot plate analgesia, EPM, and light-dark conflict to assess differential responses to OXY tolerance and withdrawal between genotypes. We did not observe any significant genotypic differences in distance traveled in response to OXY. However, Cacna1h KOs with previous OXY history travel shorter total distances during the place preference session (Genotype Main Effect: $F(1,82) = 5.511$, $p = 0.0213$; OXY-treated post-hoc: $p = 0.0219$). The effect of Cacna1h KO was specific to locomotion associated with OXY CPP, as there were no genotypic differences in OXY CPP or state-dependent learning, nor were there any significant differences OXY antinociceptive tolerance or spontaneous withdrawal in the elevated plus maze or the light/dark box.

Conclusions: Our negative findings currently fail to support a role for Cacna1h in opioid addiction model behaviors. However, we have only tested a single genetic background, and it may be useful to test Cacna1h KO on different genetic backgrounds to fully rule out its contribution to opioid behavioral responses. Further genetic mapping from our MSAAP strain survey could uncover additional genetic variants which, through in vivo validation, can inform OUD-relevant behavioral phenotypes.

Keywords: Mouse Genetics, Opioid Use Disorder, Haplotype Association Mapping, Addiction Phenotypes

Disclosure: Nothing to disclose.

P736. Advancing Psychiatric Genetics Research in Latinx Populations

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Background: There is a pressing need to engage more diverse research participants and researchers to diversify genomics studies and the genetics workforce. Collaborative and coordinated efforts will facilitate the work needed to advance the understanding of the specific genetic susceptibilities of diverse and admixed populations. These efforts must also involve significant input from the communities that are impacted so that the studies and diversification efforts will benefit the participants involved. Incorporating non-European and mixed-ancestry populations in genetic analyses can facilitate the identification of genetic effects that generalize across populations, pinpoint causal variants, and ascertain genetic variants that are population-specific. Furthermore, there are well-studied health disparities across populations, highlighting the need for more equitable genomics studies.

Methods: The Latin American Genomics Consortium (LAGC) was founded in 2019 to accelerate psychiatric genetics research in Latinx populations, particularly among Latin American countries and the US.

Results: The LAGC has ~100 active members, representing 8 different Latin American countries/territories including Mexico, Argentina, Costa Rica, Brazil, Colombia, Chile, Peru, Puerto Rico, as well as the US.

Conclusions: This initiative aims to not only address the enormous underrepresentation of non-Europeans in genomics studies, but to facilitate access to training and resources, and engage in collaborations globally.

Keywords: Genomic, Consortium, Latin America

Disclosure: Nothing to disclose.

P737. The Effect of Early Life Adversity on the Basolateral Amygdala Transcriptome

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Background: Experiencing adversity early in life can be a risk factor for the development of many psychiatric disorders, including SUD. Yet, most individuals exposed to early life stress do not go on to develop disorders. Stress that is not overwhelming can have an "inoculating" effect that promotes the development of resilience later in life. Our lab models early life adversity using limited bedding and nesting model (LBN). Previously we found this manipulation produces a resilient-like phenotype against addiction-related behaviors. Particularly in male rats, LBN reduces impulsive choice, a behavior mediated, in part, by the basolateral amygdala (BLA) behavior. We also found that this model reduces morphine self-administration in male rats only. This suggests that LBN produces an inoculating effect against addiction-related behavior in males. We therefore sought to delineate possible molecular underpinnings that promote stress-induced resilience.

Methods: To understand how early life adversity impacts the BLA, we used a rodent model of early life stress called the LBN, which mimics a low resource environment by restricting a dam's access to nesting materials during the pups first week of life. 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. On PND10 LBN animals were moved back to standard laboratory housing conditions. RNA sequencing was conducted to delineate the effect LBN had on the transcriptional profile of the BLA in adult rats (male control, $n = 4$; female control, $n = 5$; male LBN, $n = 5$; female LBN, $n = 4$). BLA tissue from adult, behaviorally naïve, rats were sequenced on an Illumina HiSeq 4000. Fastqc version 0.11.8 was used to evaluate the quality of reads with adaptors and nonpaired 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 found LBN-induced sex-specific changes in transcription. RRHO analysis revealed distinct genes upregulated and downregulated in males and females due to LBN. There was very little overlap in genes upregulated in males and females or downregulated in males in females. A large proportion of the 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. Of interest Grin1, the gene for the glutamate ionotropic receptor NMDA type subunit 1, was downregulated by LBN in females and pathway analysis found LBN -induced changes in glutamate pathways in females. In males, pathway analysis found LBN-induced changes in the MAPK pathways and several MAPKs, such as MAPK10, were upregulated in LBN compared to control males.

Conclusions: These analyses highlight unique patterns of gene expression LBN induces within the BLA in a sex-specific manner. Future work will focus on validating top targets with RNAscope and manipulating targets in a cell-type specific manner to directly link changes in gene expression to addiction-related behavior. Collectively, this work furthers our understanding of the neurobiological underpinnings of stress inoculation which may lead to advanced therapeutic techniques.

Keywords: Early-Life Adversity, Substance Use Disorder, Basolateral Amygdala

Disclosure: Nothing to disclose.

P738. How Do Real-World Contextual Factors Influence Decision-Making Preferences in Opioid Use Disorder? A 4-Week Ecological Momentary Assessment Study on the Temporal Dynamics of Optimism and Mood in Decision Making

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Background: Value-based decision-making is an integral concept to understanding addiction, including opioid use disorder (OUD). Despite availability of gold-standard treatments (i.e., medications for opioid use disorder, or MOUD), people with OUD continue to cycle between states of preoccupation-anticipation, drug use, and abstinence/withdrawal. Changes to normative cognitive processes related to valuation and decision-making, such as tolerance of risk and delay, are thought to subserve these clinically relevant transitions but empirical support for this association is lacking, particularly in patients' natural day-to-day environments. Further, psychological, and contextual factors, such as optimistic beliefs and affective states, may influence the valuation process to promote risk for reuse. However, the relationship between these factors and decision-making preferences in OUD over time remains relatively unknown. In the current study, to examine the real-word interaction of mood, optimism, and decision-making preferences, we monitored decision-making behavior on a suite of smartphone-enabled economic tasks as participants engaged in their natural environments. We anticipated that individuals with OUD, relative to healthy controls, would demonstrate preferences for riskier and more immediate rewards and that these preferences would fluctuate especially during periods of elevated mood and optimistic thinking.

Methods: Nineteen treatment-engaged OUD patients (58% male; 43.4 years ± 13.01) were recruited from two university-affiliated MOUD clinics. Eighteen healthy controls (55% male; 52.8 years ± 13.16) were recruited from the same geographic area of residence and matched to OUD subjects for sex and race/ethnicity. All subjects participated in a 4-week smartphone-based ecological momentary assessment study (704 person days; range: 7-30 days/subject). Prompts were sent 4-8 times-per-day that probed current affective (e.g., positive, and negative mood) and cognitive (e.g., optimistic thinking) states. Additionally, once-per-day, subjects completed validated behavioral economic tasks measuring preferences for real delayed and risky monetary rewards. One decision each day was selected and paid out for real at the end of each study week. The impulsive choice task probed preferences for monetary rewards that could be delivered with different delays. The risky choice task probed preferences for monetary rewards or losses that could be delivered with different known and unknown (ambiguous) probabilities. Day-to-day decision-making preferences for patients and controls were predicted from self-reported affective state and optimistic thinking. Two-way interactions were developed from these predictors (number of observations = 584, linear mixed-effects model with random intercepts and slopes for subject) with degrees of freedom computed using Satterthwaite approximation.

Results: Subjects demonstrated dynamic decision-making preferences with high variability across days for risky and ambiguous choices (SD = 0.31). While there were no significant aggregate-level group differences for risk or ambiguous choice between patients and controls, changes in risk preferences were related to fluctuations in mood and optimistic thinking. We found that when subjects reported being more optimistic, they preferred riskier offers, especially in the context of unknown/ambiguous losses (b = 0.0009, 95%CI [0.00008, 0.002], t44.74 = 1.71, $p = 0.03$). Subjects also tended to make riskier decision when they reported being in a better mood (b = -0.0009, 95%CI [-0.0001, 0.0018], t44.74 = 1.71, $p = 0.09$) with a stronger coupling between mood and risk-taking observed in patients (b = -0.0019, 95%CI [-0.0001, 0.0018], t89.22 = -2.288, $p = 0.02$). By contrast, we found that impulsive choice was increased overall in patients compared to controls (t30 = 2.61, $p = 0.01$), but was relatively stable across days and unrelated to changes in mood states and day-to-day optimistic thinking.

Conclusions: Together, our findings suggest a dynamic interaction of mood and optimism on decision making preferences, especially for risky choices, potentially identifying a mechanism for detecting and responding to specific cognitive and contextual changes associated with at-risk clinical states in OUD (i.e., reuse/relapse).

Keywords: Opioid Addiction, Value-Based Decision-Making, Ecological Momentary Assessment, Optimism, Mood

Disclosure: Nothing to disclose.

P739. Alterations in Adolescence Sleep and Circadian Rhythm as Potential Factors That Increase Risk of Substance Use Disorders

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Background: Circadian clocks regulate reward-related neuronal mechanisms and disruptions to these clocks increase substance use. In turn, drug abuse disrupts time-keeping mechanisms of circadian clocks in reward circuits, thus contributing to substance use disorders (SUDs). Adolescence is a vulnerable period for both circadian disruption and the development of SUDs because it is a transitional stage accompanied by more reward driven, impulsive, and sensation seeking behaviors. The reciprocal interaction between circadian clocks and reward pathways is a critical aspect to the development of SUDs in adolescence, but the molecular mechanisms underlying this link are not fully understood. Therefore, as a part of the Center for Adolescent Reward, Rhythms, and Sleep, our primary goal is to understand how genetically abnormal rhythms and environmental rhythm disruptions contribute to vulnerability to substance abuse during adolescence.

Methods: To understand the relationship between natural variations in circadian traits and drug addiction, we conducted multiple assays to measure circadian rhythms, addiction-related behaviors including the 5-choice serial reaction time task (5CSRTT) and intravenous self-administration using Heterogenous Stock (HS) outbred adolescent rats (P28-48). We also utilized primary skin fibroblasts from rats with longer period and lower amplitude to test potential therapeutic compounds for their ability to

modulate molecular rhythmicity in vitro. To address effects of environmental circadian disruption such as jet lag on reward functions, 5CSRTT and electrophysiological recording in neuronal tissues were conducted in rats with chronic disruptions to their light-dark cycle (12 hour every 3 days). In addition, we collected the prefrontal cortex and nucleus accumbens (NAc) following acute sleep disruption in order to measure alterations in gene expression using RNA sequencing.

Results: We found that HS rats displayed high circadian phenotypic diversity compared to conventional rat crosses presumably due to their genetic heterogeneity. This indicates that this model is a powerful tool to study complex traits such as circadian rhythms and addiction-related behaviors. We observed a wide range in circadian period and daily sleep percentage in these rats (23.76 - 24.18 hour and 32 - 56% respectively). Our rat fibroblast cultures exhibited similar period lengths as those observed in behavioral rhythms. Also, our preliminary test showed that previously identified compounds that enhance rhythms in mouse cell cultures produce similar effects on rat molecular rhythms. Interestingly, rats with chronic disruptions to their light-dark cycle took longer to acquire the 5CSRTT compared to those with fixed light-dark cycles. Furthermore, electrophysiology recordings of NAc indicated that circadian misalignment altered synaptic AMPAR levels and membrane excitability in medium spiny neurons.

Conclusions: Our preliminary data demonstrate that genetic diversity contributes to phenotypic variability in circadian rhythms and circadian misalignment during adolescence increases addiction-related behaviors in HS rats. In addition, our transcriptome study will provide further useful information for elucidating the mechanisms underlying the mutual interaction between circadian rhythms and drug of abuse during adolescence.

Keywords: Adolescence, Circadian Rhythms, Substance Use Disorder, Outbred Rats

Disclosure: Nothing to disclose.

P740. Gut Microbiome-Derived Metabolites Act as Key Regulators of Transcriptional Homeostasis and Cocaine-Seeking After Abstinence in a Rat Model of Cocaine Use Disorder

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Background: Pathological substance use disorders are devastating neuropsychiatric illnesses leading to extensive morbidity and mortality. Despite major advances in understanding the intracellular signaling cascades and neuronal signaling networks associated with prolonged drug use, there are currently no FDA-approved medications for the treatment of psychostimulant use disorders. This has led to the growth of work examining peripheral factors as potential targets for translational research. A growing body of literature has characterized the important crosstalk between the brain and the resident population of bacteria in the gut - the gut microbiome. This signaling can influence both normal brain function as well as pathogenesis in animal models of neurodegenerative illnesses as well as neuropsychiatric illnesses such as autism, depression, and addiction. While the complex mechanisms of this gut-brain communication require further investigation, robust evidence indicates that the gut microbiome produces numerous neuroactive metabolites, which can signal to the brain and modulate brain function. The strongest evidence exists for a class of bacterially-derived metabolites known as Short-Chain Fatty Acids (SCFA), which have been shown to modulate molecular, cellular, and behavioral effects in animal models of neuropsychiatric disease. Recent work from our lab has demonstrated that animals whose microbiomes are depleted with antibiotics exhibit increased place preference for cocaine and that this effect can be reversed with supplementation of SCFA metabolites. In this study, we utilized our established microbiome depletion and metabolite supplementation models to dissect the effects of gut microbiome signaling on cocaine-seeking behavior in a translational model for drug-relapse behavior. Behavioral studies are coupled with cutting edge transcriptomic and metabolomic profiling to help determine the mechanisms underlying these gut-brain effects.

Methods: Gut bacteria and their metabolites were depleted in Sprague-Dawley rats via addition of non-absorbable broad-spectrum antibiotics (neomycin, bacitracin, vancomycin) to their home cage drinking water and compared to water-treated controls. To examine the specific mechanistic contributions of SCFA, the three primary SCFA produced in the gut (butyrate, acetate, propionate) were replenished to normal physiological concentrations via addition to the home cage drinking water. Adult male rats were first trained to self-administer cocaine (FR1 0.8mg/kg/inf) followed by testing for cocaine-seeking behaviors utilizing either: (1) a within-session threshold test to evaluate motivation for cocaine at a range of doses ($n = 10$ H2O, $n = 7$ Abx) or (2) 21 days of abstinence followed by a cue-induced cocaine-seeking task to model relapse behavior ($n = 6$-7/group). Following all behavioral testing, nucleus accumbens tissue was isolated and processed for RNA-sequencing analysis. Transcriptomes were compared by differential gene expression analysis via the DeSeq2 analysis package. Functional pathways altered between groups were identified using Ingenuity Pathway Analysis and G: Profiler software packages. All experimental protocols in animal studies were approved by the Mount Sinai 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: Microbiome depletion via antibiotics did not affect cocaine acquisition on an FR1 schedule. However, when animals who were already stably self-administering cocaine underwent a within-session dose-response threshold task, microbiome-depleted subjects exhibited significantly enhanced motivation for low doses of cocaine (two-way ANOVA, main effect of Abx: $p < 0.01$). Similarly, microbiome depletion increased cue-induced cocaine-seeking following prolonged abstinence in a relapse model (*t*-test, effect of Abx: $p < 0.01$). To determine the molecular drivers of gut-brain signaling in this model, we supplemented SCFA in animals with and without a depleted microbiome. SCFA supplementation fully reversed the behavioral effects of microbiome depletion on cue-induced cocaine-seeking after abstinence. RNA-sequencing analysis further demonstrated that microbiome-depleted animals exhibited significant alterations of gene expression in networks known to affect synaptic plasticity compared to both SCFA-treated subjects and water-treated controls. Taken together, our findings suggest that the microbiome and its metabolic byproducts are critical regulators of cocaine-seeking behavior and synaptic plasticity.

Conclusions: Animals lacking a complex gut microbiome show significantly increased cocaine-seeking behaviors and altered expression of synaptic plasticity genes. In the absence of a normal microbiome, supplementation of bacterially-derived SCFA metabolites reverses behavioral and molecular changes associated with microbiome depletion. Molecular analyses reveal marked changes in activity-dependent gene expression in antibiotic-treated rats compared to controls. These findings suggest that gut bacteria via their metabolites may serve as homeostatic regulators of gene expression in the brain, and suggest the microbiome has potential as a translational research target.

Keywords: Cocaine Self-Administration, Cocaine-Seeking Behavior, Cocaine Use Disorder, Metabolites, Gut-Brain Axis

Disclosure: Nothing to disclose.

P741. Differences in Hypothalamic-Pituitary-Adrenal Axis Response to Alcohol Withdrawal in Black and White Individuals With Alcohol Use Disorder

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Background: Black individuals show an increased odds of high-risk drinking and alcohol use disorder (AUD). Furthermore, they are less likely to seek and complete treatment for AUD than White individuals. AUD has a biphasic effect on the hypothalamic-pituitary-adrenal (HPA) axis, mirrored by a shift between hyperresponsiveness to stress (during alcohol withdrawal) and hypo-responsiveness (during early abstinence). Irrespective of AUD, racial differences in HPA axis function exist: Black non-AUD individuals, compared with White non-AUD, display flatter diurnal cortisol slopes and decreased HPA axis response to a psychosocial stressor. These differences are associated with socioeconomic status (SES), early life adversity, including trauma, adult stress, and psychopathology. Although implicated in altered HPA axis function, racial minority status, is not well-studied in the context of AUD-related HPA axis dysregulation. Here, we aimed to investigate self-identified race as a possible contributing factor to HPA axis dysregulation in individuals with AUD during alcohol withdrawal.

Methods: We compared morning plasma ACTH and cortisol of 449 individuals (282 males, 167 females), specifically: treatment-seeking AUD inpatients in alcohol withdrawal (69 Non-Hispanic Black, 70 Non-Hispanic White); non-treatment-seeking AUD individuals actively drinking without clinically relevant symptoms of alcohol withdrawal (65 Non-Hispanic Black, 13 Non-Hispanic White); and controls without current AUD (127 Non-Hispanic Black, 105 Non-Hispanic White). ACTH and cortisol were collected: (a) in AUD individuals in withdrawal as a morning value on the 2nd day of admission to the NIAAA Inpatient Unit; (b) in AUD individuals actively drinking and non-AUD controls during the screening visit at the NIAAA Outpatient Clinic at 11:00 AM-12:00 PM. ACTH and cortisol were measured using FDA-cleared chemiluminescence immunoassays run on an IMMULITE® 2000 XPi (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). ANOVAs, *t*-tests and/or Chi-squared tests were carried out to compare variables among our three subsamples and between Black and White individuals within each subsample, respectively. Multiple linear regression was used to investigate the main and interaction effects on HPA axis reactivity of race, sex, age, body mass index (BMI), SES (assessed using a combination of social determinants of health, i.e., years of education, annual household income, relationship status, and current urban/rural address), early life and adult stress (measured by the Childhood Trauma Questionnaire [CTQ], Early Life Stress Questionnaire [ELSQ], and Perceived Stress Scale [PSS], respectively), depression (measured by the Montgomery-Åsberg Depression Rating Scale [MADRS]), and anxiety (measured by the State-Trait Anxiety Inventory-Trait [STAI-T]).

Results: AUD individuals in withdrawal showed HPA axis hyperreactivity with significantly higher ACTH and cortisol levels than AUD individuals actively drinking and non-AUD controls (p's $= 0.001$). ACTH levels were significantly higher in Black vs. White AUD individuals in withdrawal ($p = 0.02$), as well as in Black vs. White non-AUD controls ($p = 0.004$), whereas Black and White AUD individuals actively drinking showed comparable cortisol and ACTH levels. Cortisol levels were significantly lower in Black vs. White AUD individuals in withdrawal ($p = 0.009$), while they did not show significant racial differences within the two other groups. The regression analyses indicated that Black race, AUD withdrawal status, male sex, lower age, and higher BMI predicted higher ACTH levels (R-squared = 0.285). White race, AUD withdrawal status, higher BMI, and higher MADRS scores predicted higher cortisol levels (R-squared = 0.175). There were no significant interaction effects among variables, except for a weak interaction between Black race and STAI-T scores, which predicted higher cortisol levels. Multicollinearity was low, as all variable inflation factors were in the acceptable range (i.e., <4).

Conclusions: Black AUD individuals show a significantly lower adrenal response to withdrawal, possibly due to the blunted HPA axis stress response observed in previous studies in Black vs. White controls. Yet, they also show a significantly greater pituitary response to withdrawal. A hypothesis is that the latter observation may be linked to the ultrashort-loop positive feedback of CRH to enhance ACTH release in stress. This HPA axis dysregulation occurs independently of SES, childhood adversity, and adult stress, which did not contribute to a differential allostatic load in Black and White individuals in our sample.

Keywords: Alcohol Use Disorder, Racial Differences, Hypothalamic-Pituitary-Adrenal Axis

Disclosure: Nothing to disclose.

P742. Pharmacokinetics of Cannabidiol: A Systematic Review and Meta-Regression Analysis

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Background: Cannabidiol (CBD) has gained significant interest in recent years as a potential medication for various disorders. However, although CBD-based products and publications are growing exponentially, there remains little understanding about its pharmacokinetic (PK) properties especially across studies that have important implications for the clinical use of this cannabinoid. As such, we aimed to aggregate available evidence from multiple studies to begin to assess factors influencing CBD pharmacokinetics.

Methods: A systematic review and meta-analysis (PROSPERO ID: 269857) was conducted. In this first phase, we focused on serum/plasma PK parameters of single-dose pure CBD products in healthy adults, excluding studies on CBD-THC combinations or any co-medications, patient populations, multiple doses of CBD, or studies in urine/saliva. Following PRISMA guidelines, we systematically interrogated citations from Medline, Embase, Web of Science Core Collection, and gray literature from inception to June, 2021. Data were extracted regarding demographics and sample size, study design, CBD formulation and supplier, duration of PK sessions, measured metabolites, any reported PK parameters, particularly Cmax, AUC0-t, and Tmax. Data from different studies were converted and log-transformed as necessary for each PK parameter. Random-effects meta-regression models were used for the analysis, considering each PK parameter as the dependent outcome, and CBD dose, CBD supplier, route of administration, cannabis abstinence status, fast or fed status, and duration of PK session (specifically for AUC0-t), as predictors. We included all the predictors in each model. Quality assessment of the included studies is underway using Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group introduced by NHLBI.

Results: Fourteen studies were included, comprising 39 different treatment arms as the unit of analysis. Five treatment arms were excluded from the final analysis: two did not report fast/fed status, three did not report the average dose of CBD. The analytical sample size included 480 participants. Across individual treatment arms, CBD dose ranged between 10 to 6000 mg, Cmax [geometric mean (CV%), ng/ml] between 0.43 (112.77) to 1628

(51.4), AUC0-t [geometric mean (CV%), h*ng/ml] between 1.469 (120.49) to 8347 (34.1), and Tmax [Arithmetic mean (SD), h] between 0.05 (0.034) to 10.45 (7.057). In meta-regression, the GW Pharmaceuticals (Epidiolex) as the supplier, was associated with a higher Cmax ($p < 0.001$), AUC0-t ($p < 0.001$), and Tmax ($p = 0.002$) compared to non-GW providers. Higher CBD dose was not significantly associated with Cmax in the total sample ($p = 0.054$), but was significantly associated with a higher Cmax in subgroup analysis when evaluating GW Pharmaceuticals ($p = 0.021$) and non-GW Pharmaceuticals ($p = 0.035$) treatment arms separately. CBD dose was not significantly associated with AUC0-t or Tmax. Fed status was significantly associated with a higher Cmax ($p = 0.020$) compared to the fast status, but it was not significant for AUC0-t or Tmax. Duration of PK session was not a significant predictor for AUC0-t. The percentage of variance among studies that could be explained by the models (R2) was 86% for Cmax, 77% for AUC0-t, and 89% for Tmax.

Conclusions: Factors such as supplier/chemical composition of the CBD formulation and fast/fed condition are significant determining factors in PK parameters of CBD, to the level that significantly different doses from different CBD suppliers/chemical compositions would be needed to reach similar PK parameters and efficacy. Given the important growth of CBD research, future PK studies should consider certain consistent and unified formats to allow comparable and synthesizable information to be obtained.

Keywords: Cannabidiol, Pharmacokinetics, Meta-Analysis, New Drug Development

Disclosure: Nothing to disclose.

P743. Characterization of Transcriptionally Activate Neurons Recruited by Cocaine in the Nucleus Accumbens

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Background: Substance use disorder (SUD) is a chronic neuropsychiatric disease characterized by the motivational drive to take and seek drugs of abuse, such as cocaine. Determining the neural mechanisms that underlie cocaine reward and motivation and how they can be modulated to control cocaine-associated behavior will be central to developing evidence-based therapeutic avenues for treating SUD. While previous work has focused on the role of whole brain regions or genetically-defined cell populations, recent data show that stimuli activate only a small percentage of neurons in the nucleus accumbens (NAc), a key brain region in valence-based decision making. Here, we combined behavioral tasks, optogenetic manipulations, and genetic mouse lines that allow for labeling of transcriptionally active neurons in a temporally specific manner in order to identify, manipulate, and characterize the functional group of neurons that are activated by cocaine and determine their role in drug taking.

Methods: Using transgenic mice (Arc-CreERT2) that allows for temporally specific labelling of transcriptionally active neurons, we expressed Cre-dependent viruses, such as channelrhodopsin and diphtheria toxin, in cocaine-activated neurons in the NAc. We combined this approach with operant tasks to define the necessity and sufficiency of these cocaine-activated neurons to control reinforcement learning for drug and non-drug stimuli. We also used electrophysiological techniques to interrogate the physiological properties of these neurons. Through these experiments, we defined the role of cocaine-activated neurons in the NAc in encoding cocaine-associated information and guiding learning and motivated behavior to determine how cocaine acts on the NAc to drive progression to substance use disorder.

Results: We find that cocaine-activated neuronal populations in the NAc core are physiologically distinct from those active during a saline injection, demonstrating that cocaine selects for a group of neurons with unique physiological properties. Further, we demonstrate that activated neurons in the NAc are sufficient to drive reinforcement on their own but are unable to modulate learning or performance in reinforcement tasks for natural rewards or negative reinforcers. Lesioning these cocaine-activated neurons does not impact cocaine self-administration or modulate behavioral responses in other types of operant learning, suggesting that this initially cocaine-activated ensemble of neurons may not play a key role in driving progression to substance use disorder. Finally, we demonstrate that a subset of neurons recruited to chronic cocaine are also able to drive reinforcement, but do not modulate other reinforcement behaviors and are also not necessary for cocaine self-administration.

Conclusions: Together, we find that following cocaine exposure, transcriptionally active neurons in the NAc core are physiologically unique and are sufficient to drive reinforcement. However, despite these unique physiological properties, these cocaine-recruited neurons do not alter behavior associated with other primary reinforcers. This suggests that neurons in the NAc may be innately reinforcing, but that either there is a different set of neurons that drives the progression to drug addiction, or there is plasticity within this initial set of neurons that cannot be fully replicated under these experimental conditions that drives drug taking and seeking behaviors. Further, lesioning the group of neurons recruited by chronic cocaine exposure is insufficient to modulate cocaine self-administration acquisition or performance. These data are critically important as they shed new light on the fundamental mechanisms underlying cocaine use disorder and raise the question of what role ensembles in the NAc play and how these groups of neurons may be implicated in dysregulated behaviors seen in SUD.

Keywords: Cocaine Use Disorder, Nucleus Accumbens, Cocaine Self-Administration, Operant Behavior, Optogenetics

Disclosure: Nothing to disclose.

P744. Neuronal Nsun2 Deficiency Causes Epitranscriptomic Dysregulation of Gly-Trnas and Proteomic Shifts Impacting Synaptic Function and Behavior

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Background: Protein translation is a critical component of brain function and aberrant translational processes have been implicated in psychiatric disease. Transfer (t)RNAs, non-coding cloverleaf shaped RNAs that are key players in ribosomal protein synthesis, are barely explored in brain. Nsun2, a mammalian tRNA cytosine methyltransferase, is expressed at high levels in brain and has been linked to neurodevelopmental defects in humans and mice. Previous work has shown that loss of tRNA cytosine methylation produces deficits in protein synthesis in non-neuronal cells, but the role of tRNA methylation in adult neuronal function has not been explored.

Methods: We used conditional ablation and transgene-derived overexpression of Nsun2 in the male and female mouse prefrontal cortex (PFC) to manipulate tRNA methylation levels postnatally. We used targeted RNA bisulfite sequencing and tRNA sequencing to assess tRNA methylation/expression, LS-MS/MS to measure

protein expression, electrophysiological recordings to assess synaptic transmission, and behavioral testing for complex behaviors including cognition, behavioral despair, and addiction-related behaviors.

Results: Results showed that complex behaviors relating to emotion, cognition, and addiction, are highly sensitive to bi-directional changes in Nsun2 in prefrontal cortex (PFC) neurons. Further, Nsun2-deficient cortex showed a selective deficit in multiple glycine tRNAs which resulted in codon-specific shifts in the proteomic landscape, with deficits in glycine-rich neuronal proteins that were associated with impaired neurotransmission and altered behavioral phenotypes.

Conclusions: tRNA methylation is a critical process for regulating synaptic plasticity and behavior through proteomic changes in the mature cortex. This data suggests that there is another mechanism, aside from glycinergic receptors, by which glycine could critically regulate brain function and complex behaviors, suggesting potential for novel therapeutic avenues in psychiatry.

Keywords: Epigenetics, Non-Coding RNA, Opioid Addiction

Disclosure: Nothing to disclose.

P745. Opioids and Risky Behavior: Focus on the Rodent Prefrontal Cortex

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Background: Drug overdoses have steadily increased in recent years with most cases being attributed to opioid misuse. Opioid addiction is associated with impaired risk-related decision-making. However, it remains unclear how the brain processes risk in the pursuit of opioid use to alter motivated behavior during conflict situations. The prelimbic area of the medial prefrontal cortex (PL) has been associated with executive functioning and behavioral responses to rewarding and aversive stimuli. We therefore hypothesized that repeated opioid exposure would suppress activity in PL and increase risk-taking behavior.

Methods: To test our hypothesis, we designed an approach-avoidance conflict model that involves the presentation of an innate fear-inducing predator odor. Using a biased opioid conditioned place preference (CPP) model, we injected adult male Long-Evans rats with saline ($n = 36$) or morphine ($n = 24$) and exposed them to their least-preferred side of the chamber on alternating days over a 10-day period. After 72 h of forced abstinence, rats were exposed to a 10-min preference test to assess the amount of time rats spent in each side of the chamber. Immediately after preference testing, we added predator odor (cat saliva) to the previously drug-paired side to produce a motivational conflict and re-exposed the animals to the chamber for a 10-min conflict test.

Results: During the preference test, morphine-treated rats showed a higher preference for the drug-paired side when compared to the saline group (Repeated-measures ANOVA followed by Tukey post-hoc test, $p < 0.001$). During the conflict test, the saline group showed increased avoidance to the cat odor ($p < 0.001$). In contrast, the morphine group continued to enter the drug-paired side despite the presence of the cat odor ($p = 0.45$), demonstrating that morphine conditioning causes an increase in risk-taking behavior. Single-unit recordings from PL neurons revealed a significant number of cells with suppressed firing rates after acute administration of morphine (22 %, 19/86), but not saline (6 %, 5/64; Fisher's exact test, $p = 0.011$). On the last conditioning day, morphine administration did not induce changes in PL firing rates as compared to saline ($p = 0.397$), suggesting that PL neurons adapt to the effects of repeated morphine over time. During the conflict test, distinct populations of PL neurons were either excited ($n = 28$ neurons, $p = 0.011$) or inhibited ($n = 14$ neurons, $p = 0.0061$) when saline rats entered the cat odor-paired side as compared to when they entered the same side during the preference test. Interestingly, this change in neural activity was not observed in the morphine group during the conflict test.

Conclusions: Taken together, our results demonstrate that opioid-induced CPP is associated with reduced inhibition of PL activity following repeated administrations of morphine. In addition, after repeated opioid exposure, PL neurons failed to respond to threat stimuli, and this pattern of neuronal activity occurred alongside increased risk-taking behavior during approach-avoidance conflict.

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 Animal

Keywords: Prelimbic Cortex, Recordings, Morphine, Approach-Avoidance, Reward-Seeking Behavior

Disclosure: Nothing to disclose.

P746. Genome-Wide Association Study of Problematic Opioid Prescription Use in 132,113 23andMe Research Participants of European Ancestry

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Background: Rates of opioid use disorder (OUD) constitute an urgent health crisis. Ample evidence indicates that risk for OUD is heritable. As a surrogate (or proxy) for OUD, we explored the genetic basis of using opioids 'not as prescribed'. We hypothesized that misuse of opiates might be a heritable risk factor for OUD.

Methods: To test this hypothesis, we performed a genome-wide association study (GWAS) of problematic opioid use (POU; 'ever taking opioid prescriptions not as prescribed') in 132,113 23andMe research participants of European ancestry (Ncases = 27,805).

Results: Our GWAS identified two genome-wide significant loci (rs3791033, an intronic variant of KDM4A; rs640561, an intergenic variant near LRRIQ3). POU showed a positive genetic correlation with opioid dependence and OUD, as measured in the largest available GWAS (rg = 0.58-0.80). We also identified numerous additional genetic correlations with POU, including alcohol dependence (rg = 0.74), smoking initiation (rg = 0.63), pain relief medication intake (rg = 0.49), major depressive disorder (rg = 0.44), chronic pain (rg = 0.42), insomnia (rg = 0.39), and loneliness (rg = 0.28). Although POU was positively genetically correlated with risk-taking (rg = 0.38), conditioning POU on risk-taking did not substantially alter the magnitude or direction of these genetic correlations, suggesting that POU does not simply reflect a general tendency for risky behavior. Lastly, we performed phenome- and lab-wide association analyses, which uncovered additional phenotypes that were associated with POU, including respiratory failure, insomnia, ischemic heart disease, and metabolic and blood-related biomarkers.

Conclusions: We conclude that opioid misuse can be measured in population-based cohorts and provides a cost-effective

complementary strategy for understanding the genetic basis of OUD.

Keywords: Prescription Opioids, Opioid Addiction, GWAS, Human Genetics

Disclosure: Nothing to disclose.

P747. Upregulation of MICRORNA-222 in the Amygdala Contributes to Adult Anxiety and Excessive Alcohol Drinking After Adolescent Alcohol Exposure

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Background: Adolescent binge drinking alters normal neurodevelopmental pathways, increasing an individual's risk of developing alcohol use disorder (AUD) and comorbid anxiety in adulthood. Non-coding RNAs, including microRNAs (miRNAs) and enhancer RNAs (eRNAs), cooperatively regulate neuronal gene expression, and their expression can be altered by early-life alcohol exposure. Here, we used miRNA microarray to identify miR-222 as a candidate that may, as a result of adolescent alcohol exposure, regulate gene expression in the amygdala to induce behavioral phenotypes of anxiety and increased alcohol consumption in adulthood.

Methods: Male adolescent Sprague-Dawley rats were exposed to 2g/kg ethanol (AIE) or intermittent n-saline (AIS) on a 2-day on/off schedule via intraperitoneal injection (i.p.) during postnatal days (PND) 28-41. Once the rats matured to adulthood, we collected brain tissue and performed an unbiased miRNA microarray. In other cohorts of adult AIE/AIS rats, we performed infusion of a miR-222 mimic, a Fos LNA, and a miR-222 antagomir into the central nucleus of the amygdala (CeA), and their subsequent behavioral phenotypes were evaluated. Brain tissue was collected for measurement of Fos mRNA and eRNA expression. cFOS protein levels in the amygdala were measured using gold immunolabeling. Between 5-9 rats were assigned to each group. Statistical analyses were performed using Student's *t*-test, two-way ANOVA, and repeated measures ANOVA with post-hoc comparison using Tukey's test.

Results: miRNA microarray data showed that miR-222 expression was significantly higher in the amygdala of AIE rats compared to those of AIS controls, which was validated by qPCR. miR-222 target gene analysis revealed a high-confidence binding site in the Fos 3'-UTR, and downregulation of both Fos mRNA and cFos protein was observed in the AIE adult amygdala. The expression of Fos eRNA-2, a highly conserved activity-regulated enhancer, was also downregulated in the of amygdala of AIE adult rats compared to AIS rats. Infusion of either a miR-222 mimic or a locked nucleic acid inhibitor of Fos eRNA-2 into the CeA of alcohol-naïve animals resulted in increased anxiety-like behavior and decreased Fos mRNA expression in the amygdala. Interestingly, miR-222 antagomir infusion directly into the CeA of AIE adult rats reversed the AIE-induced increases in anxiety-like behaviors and voluntary alcohol consumption and normalized Fos mRNA and eRNA expression in the amygdala.

Conclusions: Adolescent alcohol exposure disrupts an epigenetic circuit in the amygdala by increasing miR-222 targeting of Fos mRNA and decreasing the transcription of a conserved Fos enhancer, which increases an individual's risk of developing psychopathology in adulthood (Supported by the NIAAA UO1AA-019971, U24AA-024605 (NADIA project), P50AA022538 grants and senior career scientist award from department of Veterans Affairs to SCP)

Keywords: MicroRNA, Adolescent Alcohol, Amygdala, Anxiety, c-Fos

Disclosure: Nothing to disclose.

P748. Relationships Between Childhood Trauma, Hippocampal-Dependent Context Processing, and Opioid Misuse

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Background: Opioid Use Disorder (OUD) is serious, prevalent, and costly. Reliable indicators of who will take opioids as prescribed and who will develop an OUD remain elusive. Ample evidence has linked childhood trauma to the development of OUD later in life, but underlying mechanisms have not been identified. Hippocampal function and associated context dependent learning and memory (CDLM) are impacted by childhood trauma, and deficits in CDLM have been observed in OUD. We propose that CDLM processes mediate the link between childhood trauma and OUD.

Methods: In this pilot study, we examined links between childhood trauma, CDLM performance, and opioid misuse behaviors in 23 adults taking prescription opioids. Participants completed questionnaires and tasks in a virtual format. Childhood trauma was retrospectively assessed using the Childhood Trauma Questionnaire and opioid misuse was assessed with the Current Opioid Misuse Measure. CDLM was assessed with the Mnemonic Similarity Task and the Context Discrimination Task.

Results: Childhood trauma was significantly associated with poorer CDLM performance, $r(17) = -.510$, $p = .036$. Childhood trauma was also associated, at a trend level, with increased opioid misuse, $r(17) = .458$, $p = .065$. Increased opioid misuse was associated with poorer CDLM performance, $r(21) = -.447$, $p = .042$.

Conclusions: These preliminary findings in a small sample of adults taking prescription opioid medications support further study of relationships between childhood trauma, CDLM, and opioid misuse. Future work will examine these factors as predictors of OUD in a larger study of patients receiving prescription opioids. Our goal is to develop neurocognitive approaches to identify and protect at-risk youth, perhaps by strengthening hippocampal-dependent CDLM pathways before substance use is initiated. We hope, ultimately, to reduce the public health consequences of opioid misuse and other substances later in life.

Keywords: Childhood Trauma, Context, Memory, Hippocampus, Opioid Use Disorder

Disclosure: Nothing to disclose.

P749. Cross-Validated Prediction Identifies Dissociable Neural Networks of Cannabis Severity Versus Subsequent Treatment Outcome

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Background: Cannabis use is the most widely used illicit drug worldwide1 and rates of daily or near daily use within the United States have nearly doubled in recent years. While cannabis use (CU) treatment outcomes vary widely across individuals, little is known about brain-based predictors of CU treatment outcomes. Here, for the first time, we extend our prior work—demonstrating robust, substance-specific neuromarkers of opioid- and cocaine-

use treatment outcomes— to identify a neuromarker of CU treatment outcomes using a wholly data-driven approach.

Methods: Fifty-eight individuals with cannabis-use disorder participated in neuroimaging at the start of treatment. Neuroimaging data from reward and cognitive tasks were used to compute functional connectivity matrices (two per participant) and these were entered into connectome-based predictive models (CPMs) to identify networks predictive of baseline addiction severity and subsequent biologically confirmed cannabis abstinence over three months. CPMs were run using 10-fold cross-validation with 100 iterations and permutation testing was used to determine statistical significance. Model generalizability to other substances was tested in an external sample of 53 polysubstance users, and cannabis network strength was compared to a group of control participants ($n = 46$).

Results: CPMs of both reward and cognitive task data successfully identified networks predictive of cannabis abstinence during treatment (reward: $r = 0.39$, $p = 0.004$; cognitive: $r = 0.35$, $p = 0.005$). Combining reward and cognitive task data also successfully predicted cannabis abstinence, with higher accuracy relative to either task alone ($r = 0.44$, $p = 0.002$). Out-of-sample analyses indicated that the identified cannabis abstinence network did not generalize to predicting abstinence from other substances, consistent with prior work indicating substance-specific neuromarkers of abstinence. CPMs of combined task data also predicted baseline addiction severity, as measured by the Addiction Severity Index ($r = 0.43$, $p < 0.001$). Consistent with other CPM work, identified networks for both analyses were complex and included both cortical and subcortical connections. Despite this complexity, the spatial extent of both abstinence and severity networks together included only 758 edges (584 abstinence, 174 severity), or less than 2.2% of possible connections. Spatial overlap between networks was minimal and included 9 edges. Finally, healthy controls displayed intermediate cannabis network strength relative to treatment responders and non-responders.

Conclusions: These data indicate dissociable neural networks of cannabis severity versus subsequent treatment outcome. Contrary to classical conceptions, these findings further add to a growing body of evidence indicating unique, substance-specific predictors of addiction treatment outcomes.

Keywords: Cannabis Use Disorder, Biomarkers, Neuromarkers, Treatment Prediction

Disclosure: Nothing to disclose.

P750. Exposure to Ethanol Under Food Deprivation Enhances and Under Opioid Blockade Inhibits Alcohol Self-Administration

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Background: Alcohol use disorder (AUD) produces a tremendous burden to those suffering from the disorder, to those that aid in their treatment, and to society. A complex disorder involving behavioral, genetic, and neurochemical mechanisms, AUD interacts with basic motivational and nutritional systems that change because of the intense experience with ethanol (EtOH). Food deprivation enhances EtOH consumption and chronic EtOH intake adversely affects nutrient intake and metabolism. Endogenous opioids systems also play a major role in EtOH intake and reward processes as well as these processes with food intake. We have found that the underlying motivational system during initial experiences with EtOH may affect alcohol self-administration and the progression to AUD. This research evaluates the role in food deprivation and opioid mechanisms in the motivation to consume EtOH.

Methods: Rodents were given their first exposure to EtOH or saline under two motivational conditions: A) Rats (Sprague Dawley male, $n = 13$) were either food deprived or allowed ad lib food and water for 24 hr. before access to 2cc of 3% EtOH on three occasions. These sessions were separated by three days when the animals were allowed ad lib. food and water. All animals were then given a two-bottle choice between EtOH or water during which EtOH concentrations increased every three days (1, 3, 5, 7 and 10%). B) C57BL/6 mice (C57 male, $n = 20$) where given naloxone HCl (5 mg/kg) or saline vehicle immediate after EtOH exposure (2cc of 3%) on three occasions (food was withheld until the EtOH was consumed). Following this exposure to EtOH, the animals were given a two-bottle choice between 10% EtOH and water for 1hr.

Results: A) Food Deprivation paired with EtOH drinking produced an increase in EtOH consumption over control during the acquisition of 2-bottle choice across increasing concentration ($t = 3.45$, $p < .05$) B) Naloxone-paired pre-exposure decreased EtOH consumption in comparison to non-paired consumption during the 2-bottle choice of EtOH ($F(3, 21) = 13.64$, $p < 0.05$).

Conclusions: The exposure of EtOH under conditions of food deprivation increased voluntary self-administration of EtOH. The increased drive associated with the deprivation of food may have increased the stimulus salience and incentive value of the EtOH leading to enhanced preference. On the other hand, the pairing of naloxone with initial EtOH exposure decreased voluntary self-administration. The blockade of opioid receptors may have decreased EtOH incentive value leading to diminished preference. These data suggest that the reinforcing effects of EtOH are influenced by motivational factors involving drive and opioid mechanisms that may lead to enhanced treatment of AUD.

Keywords: Alcohol Preference, Food Deprivation, Naloxone, Stimulus Enhancement, Stimulus Inhibition

Disclosure: Nothing to disclose.

P751. Serotonin 2A Receptors in the Rat Claustrum Inhibit Cingulate Cortex Neurons to Modulate Cognitive Flexibility

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Background: The claustrum (CLA) has long been speculated to be involved in a variety of brain functions including attention salience and sensorimotor integration. The CLA is also the most densely innervated structure in the brain and has the highest expression of the serotonin 2A receptor (5HT2AR). The CLA, as well as 5HT and the 5HT2AR, have all been separately proposed to play a role in substance use and cognitive flexibility, the ability to adapt behavioral strategies to environmental changes. Here, we examine the impact of serotonin 2A receptor signaling on neuronal excitability within the claustrum in the context of cocaine-induced deficits in a cognitive flexibility, set shifting task.

Methods: Electrophysiological slice recordings were performed from retrogradely labeled CLA neurons projecting to the anterior cingulate cortex (ACC). Spontaneous excitatory (sEPSCs) and inhibitory (sIPSCs) post-synaptic currents were analyzed along with measures of cell excitability, including resting membrane potential, membrane capacitance, and action potential firing rate. Recordings were conducted following evaluation of behavioral performance on a set-shifting task with and without history of cocaine self-administration (extended access, 6hr/day).

Results: Retrograde virus injected into the ACC labeled a large population of CLA neurons with the label closely following anatomical outline of the CLA. 5HT application resulted in

significant decreases of sEPSC frequency and amplitude accompanied by membrane hyperpolarization and decreased firing of CLA neurons projecting to ACC. In contrast, 5HT induced robust increases in sIPSC frequency and amplitude accompanied by sIPSC bursts. Blockade of the 5HT2A receptors with ketanserin eliminated the synaptic effects of 5HT, indicating a regulatory role of the 5HT2AR in claustrocortical signaling. Our behavioral experiments showed that both cocaine and serotonin-releasing agent, MMAI, had profound negative effects on cognitive flexibility performance.

Conclusions: These findings provide the first physiological evidence that a large population of CLA-ACC neurons is under strong inhibitory control from 5HT and the 5HT2AR. The overall inhibitory effect on neuronal output appears driven by synergistic bi-directional regulation of inhibitory and excitatory synapses by 5HT2A receptors. On-going experiments examine whether these effects account for the cocaine-induced cognitive deficits.

Keywords: Claustrum, Electrophysiology, Serotonin, Cocaine, Cognitive Flexibility

Disclosure: Nothing to disclose.

P752. Prefrontal-Habenular Track Abnormalities Associated With Recent Drug Use in Human Cocaine Addiction

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Background: A fundamental feature of drug addiction encompasses maladaptations in the systems that regulate reward valuation and salience attribution. Emerging evidence from animal models implicates the lateral habenula (Hb), an epithalamic hub for corticolimbic communication, as a key neural substrate of reward functions that are impaired in psychostimulant dependence. Specifically, while the prefrontal cortex (PFC) supplies top-down control over responses to drug-predictive cues, neural adaptations in the lateral Hb's encoding of negative reinforcers are hypothesized to trigger aversive states that are characteristic of withdrawal, potentially precipitating relapse and bingeing. However, direct translational evidence for the role of this circuitry in human drug addiction is still warranted. We aimed to bridge this gap using noninvasive tract reconstruction to map PFC-Hb anatomical connections in a population of healthy and cocaine-addicted individuals.

Methods: We performed diffusion MRI tractography in 31 individuals with cocaine use disorder [CUD, including 16 urine-positive (CUD +) and 15 urine-negative (CUD-) for cocaine at day of scanning, as a measure of recency of use] and 28 healthy controls (CTL). Clinical interviews were conducted to assess lifetime and recent drug use measures. PFC-Hb projections were segmented into component fiber bundles (subsections): the stria medullaris (SM) and the anterior limb of the internal capsule (ALIC). Since the ALIC contains multiple parallel fiber tracts, we constructed additional tracks between the PFC and ventral tegmental area as well as the anterior thalamus as control regions to target the superolateral medial forebrain bundle and anterior thalamic radiations, respectively, and assess the specificity of results to the Hb. White matter microstructure was analyzed using diffusion tensor imaging to obtain mean fractional anisotropy (FA), an index representing the orientational coherence of fibers in the tracks of interest, as well as the total mean diffusivity (MD) and diffusivity in the axial (AD) and radial (RD) directions. Differences in DTI metrics were tested using 3 (Group: CTL, CUD + , CUD-) × 2 (Subsection: SM, ALIC) × 2 (Side: left, right) ANOVAs, and statistical tests were considered significant at $p < .05$ with Bonferroni correction for multiple comparisons.

Results: FA was reduced overall in PFC-Hb tracks in CUD compared with CTL (Group main effect $p < .001$). This was qualified by a significant Group × Subsection interaction effect ($p < .001$), in which CUD + < CTL = CUD- in the SM and CUD- < CTL = CUD + in the ALIC. Importantly, the right SM FA correlated negatively with cocaine abstinence duration ($r2 = 0.21$) and with choice preference for viewing drug over food images ($r2 = 0.21$; driven by the CUD group, $r2 = 0.20$). Additionally, there was a significant Group main effect for MD in which CUD- > CTL = CUD + . No significant Group or interaction effects were observed for AD or RD. Further, there were no significant group differences in DTI metrics in the control ventral tegmental area or anterior thalamus tracks, supporting the specificity of the results in the Hb.

Conclusions: White matter abnormalities in PFC-Hb projections were observed in cocaine-addicted individuals, and their severity was linked to both objective (urine toxicology) and self-reported (abstinence duration) measures of drug use recency. Distinct subregions of these tracks may be associated with the severity of recent drug use (in the SM), or with longer-term or predisposing factors (in the ALIC). These results support further interrogation of the Hb's role in addiction and cognitive impairments related to drug seeking and relapse. Subsequent analyses will include functional MRI during a drug choice task to assess the role of the Hb in drug-biased responses.

Keywords: Diffusion Tensor Imaging (DTI), Lateral Habenula, PFC, Cocaine Addiction

Disclosure: Nothing to disclose.

P753. Cross-Ancestry Meta-Analysis of Opioid Use Disorder Uncovers Novel Associated Loci

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Background: Opioid use disorder (OUD) affects more than 2 million Americans. Elucidation of the genetic risk for OUD could enhance prevention, diagnosis, and treatment efforts. Despite an estimated twin heritability of ~50%, the largest genome-wide association study (GWAS) of OUD to date, with 10,544 mostly-MVP cases, revealed only one genome-wide significant (GWS) locus, OPRM1, which was limited to European Americans (EAs).

Methods: We expanded our sample size by using the latest release of MVP data and a less stringent phenotypic definition in order to identify novel loci. We performed a cross-ancestry meta-analysis of OUD in the MVP with 31,480 cases (phenotype definition requiring 1 + ICD9/10 code for OUD) and 394,484 opioid-exposed controls (any outpatient opioid prescription fill). For comparison, we performed GWAS using two other traits: a stringent definition (1+ inpatient or 2+ outpatient codes; *N* cases = 23,552) and opioid dependence (1 + ICD9/10 code for dependence; *N* cases = 28,190).

Results: Ten loci exceeded genome-wide significance in the cross-ancestry meta-analysis of OUD (less stringent), 9 of them novel. Three additional loci were identified in ancestry-specific analyses (1 in African American (AA; *N* = 88,502; 1 in EA (*N* = 302,596); 1 in Latinx American (LA; *N* = 34,865). In addition to the previously identified exonic variant in OPRM1 which was still the lead SNP genome-wide ($p = 9.72x10\text{-}11$), intronic variants were identified in RABEPK, FBXW4, NCAM1, and KCNN1.

Conclusions: Many of these genes have prior associations with other diseases, including psychiatric disorders such as bipolar disorder and schizophrenia. Genetic correlation analyses identified significant positive genetic correlations with schizophrenia, ever vs never smoking, and cross disorder. Gene-based association analyses identified 3 genes in EAs: OPRM1, DRD2, and FTO. With a case sample size triple that of the largest previous GWAS, we identified 12 novel loci for OUD, including ancestry-specific loci. These findings will increase our understanding of the biological pathways involved in OUD, which will inform preventive, diagnostic, and therapeutic efforts.

Keywords: Opioid Use Disorder, Genome-Wide Association Study, Novel Therapeutics

Disclosures: Dicerna Pharmaceuticals: Advisory Board (Self)

Sophrosyne Pharmaceuticals: Consultant (Self) American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which was supported in the last three years by AbbVie, Alkermes, Dicerna, Ethypharm, Indivior, Lilly, Lundbeck, Otsuka, Pfizer, Arbor, and Amygdala Neurosciences:, Other Financial or Material Support (Self)

PCT patent application #15/878,640 entitled: Patent (Self)

P754. Astrocytes in the External Globus Pallidus Coordinate Flexibility of Action Strategy

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Background: The external globus pallidus (GPe) is an integrative hub and gateway for behavioral flexibility in reward-related behaviors. However, it remains unknown whether enriched astrocytes in the GPe guide behavioral flexibility.

Methods: We utilized the two operant behavior tasks with either effort- or time-based reward (10 ul of 20% sucrose solution) delivery to establish goal-directed and habitual action selections. Then, we employed in vivo calcium imaging to probe temporal dynamics of GPe astrocytes during goal-directed and habitual learnings. Using the machine learning approach, we validated whether GPe astrocyte dynamics can predict the type of behavior task in mice. Lastly, we determined whether the promotion of the calcium signaling in GPe astrocytes adapted by habitual learning attenuates the habitual reward-seeking behaviors.

Results: GPe astrocytes were substantially silenced during habitual learning compared to goal-directed learning. In the timescale of action events, GPe astrocyte activities were increased immediately after termination of reward-taking behavior before the next action. However, during habitual learning, this increase was not notable during goal-directed learning. Moreover, support vector machine (SVM) analysis demonstrated that GPe astrocytes dynamics predicted whether mice perform goal-directed or habitual behaviors. Interestingly, chemogenetic activation of GPe astrocytes which dampened GPe neuronal firings, manifested goal-directed behavior from habit. Furthermore, brief, and repeated attentional stimulations recapitulated the effect of chemogenetic activation of GPe in intervening the habitual reward-seeking behaviors with increased GPe astrocyte activities.

Conclusions: Our findings reveal a novel insight that GPe astrocytic activation attenuates habitual behavior and improves behavioral flexibility, which may provide a potential therapeutic target for decision-making-related disorders, such as obsessive-compulsive disorder and addiction.

Keywords: Astrocytes, Globus Pallidus, Habits, Goal-Directed Behaviors, Calcium Imaging

Disclosure: Peptron: Advisory Board (Self)

P755. Effects of Two Doses of Smoked Cannabis (Tetrahydrocannabinol-THC) on mRNA Responses in Peripheral Blood Mononuclear Cells

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Background: There is evidence from animal and in-vitro studies that THC can affect cannabinoid receptors (1 and2) in brain and periphery and influence immunological makers, and limited evidence from animal studies that it may affect epigenetic related methylation processes. THC ingestion has also been reported as a trigger for inducing schizophrenia in venerable individuals. This is less direct work on these effects in human cannabis smokers investigating these types of chemical biomarkers in peripheral blood cells. The present study further evaluated whether smoked cannabis in human subjects produced changes in cannabinoid receptors, biomarkers for DNA methylation cycle and immuno-related gene mRNA expression.

Methods: 23 Subjects participated in an experiment in which they smoked cannabis cigarettes with one of two doses of marijuana (5.3% or 13.4% THC) or placebo (0.02%) and were evaluated driving abilities. Blood samples were drawn at baseline and several times after smoking. Plasma and WBC (PMCs) were separated and stored at -80°C until further analysis. Samples were analyzed for mRNA content for cannabinoid receptors 1(CBR1) and 2(CBR2), methylation and demethylating enzymes (DNMT, TET), glucocorticoid receptor (NRC3) and immunological markers (IL1B, TNFα) by qPCR using TaqMan probes. The results were correlated with THC whole blood levels and TCOOH baseline levels. Statistical analyzed used analysis of variance and covariance and *t*-test, or non-parametric equivalents for those values which were not normally distributed.

Results: There were no difference in background baseline characteristics of the subjects except that the higher dose THC group was older than the low dose and placebo groups, and the low dose THC group had higher baseline CBR2 mRNA levels. Both the 5.9 and 13.4 THC groups showed increased THC levels over the next 2 hours and then decreased toward baseline; the 13.4 THC dose still showed a higher THC levels than placebo at four hours. However, there were no significant differences between THC levels between the 5.9 and 13.4 doses at any time point. At the 4-hour time point after drug administration the 13.4% THC group had higher CBR2 ($P = .021$) and DNMT3A ($P = .027$) mRNA levels than the placebo group and DNMT1 mRNA levels showed a trend in the same direction ($P = .056$). The higher13.4 THC group had significantly higher CBR2 mRNA levels than the 5.9 dose group at several post drug administration time points, and showed trends for difference in effects for between 5.9 and 13.4 THC groups for other mRNAs. TET3 mRNA levels were higher in the 13.4 THC group at 55 minutes post-drug ingestion. When the high and lower dose THC groups were combined, none of the differences in mRNA levels from placebo remain statistically significant. Changes in THC plasma levels were not related to changes in mRNA levels.

Conclusions: Over the time course of this study CBR2 levels increased in human PMCs in the high dose THC group but where not accompanied by changes in immunological markers. The changes in DNMT and TET mRNAs suggest potential epigenetic effects of THC in human PMCs. Increases in DNMT metylating enzymes have been linked to some of the pathophysiological process in schizophrenia and, therefore, should be further

explored as one of the potential mechanisms linking cannabism use as a trigger for schizophrenia.

Keywords: THC, Dnmt, Canabinoid Receptors, Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P756. Nr4a1 as a Novel Therapeutic Target in Cocaine Addiction

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Background: Craving and relapse are key symptoms of addiction that occur across abstinence. How do the pathological effects of drug taking persist and even worsen once the drug signal is lost? We recently discovered that the transcription factor, nuclear receptor subfamily 4 1 (Nr4a1/Nurr77), regulates persistent gene expression in the nucleus accumbens (NAc) across abstinence after mouse cocaine self-administration (SA). We find that CRISPR activation of Nr4a1 expression in the nucleus accumbens (NAc) during abstinence reduces cocaine reward behavior, suggesting that Nr4a1 acts homeostatically to reverse the effects of cocaine. We recently began to explore the cell-type specific nature of this regulation, given evidence of a cell-type specific role of Nr4a1. For example, recent studies point to a direct pathway (Drd1 +) specific function of Nr4a1 given its activation of Drd1-specific pERK signaling. We aim to define the specific striatal neuronal population in which Nr4a1 regulates persistent transcription to attenuate reward behavior.

Methods: All experiments are conducted in 8-12 week old, C57B/L6 or transgenic, male and female mice. We applied 'isolation of specific neuronal cell types' (INTACT) coupled to 'cleavage under targets and release using nuclease' (CUT and RUN) to determine Nr4a1 occupancy, chromatin landscape, and expression of Nr4a1 target genes in specific cell types. To determine the NAc cell type responsible for Nr4a1 regulation of cocaine behavior, we activated or inhibited Nr4a1 (CRISPRa/i) in either of two medium spiny neuron (MSN) subtypes and measured cocaine conditioned place preference, SA acquisition and extinction/reinstatement.

Results: All experiments were carried out one to three times, and data replication was observed in instances of repeated experiments, or otherwise reported. Main and interaction effects were considered significant at $P < 0.05$. We profiled chromatin and gene expression in indirect and direct pathway MSNs. A2a- or Drd1-Cre; LS1L-Sun1-GFP mouse striata ($n = 3$) were subjected to INTACT, followed by H3K27me3 or H3K4me3 CUT and RUN. Spearman correlations were calculated between A2a+ replicates and published datasets of H3K27me3 ChIP-Seq from whole NAc and from CamK2a+ excitatory forebrain neurons. A2a + H3K27me3 peaks more closely correlated with bulk NAc MSNs ($rs = 0.79$) than forebrain excitatory neurons ($rs = 0.38$). To determine accuracy of CnR in each cell type, K27me3+ from A2a+ MSNs were overlapped with a published cell-type-specific gene lists (PMID: 31171808). K27me3 in A2a+ MSNs was enriched in a greater percentage of Drd1-specific genes (2.05%) than A2a-specific genes (0.17%), consistent with the repressive role of H3K27me3 in gene expression. To regulate Nr4a1 expression in specific MSN subtypes, we engineered Cre-dependent dCas9 expression plasmids to express in A2a- or Drd1-Cre;LSL-Sun1-GFP mice. NAc was stereotaxically and unilaterally transfected (JetPEI) with either control non-targeting (NT) or Nr4a1-sgRNA and dCas9-VP64. Nuclei were isolated using INTACT and gene expression was quantified by qRT-PCR. Nr4a1 CRISPRa activated Nr4a1 and Cartpt expression specifically in Cre+ indirect pathway MSNs (A2a +) relative to NT (2-way ANOVA, Effect of sgRNA: Nr4a1 F (1, 10) = 12.05 $P = 0.0060$). To validate the specificity of our approach, we measured A2a, and Drd1 expression. Cre+ nuclei isolated from A2a-Cre mice, were enriched in A2a and depleted in Drd1 relative to Cre- nuclei (A2a 2-way ANOVA, Effect of Cre, F (1, 8) = 14.23 $P = 0.0054$; Drd1 2-way ANOVA, Effect of Cre, F (1, 8) = 107.2 $P < 0.0001$). Nr4a1 CRISPR activation in A2a+ MSNs greatly attenuated cocaine conditioned place preference (CPP) ($n = 3$-6 per group; 2-way ANOVA Effect of Drug: F (1, 7) = 0.005593, $P = 0.9425$; Effect of sgRNA: F (1, 7) = 21.65, $P = 0.0023$).

Conclusions: Through the application of cocaine SA, INTACT/CUT and RUN + RNA-Seq and CRISPR epigenetic editing, we found that Nr4a1 activation in direct pathway MSNs is sufficient to attenuate or even reverse cocaine reward behavior.

Keywords: Cocaine Self-Administration, Epigenetics, CRISPR

Disclosure: Nothing to disclose.

P757. Intergender Social Interaction and Elevated Brain Gene Expression Levels in Dopamine Receptor Drd4 are Associated With Higher Levels of Ethanol Intake in Mice

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Background: There is considerable evidence that social environments, including sex pairing-mediated social interaction, affect ethanol drinking in humans and in animals. Differences in gene expression are also thought to play a role in modulating individual's level of ethanol consumption, although much less is known about the involvement of specific genes in the context of social interaction-related ethanol drinking.

Methods: CD-1 mice (Drinkers) were given continuous access in the two-bottle free choice paradigm (ethanol vs. water), while housed in their home cage in the presence of a proximal mouse companion (Cagemate) of the same or opposite sex. Since the amount of ethanol consumption varied substantially among different individuals, we compared mice with high ethanol consumption (High Drinkers) to mice with low ethanol consumption (Low Drinkers) and analyzed steady-state mRNA levels of dopamine receptor gene Drd4, with sex pairing between Drinker and Cagemate as an independent variable.

Results: The expression of dopamine receptor gene Drd4 differed significantly with sex pairing-mediated effects on ethanol intake. The differences of gene expression levels were most pronounced in Drinkers paired with Cagemates of opposite sex.

Conclusions: These findings suggest that voluntary ethanol intake may be modulated by social interaction with sex pairing as an influencing factor, and that dopamine neurotransmitter system may play a role in the underlying regulatory mechanisms.

Keywords: Social Interaction, Sex Pairing, Ethanol Intake, Gene Expression

Disclosure: Nothing to disclose.

P759. Deep Brain Stimulation of the Nucleus Accumbens Shell Increases GluR1/GluA1 in the Central Nucleus of the Amygdala and Does Not Decrease Cocaine Self-Administration in Cocaine-Dependent Rats

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Background: Cocaine addiction is a major public health problem. Despite decades of intense research, no effective treatments are available. Both preclinical and clinical studies of drug addiction

strongly suggest that the nucleus accumbens (NAcc) is a viable target for deep brain stimulation (DBS). Although previous studies have shown that DBS of the NAcc decreases cocaine seeking and reinstatement, the effects of DBS on cocaine intake in cocaine-dependent animals have not yet been investigated.

Methods: Rats ($n = 14$) were made cocaine-dependent by allowing them to self-administer cocaine in long-access sessions (6 h, 0.5 mg/kg/infusion). The effects of high-frequency DBS of the NAcc shell on cocaine intake was then studied. Furthermore, cocaine-induced locomotor activity, irritability-like behavior during cocaine abstinence and the levels of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits 1 and 2 (GluR1/GluA1 and GluR2/GluA2) after DBS were investigated.

Results: Contrary to our expectations, DBS of the NAcc shell induced a slight increase in both cocaine self-administration and cocaine-induced locomotor activity. In addition, 18 h into cocaine withdrawal, we found that DBS decreased irritability-like behavior. We also found that DBS-induced a robust increase in both cytosolic and synaptosomal levels of GluR1, but not GluR2, specifically in the central nucleus of the amygdala but not in other brain regions.

The escalation of cocaine self-administration ($n = 14$) was analyzed using one-way repeated-measures analysis of variance (ANOVA) or Student's paired *t*-test (two-tailed). The effect of DBS and sham treatments on the escalation of cocaine self-administration was analyzed using either two-way repeated-measures ANOVA or Student's paired *t*-test (two-tailed) using the average intake in the two OFF/ON sessions. The effect of DBS on irritability-like behavior was analyzed using unpaired *t*-test (two-tailed). Cocaine-induced locomotor activity was analyzed using two-way repeated-measures ANOVA. Differences in protein levels of GluR1 and GluR2 were analyzed using two-way ANOVA followed by Bonferroni's multiple-comparison post hoc test. The Western blot data were analyzed using two-way ANOVA followed by Bonferroni's multiple-comparison post hoc test. Values of $p < 0.05$ were considered statistically significant.

Conclusions: These preclinical results with cocaine-dependent animals do not support high-frequency DBS of the NAcc shell as a therapeutic approach for the treatment of cocaine addiction in active cocaine users. However, the decrease in irritability-like behavior during cocaine abstinence, together with previous findings showing that DBS of the NAcc shell reduces the reinstatement of cocaine seeking in abstinent animals, warrants future investigations of DBS as a treatment for negative emotional states and craving during abstinence.

Keywords: Cocaine Addiction, DBS, Withdrawal, Neuromodulation, Amygdala

Disclosure: Nothing to disclose.

P760. A Rise in Cortisol Following Smoking Cue Exposure is Associated With Greater Cue-Induced Brain Reactivity

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Background: Smoking cue reactivity drives the motivation to smoke and is associated with poorer treatment outcome (Ferguson 2009). There have been a wealth of studies evaluating the neurobiological and physiological underpinnings of cue reactivity and their relation to treatment outcome. For instance, we previously have shown that enhanced insula reactivity to smoking cues predicts relapse vulnerability (Janes et al. 2010; 2017), while others have reported a similar link between relapse and a greater drug cue-induced cortisol response (Fatseas 2011). The current work aims to fill a gap by evaluating the association between a cue-related rise in cortisol and brain reactivity to smoking cues in nicotine dependent individuals.

Methods: Our sample was drawn from a larger project investigating brain changes following a smoking cessation attempt. Participants were included if they smoked daily for the past 6 months and excluded if they had been diagnosed with psychiatric or neurological disorders. The final sample included 27 nicotine-dependent participants (9 women and 18 men, mean age [SD] = 27.0[5.9]) who smoked for an average of 10.2[6.7] years, with an average of 11.4[6.1] cigarettes per day. Participants underwent a 90-minute scanning session that included anatomical, resting state, and functional neuroimaging of an event-related cue reactivity paradigm containing smoking cues, neutral cues, target images, and a fixation cross. Salivary cortisol levels were obtained immediately before and after the scanning session.

FSL was used to model the BOLD response to smoking and neutral cues. At the group level, whole brain BOLD activation for the smoking > neutral cues contrast was correlated with the change in salivary cortisol (post-scan minus pre-scan) using a mixed effects model (FLAME 1). Cluster level thresholding was set to $z = 3.1$, $p = 0.01$. Ad hoc analyses used linear regression to determine whether time-of-day was associated with baseline cortisol levels or change in cortisol.

Results: Increases in BOLD activation in the right anterior insula and right dorsolateral prefrontal cortex (DLPFC) when viewing smoking vs. neutral cues were positively correlated with the pre-to-post-scan increase in salivary cortisol. On average, we saw no statistically significant increase in salivary cortisol response when comparing post to pre-scanning levels. Time of day of the scan had no effect on baseline cortisol levels ($R = 0.23$, $p = 0.27$) or change in cortisol ($R = -0.013$, $p = 0.95$).

Conclusions: The current findings show that a rise in cortisol following smoking cue-exposure is associated with elevated smoking cue-induced brain activation in the right insula and DLPFC. This finding moves the field forward in a couple of ways. First, by bridging two independent prior studies showing that both a cue-induce increase in insula activity (Janes et al. 2010; 2017) and cortisol (Fatseas 2011) relate to poorer treatment outcome. The present work goes as step further by suggesting a link between interoceptive processing of smoking cues, as mediated by the insula, and a quantifiable physiological response. Taken together, these findings indicate that these physiological neurobiological elements are acting in concert to facilitate the motivation to smoke.

Keywords: Substance Abuse, Cortisol, Insula, DLPFC, Cue Reactivity

Disclosure: Nothing to disclose.

P761. Behavioral Signature Identified for Relapse Vulnerability in a Rodent Model of Cocaine Use Disorder

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Background: Relapse is a dynamic, essential barrier to recovery in substance use disorders. Relapse is often precipitated by exposure to drug-associated cues and has been tied to impulsive behavior, particularly in cocaine use disorder. Motor impulsivity is characterized by impulsive action or the inability to withhold a premature response. Here, we tested the hypothesis that phenotypic levels of motor impulsivity may predict drug-seeking behavior following extended abstinence from cocaine.

Methods: Naïve male Sprague-Dawley rats ($n = 48$) were trained to stability on the 1-choice serial time reaction (1-CSRT) task and phenotyped as high impulsive (HI) or low impulsive (LI).

Rats were then trained to self-administer (SA) cocaine (0.75 mg/kg/inf) until stability on a fixed ratio 5 schedule of reinforcement, followed by reinitiation of stable 1-CSRT task performance. On the day corresponding to 30 days of abstinence from cocaine SA, rats underwent a drug-seeking test session in which lever presses were reinforced with the discrete cue complex previously paired with drug infusion.

Results: Acquisition of cocaine SA and the cumulative levels of cocaine intake observed did not differ in HI vs. LI rats. Rats identified as HI or LI retained their original motor impulsivity phenotype during 30-days of abstinence from cocaine SA, with HI rats exhibiting increased lever presses for cocaine-associated cues relative to LI rats.

Conclusions: These data suggest that antecedent levels of motor impulsivity are not a major driver of cocaine intake under the present conditions, but motor impulsivity is predictor of cocaine-seeking during extended abstinence. Importantly, these results demonstrate the efficacy of the motor impulsivity endophenotype in predicting relapse-like behaviors. Identification of motor impulsivity may provide more accurate and tailored diagnoses and/or treatments for patients, which could improve treatment outcomes, especially prevention of relapse.

Keywords: Cocaine, Impulsivity, SUD-Like Phenotypes

Disclosure: VidaLibreBio, Inc: Contracted Research (Self)

P762. Associations Between Sex Hormone Levels, Alcohol Dependence and Depression Related Phenotypes in UK Biobank

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Background: Sex and gender-related differences are associated with differences in alcohol consumption and risk for development of alcohol dependence (AD). Depression and anxiety spectrum disorders are also common among alcohol users and contribute to AD risk, progression and treatment outcomes in sex-specific manner. However, biological mechanisms underlying these complex relationships remain poorly understood, largely due to the limited number of samples powered to study such associations. Here, we present analyses of associations between sex hormone (testosterone and estrogen) levels and AD and depression related phenotypes in a large community-based sample collected by UK Biobank. We also considered the role of sex hormone binding globulin (SHBG) and albumin levels in these phenotypes.

Methods: AD cases ($N = 2\ 900$) were defined by having any ICD-9 or ICD-10 codes of AD, alcohol withdrawal, or alcohol-induced mental issues. Controls ($N = 448\ 918$) were having none of those ICD9 or ICD-10 codes or codes associated with alcoholic organ damage. We also exclude subjects with AUDIT score of 8 or above. The "narrowly defined" depression phenotype was identified by presence of ICD-9 or ICD-10 diagnosis of depressive disorder. In addition a "broad depression" phenotype defined according to Howard et al. (2018), i.e., self-report of ever seeking help from general practitioners or psychiatrists for nerves, anxiety and tension or depression was included in the analyses. Serum levels of total testosterone (TT), total estradiol (TE2), SHBG, and albumin were measured by chemiluminescent immunoassays performed on Beckman Coulter Uicel Dxl 800 system. Free and bioavailable testosterone were calculated based on assumed binding to SHBG and/or albumin.

For each hormone/protein, we compared sex hormone levels between patients with and without AD using linear regression. We also assessed whether these associations were moderated by depression phenotypes by including interaction terms. All analyses were stratified by sex and adjusted for age and BMI.

Results: The frequency of broad depression was higher in females than in males and nearly twice higher in AD cases compared to controls in both males (67.5% vs 35.0%) and females (82.8% vs 46.2%). The frequency of ICD-10 depression was even higher (8-fold and 6-fold) among AD cases compared to controls (67.1% vs 8.2% in males and 89.7% vs 14.4% in females). Interestingly, in AD cases prevalence of broad depression and ICD depression were similar (males: 67.5% vs 67.1%; females: 82.8% vs 89.7%), while in controls the prevalence of ICD depression was much smaller than broad depression (males: 8.2% vs 35.0%; females: 14.4% vs 46.2%). Due to the limited number of individuals with ICD depression, hormone association analyses were conducted only with broad depression phenotype. AD males had significantly higher TT ($p < 0.001$) and TE2 ($p < 0.001$) levels but lower bioavailable testosterone (BioT; $p < 0.001$) and estradiol (BioE2; $p < 0.001$) levels than the male controls. In females overall, AD subjects had significantly higher TT, free testosterone (FT), and BioT levels ($p < 0.001$, $p = 0.003$, and $p = 0.014$, respectively) but lower TE2 level ($p = 0.010$) levels than female controls. In both sexes, higher SHBG ($p < 0.05$) and lower albumin ($p < 0.001$) levels were found in the AD subjects. These differences were all statistically significant after adjusting of age and BMI.

The levels of FT, BioT and albumin were significantly affected by the interaction effect between AD and broad depression in males. Males with comorbid AD and broad depression had much higher levels of FT and BioT but lower SHBG levels than males with AD only, broad depression only, or without either condition (AD*BDep: $\beta = 10.38$, $p = 0.002$ and $\beta = 328.23$, $p = 0.001$, respectively). TT levels were negatively associated with broad depression ($\beta = -0.115$, $p < 0.001$).

In contrast, no significant interaction effects on hormone or protein levels were found in females, but AD and broad depression exerted significant independent effects on testosterone and estradiol levels. In particular, broad depression was negatively associated with TE2, free estradiol (FE2), and BioE2 levels ($\beta = -17.59$, $p < 0.001$; $\beta = -0.274$, $p = 0.005$; $\beta = -8.89$, $p < 0.001$, respectively). TT and FT levels were associated with broad depression in opposite direction ($\beta = -0.008$, $p < 0.001$ and $\beta = 0.184$ $p = 0.005$, respectively).

Conclusions: We found significant sex-specific associations between circulating levels of testosterone and estradiol and AD in both males and females. The SHBG levels were significantly higher and albumin levels significantly lower in AD subjects. Furthermore, the associations between testosterone levels and AD were found to be moderated by broad depression in males, in which subjects with AD and broad depression had the highest FT and BioT levels. In females, we found broad depression (i.e., treatment-seeking depression/anxiety) was associated with lower TE2, FE2, BioE2 levels regardless of AD. Overall, these findings support the importance of estradiol in regulating depression/anxiety in females but not in males and the significance of testosterone in regulating depression/anxiety in AD males but not in AD females. Future research is needed to replicate these findings and uncover the biological mechanisms underlying these associations.

Keywords: Sex Hormones, Alcohol Dependence, Depression

Disclosure: Nothing to disclose.

P763. The Interrelationship Between Anxiety and Binge Drinking in the National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA) Study

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Background: Anxiety (e.g., generalized anxiety disorder, social anxiety, phobias) is the most common mental disorder of adolescence, experienced by over 30% of youth and frequently co-occurs with alcohol use. Most studies examining neural characteristics in youth with anxiety traits have been small and larger studies clarifying the interrelationship between anxiety and alcohol use in humans are lacking. The National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA) provides an ideal resource to examine these neural characteristics. It is designed as a comprehensive study ($N = 831$) that recruited 12-21 year-olds and follows them for 10 years, annually measuring substance use, psychiatric functioning, and brain structure via magnetic resonance imaging (MRI). Some youth in the sample transition into anxiety and/or heavy drinking after a healthy baseline. Data from this study will help explain the interrelationships over time between anxiety and binge drinking in adolescence.

Methods: We used parallel process latent growth curve modeling to examine trends in directionality between adolescent anxiety and binge drinking, using annual Achenbach System of Empirically Based Assessment (ASEBA) measures of anxiety and Customary Drinking and Drug Use Records (CDDR) of monthly binge drinking (4+ drinks for females/5+ drinks for males within an occasion; also known as heavy episodic drinking or HED) from baseline to year 5 follow-up (ages 12-21). Parallel process latent growth curve modeling (PPGM) are a multivariate extension of latent growth models that examine concurrent levels and change-over-time across multiple measures. This approach allowed estimation of average anxiety and HED growth trajectories; trajectories for each individual within the sample; and to assess correlations between these change processes. We evaluated model fit using the adjusted $\chi 2$ difference test, where a non-significant result was an indication of adequate model fit. We supplemented this test with a number of alternative fit indices, including the comparative fit index (CFI), the Tucker-Lewis index (TLI), the root mean square error of approximation (RMSEA), and the standardized root-mean square residual (SRMR). In our comprehensive final model, we included race as a covariate to control for confounding effects of racial majority identity. All analyses were conducted in *R* using lavaan.

Results: We found there to be no significant linear and/or quadratic relationship between ASEBA Anxiety raw scores and CDDR monthly binge drinking in the NCANDA study from baseline to 5 years.

Conclusions: Our work provides a novel investigation of the interrelationship between ASEBA Anxiety raw scores and CDDR monthly binge drinking in the NCANDA study. This is in line with other studies that have found no clear association between anxiety and substance use. This may highlight the importance of primary treatment of binge drinking before addressing co-morbid anxiety disorders.

Keywords: Adolescent Binge Drinking, Adolescent Anxiety, NCANDA, Longitudinal Study

Disclosure: Nothing to disclose.

P764. The Dual Orexin Receptor Antagonist Suvorexant Reduces Cocaine Behaviors at Non-Sedating Doses in a Rat Model of Addiction

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Background: The orexin (hypocretin) receptor system has emerged as a promising target for novel therapies to treat cocaine use disorder. The dual orexin receptor antagonist suvorexant is FDA-approved for the treatment for insomnia and has a strong safety/tolerability profile, and thus could be readily repurposed. Although there is some evidence in rat to indicate that suvorexant can reduce cocaine-related behaviors, this has not been tested in a model of cocaine self-administration that promotes a multifaceted addiction-like phenotype. Moreover, the dose-response profile of suvorexant when administered via a clinically relevant route (p.o.) in rat has not been examined, nor is it known if there are sedative or other off-target effects at doses required to reduce cocaine behaviors. Thus, here we tested the efficacy of suvorexant (delivered p.o.) on the expression of addiction phenotypes in rats with a history of intermittent cocaine self-administration. We also tested for any off-target effects of suvorexant using assays of general activity and psychomotor vigilance.

Methods: Male ($n = 16$) and free-cycling female ($n = 18$) Long Evans rats were assessed for baseline economic demand for cocaine using a within-session threshold procedure. Rats were then given daily intermittent access to cocaine (5min access every 30min for 6h) for 2 weeks before being reassessed for economic demand and compulsive (punished) responding for cocaine. The effect of suvorexant (0, 3, 10, 30mg/kg; oral gavage) on demand and compulsive (punished) responding was assessed. Potential sedative effects of suvorexant were assessed on locomotor reactivity and operant psychomotor vigilance tasks.

Results: In both male and female rats, intermittent access to cocaine was associated with a significant decrease in demand elasticity for cocaine (increased motivation). In male rats, this enhanced motivational profile was significantly attenuated by suvorexant (10, 30mg/kg), as was compulsive (punished) responding for cocaine. At the minimum effective dose of suvorexant (10mg/kg), no sedative effects were observed on the locomotor activity test in male rats; examination of suvorexant's effects on the psychomotor vigilance task is ongoing. Data relating to efficacy and potential sedative effects in female rats is also forthcoming.

Conclusions: In male rats that transitioned to an addiction-like state, suvorexant was effective at reducing key addiction endophenotypes at doses that are non-sedating, supporting the potential repurposing of this compound for the management of cocaine use disorder. Ongoing studies are testing for any effects of the minimum effective dose on cognitive/psychomotor outcomes, as well as the efficacy of suvorexant in reducing addiction behaviors in females. These studies may also be informative for the repurposing of other FDA-approved dual orexin receptor antagonists for the management of substance use disorders, including opioid use disorder.

Keywords: Dual Orexin Receptor Antagonist, Cocaine, Addiction, Insomnia, Neuropeptides

Disclosure: Nothing to disclose.

P765. Homogenate and Synaptic Proteomics Reveals Alterations in Immune, Mitochondrial, and Dopamine Signaling in the Brains of People With Opioid Use Disorder

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Background: Prevalence rates of opioid use disorder (OUD) have increased dramatically, accompanied by a surge of overdose deaths. Additionally, most patients being treated for OUD relapse within the first year of treatment. While OUD has been extensively studied in preclinical models, an understanding of the molecular alterations that occur in the brains of people chronically use opioids and who are diagnosed with OUD remains limited.

Corticostriatal circuity in the brain is heavily implicated in opioid reward, craving, and relapse, including the dorsolateral prefrontal cortex (DLPFC) and nucleus accumbens (NAc). To address this limitation, we used proteomics to investigate alterations in proteins in DLPFC and NAc homogenates and synaptosomes from postmortem brains in people with OUD.

Methods: Both DLPFC and NAc were collected from postmortem brains of unaffected comparison subjects ($n = 20$) and subjects with OUD ($n = 20$). Between unaffected and OUD cohorts, subjects were matched on postmortem interval, RNA integrity, age, sex, and other criteria. In the OUD cohort, subjects were both diagnosed with OUD and known to be active opioid users at time of death. Targeted mass spectrometry was used to measure protein levels in DLPFC and NAc homogenate and synaptosome preparations. Experimenters were blinded to diagnosis for sample preparation and proteomics experiments. Protein expression analyses between unaffected and OUD subjects was conducted using regression modeling (Limma) with covariates (e.g., age, sex, postmortem interval). Protein differential expression (DE) analyses used fold-change (FC greater than plus/minus, 1.2) and *p*-value ($p < 0.05$) thresholds. For DE proteins in OUD, we conducted pathway enrichment, gene set enrichment analyses (GSEA), and protein-protein interaction (PPI) and transcription factor (TF) analyses. Integrative analyses between transcriptomics (RNA-seq) and proteomics data in brain regions from same subjects was also used to identify isoform-specific and peptide-specific enrichments in DLPFC and NAc.

Results: Between unaffected and OUD subjects, we identified 61 DE proteins (10 decreased and 51 increased in OUD) in DLPFC homogenates. Enrichment analyses in the DLPFC of OUD subjects included pathways related to metabolism, synaptic vesicle signaling, and glucocorticoids ($p < 0.05$). In NAc homogenates, we identified 29 DE proteins in OUD subjects (22 decreased and 7 increased). Enrichment analyses in the NAc identified pathways related to cytoskeleton, immunity, nuclear receptor signaling, and dopamine signaling ($p < 0.05$). In synaptosomes, GSEA identified innate immunity, RNA-binding, and oxidative phosphorylation pathways as enriched in the DLPFC of OUD subjects (143 DE synaptic proteins), while pathways related to glial cell activation, metabolism, and lactate signaling were enriched in the NAc (58 DE synaptic proteins). PPI and TF analyses predicted involvement of HIF, PPAR, and SP1 family of proteins, along with RNA-binding proteins, in protein interaction networks in DLPFC and NAc of OUD subjects ($p < 0.01$). Using integrative approaches of transcriptomics and proteomics datasets from the same subjects, we also found isoform- and peptide-specific alterations in DLPFC and NAc associated with OUD.

Conclusions: Our findings begin to uncover the molecular alterations in corticostriatal circuitry in the brains of people with OUD. Overall, our further suggest connections between the brain's immune system, cellular metabolism, and synaptic function in chronic opioid use and OUD in the human brain.

Keywords: Opioid Use Disorder, Opioid, Opiate Addiction, Human Postmortem Brain, Proteomics

Disclosure: Nothing to disclose.

P766. Sex Differences in Corticolimbic Kappa Opioid Receptor Availability Among Individuals With Alcohol Use Disorder Compared to Healthy Controls

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Background: Alcohol abuse 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 Women and Alcohol]. Alcohol has been shown to interact with numerous neurotransmitter systems to exert its pharmacological effects. The endogenous kappa opioid receptor (KOR) system has been strongly implicated in positive and negative reinforcing effects of alcohol, negative affect, and stress [Bruchas et al. 2010 Brain Res]. Dysregulation of the KOR system by chronic alcohol use contributes to individual differences in alcohol use behaviors [Kosterlitz et al. 1989 NIDA Res Monogr] and blockade of KORs decreases alcohol intake in dependent but not in nondependent rats [Walker and Koob 2008 NPP]. A large body of work shows that women are more likely to drink to regulate negative affect/stress, while men are more likely to drink for positive reinforcement [Logrip et al. 2018 Alcohol]. These differences may be attributed to neuroadaptations in the KOR system, particularly in the coupling of amygdalo-frontal cortex region projections, which are critical for negative affect/stress regulation [Tejada et al. 2015 NPP]. Currently approved alcohol use disorder (AUD) medications have a relatively low efficacy, were initially developed with male samples and, do not consider sex-specific targets. We previously observed that individuals with AUD had lower corticolimbic levels of available KOR than healthy control (HC) subjects (Vijay et al. 2018 NPP]. We also observed that healthy males had higher levels of available KOR than healthy females [Vijay et al. 2016 AJNMMI], suggesting that men and women with AUD should be compared to their sex-matched HC counterparts. The goal of this study is to examine the KOR system in women and men with AUD compared to their HC counterparts. Based on preclinical and human behavioral studies, and our prior work mentioned above, we hypothesized that men with AUD will have lower KOR than HC men in striatum, amygdala, and frontal cortex and that women with AUD vs. HC women will have lower KOR in amygdala, but show no differences in frontal cortex and striatum.

Methods: Fifty-two individuals with AUD (19 females) and 25 HC subjects (9 females) underwent positron emission tomography (PET) scans with [11C]LY2795050, a selective, high-affinity, KOR antagonist radiotracer with favorable kinetics for imaging KOR in vivo [Kim et al. 2013 JNM]. Partial-volume correction was applied to all AUD and HC subject PET data to correct for potential atrophy. Volume of distribution (VT) of the tracer was estimated regionally as a measure of KOR availability. VT values of AUD versus HC were compared for 3 a priori regions of interest based on their behavioral involvement–striatum (reinforcement), amygdala (negative affect) and frontal cortex (negative affect/stress regulation). Independent-samples *t*-tests were used to compare VT values for men with AUD vs. HC men and, women with AUD vs. HC women.

Results: We found preliminary evidence of AUD-related differences in KOR availability between sexes. KOR availability was significantly lower in men with AUD compared to HC men in striatum ($p = 0.038$, Cohen's $d = 0.374$) and amygdala ($p = 0.013$, Cohen's $d = 0.597$), and trending in the same direction in frontal cortex ($p = 0.069$, Cohen's $d = 0.420$). Women with AUD had lower KOR availability than HC women in amygdala ($p = 0.008$, Cohen's $d = 0.319$), but did not show differences in KOR availability in striatum ($p = 0.973$, Cohen's $d = 0.302$), and frontal cortex ($p = 0.787$, Cohen's $d = 0.370$).

Conclusions: Consistent with our hypotheses, KOR availability was lower in men with AUD than HC men in striatum and amygdala, and lower, but not statistically significant, in frontal cortex. Women with AUD vs. HC showed lower KOR availability in amygdala, but no differences in frontal cortex and striatum. These data suggest that men and women with AUD have different patterns of KOR neuroadaptations compared to HCs. In women, the pattern of amygdalo-frontal decoupling of KOR may explain why women drink to regulate negative affect. These findings point towards a possible neurobiological basis for sex differences in

alcohol use behaviors and potential differences in clinical effectiveness of opioid receptor-targeted medications. We are currently examining imaging-based biomarkers of stress in an expanded sample with more women.

Keywords: Alcohol Use Disorder, Sex Differences, Kappa Opioid Receptor, Positron Emission Tomography (PET), Corticolimbic

Disclosure: Nothing to disclose.

P767. Investigating Brain Astrocyte Status in Alcohol Use Disorder: A Positron Emission Tomography Study With the Monoamine Oxidase B Radiotracer [C-11]SL25.1188

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Background: Alcohol use disorder (AUD) relapse rates are high and current pharmacotherapies are limited. In this regard, astrocytes have been identified as an innovative therapeutic target for AUD. Monoamine oxidase-B (MAO-B) is a putative marker of astroglia levels, however, there is a lack of in vivo studies investigating MAO-B in AUD. Our objective was to establish whether MAO-B levels, as inferred from binding of a positron emission tomography (PET) radiotracer, [C-11]SL25.1188, would be elevated in living brain in AUD in early abstinence and later abstinence from alcohol, as suggested by some preclinical and post-mortem data.

Methods: In this pilot study, treatment-seeking patients with a DSM diagnosis of AUD received one PET scan with the MAO-B radiotracer [C-11]SL25.1188 after 3–7 days of abstinence ($n = 15$; M/F 10/5; 49 ± 10 years) with a subgroup of four participants completing a second scan at 2–4 weeks of monitored abstinence from alcohol. Participants were not asked to abstain from cigarette use, if applicable. Healthy controls ($n = 14$; M/F 8/6; 42 ± 13 years) completed one scan. [C-11]SL25.1188 total distribution volume (VT) in cortical and subcortical brain regions was compared between AUD and controls, as well as within-subjects in early and later abstinence from alcohol.

Results: A repeated measure (rm-) ANOVA revealed that [C-11]SL25.1188 VT did not significantly differ between controls and AUD in early abstinence ($F(1,27) = 0.22$, -4%, $p = 0.63$). However, AUD subjects who smoked cigarettes ($n = 5$) had, as expected from the literature, significantly reduced [C-11]SL25.1188 VT compared to controls ($F(1,17) = 19.5$, -36%, $p < 0.001$) and non-smoking individuals with AUD ($F(1,13) = 24.6$, -43%, $p < 0.001$). Taking smoking into consideration, a rm-ANCOVA revealed trends for a marginal effect of time of abstinence ($F(1,2) = 10.4$, $p = 0.09$, +3%), and a marginal time x smoking interaction ($F(1,2) = 11.4$, $p = 0.08$, smoker ($n = 1$) +28%, non-smokers ($n = 3$) -6%). [C-11]SL25.1188 VT did not differ between non-smoking controls and non-smoking AUD ($F(1,20) = 0.95$, +6%, $p = 0.34$).

Conclusions: Our exploratory study is limited by the small sample size. To the extent that astrogliosis status can be inferred from SL binding by PET, our data suggest that brain astroglial status might be normal in AUD in early and slightly longer abstinence, however there may be an effect of cigarette smoking. We also replicate the previous finding of low brain MAO-B binding in current cigarette smokers (Fowler et al. 1996). Robust MAO-B inhibition in smoking AUD might alter reinforcing properties of alcohol. Continuous recruitment of AUD participants in our ongoing study may well provide findings that will modify these tentative conclusions. Supported by U.S. NIH NIAAA 026680 and Canada CIHR GSD-159264.

Keywords: Monoamine Oxidase B, Positron Emission Tomography (PET), Alcohol Use Disorder

Disclosure: Nothing to disclose.

P768. Time-Dependent Role of Dorsal Hippocampus in Context-Cocaine Memory Reconsolidation

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Background: In the course of chronic cocaine use, associations form between the motivational effects of cocaine and environmental stimuli in which these effects are experienced. Re-exposure to cocaine-predictive environmental stimuli leads to the retrieval and destabilization of corresponding context-cocaine memories. As a result, labile context-cocaine memories need to be re-stabilized into long-term memory stores through the protein synthesis-dependent process of memory reconsolidation in order to be maintained over time. Importantly, interference with memory reconsolidation weakens cocaine-associated memories and reduces cocaine seeking-behaviors in preclinical models of drug relapse.

The dorsal hippocampus (DH) is a brain region that plays a critical role in context-cocaine memory reconsolidation. Inhibition of neural conductance or Src-family tyrosine kinase activity in the DH immediately after retrieval-induced memory destabilization disrupts subsequent cocaine-seeking behaviors. Inhibition of protein synthesis in the DH fails to alter memory reconsolidation, but neural conductance in the DH is necessary for protein synthesis-dependent memory reconsolidation in the basolateral amygdala. We have hypothesized that the DH maintains or provides access to labile context-cocaine memories prior to their re-stabilization in the basolateral amygdala during reconsolidation. If so, the functional contribution of the DH is likely limited to the early stages of cocaine-memory reconsolidation. We evaluated the time-dependent involvement of the DH in cocaine-memory reconsolidation using temporally precise optogenetic manipulations.

Methods: Rats received bilateral intra-DH infusions of AAV5-hSyn-eNpHR3.0-eYFP-WPRE-PA (eNpHR3.0, 5.5 x 1012 vg/ml) or AAV5-hSyn-eYFP (eYFP, 3.5 x1012 vg/ml) (0.7 µl/hemisphere), indwelling optic fiber implants aimed at the DH, and a jugular catheter implant. Rats were trained to press a lever for intravenous cocaine infusions under a fixed ratio 1 schedule. Training sessions took place in a distinct environmental context for 2 hours/day over 10 days. The rats then received extinction training sessions in a different context for 2 hours/day over 7 days. Twenty-four hours after the last extinction training session, rats were either re-exposed to the previously cocaine-paired context for 15 minutes to trigger the destabilization of cocaine memories or remained in their home cages. Rats in each group then received laser-light stimulation (light-ON; 532nM; 10 mW; 5s on 5s off, 1-hour duration) or no laser light stimulation (light-OFF) immediately after or 1-hour after the memory retrieval session or home cage stay (8 groups total; $n = 6$-8/group). On the next day, daily extinction training sessions resumed until lever presses declined to a predetermined extinction criterion (~2 days). During this time, potential effects of optogenetic manipulations on extinction memory strength were assessed based on non-reinforced lever presses in the extinction context. Lastly, rats were re-exposed to the cocaine-paired context to assess the effects of the optogenetic manipulations on context-cocaine memory strength. The effectiveness of the optogenetic constructs was verified in a separate cohort of rats. Brain tissue was collected from these rats

immediately after a 15-min memory retrieval session and either the light-ON or light OFF manipulation ($n = 6$-7/group). C-Fos immunoreactivity was quantified in GAD67- and CaMKII-immunoreactive cell populations in the dorsal cornu ammonis (dCA3) region, just ventral to the tip of the optic fiber tracts.

Results: There were no pre-existing differences between the experimental groups in lever responding during the self-administration, extinction, memory retrieval, and post-treatment extinction sessions. Inhibition of eNpHR3.0-expressing DH neurons immediately ($p = 0.02$), but not one hour ($p = 0.48$), after memory retrieval attenuated cocaine-seeking behavior in the cocaine-paired, but not the extinction, context at test. In contrast, inhibition of eNpHR3.0-expressing DH neurons without memory retrieval (i.e., after home cage stay) did not alter cocaine-seeking behavior ($p = 0.64$). Similarly, light-ON manipulation of eYFP-expressing DH neurons immediately after memory retrieval ($p = 0.49$) did not alter cocaine-seeking behavior at test. The light-ON manipulation in the eNpHR3.0-expressing group reduced c-Fos expression in the dCA3 compared to the light-OFF manipulation ($p = 0.04$), whereas it had no effect on in the eYFP-expressing group. These effects in the eNpHR3.0-expressing group were observed in both GAD67- ($p < .0001$) and CaMKII-immunoreactive ($p = 0.05$) dCA3 cell populations.

Conclusions: Optogenetic inhibition of the DH reduced context-cocaine memory strength, as measured based on the magnitude of cocaine-seeking behavior in the previously cocaine-paired context, in a time-dependent fashion, when it was applied during the first, but not the second, hour of cocaine-memory reconsolidation. DH engagement was required for context-cocaine memory retrieval, and the changes in behavior did not reflect nonspecific effects of virus expression or laser light exposure on cell health. Together, these findings suggest that the critical contribution of the DH is limited to early-stage memory reconsolidation, consistent with the idea that the DH may support the maintenance of labile context-cocaine memories prior to their re-stabilization in long-term memory stores.

Keywords: Cocaine Self-Administration, Memory Reconsolidation, Dorsal Hippocampus, Optogenetics

Disclosure: Nothing to disclose.

P769. Alcohol Use Severity Relates to Anterior Cingulate Activity During the Classic but Not the Alcohol Stroop

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Background: Alcohol use disorder (AUD) is characterized by a persistent consumption of alcohol despite negative consequences. Individuals with AUD have difficulty abstaining from alcohol in part because stimuli associated with alcohol capture attention and drive use (Cox et al. 2006). For example, individuals with AUD display greater cue-induced behavioral interference during the alcohol Stroop (Lusher et al. 2004). However, it is unclear whether this increased alcohol cue-induced interference is due to the interference of salient, self-relevant drug-related stimuli or is due to a more general cognitive control deficit. The current study addresses this gap by evaluating brain reactivity using an alcohol and classic (i.e., color-word) Stroop, which assesses both alcohol and non-alcohol-related interference. During both tasks, we measured brain reactivity in the rostral and dorsal anterior cingulate (rACC and dACC) due to their involvement in affective and cognitive interference, respectively (Mohanty et al. 2007). We also related brain reactivity in both ACC regions with AUD severity to determine whether brain reactivity during the alcohol or classic Stroop is more strongly linked to addiction severity.

Methods: 46 participants (32 male, 13 female, 1 transgender; mean age [SD] = 40.8 [11.0]) were recruited as a part of an ongoing double-blind, randomized, controlled treatment study of an anticonvulsant medication for AUD. The current analysis used data from the pre-treatment baseline collection period. Participants completed the Alcohol Dependence Scale (ADS) and an fMRI scan. During the fMRI scan, participants completed a modified Stroop task that incorporated both the alcohol Stroop and the classic color-word Stroop. fMRI data were analyzed in FSL, and beta weights for the rACC and dACC regions of interest were extracted for the alcohol Stroop (alcohol > neutral) and classic Stroop (incongruent > congruent) contrasts of interest. AUD Severity was correlated with rACC and dACC activity for each contrast.

Results: Participants displayed behavioral interference for both the alcohol and classic Stroop, with significantly slower reaction times for alcohol trials versus neutral trials ($t = 7.85$, $p < 0.001$) and incongruent trials versus congruent trials ($t = 8.78$, $p < 0.001$). There was significantly more rACC activity during the alcohol Stroop (alcohol > neutral contrast) relative to the classic Stroop (i.e., incongruent > congruent contrast) ($t = 2.80$, $p = 0.01$). dACC activity did not significantly differ between the alcohol and classic Stroop ($t = -0.08$, $p = 0.94$). AUD severity was associated with increased dACC activity during the classic Stroop ($r = 0.31$, $p = 0.03$) but not the alcohol Stroop ($r = -0.05$, $p = 0.75$). Stroop performance (i.e., reaction time and accuracy) were not associated with rACC or dACC activity or AUD severity (all p's > 0.05).

Conclusions: In a sample of adults with AUD, both the alcohol and classic Stroop resulted in behavioral interference. We found that the rACC displayed increased activity to the alcohol Stroop relative to the classic Stroop, whereas the dACC did not. Consistent with the current findings, increased rACC activation is linked to affective interference during emotional Stroop paradigms (Mohanty et al. 2007). More broadly, the rACC is a key node of the default mode network, which supports self-referential processing (Qin and Northoff, 2011), and the activation of this network is anticorrelated with cognitive task performance (Frida et al. 2005). The current findings suggest that when individuals with AUD complete the alcohol Stroop, regions implicated in self-referential processing show enhanced activation, which may contribute to cue-related affective interference. In addition, we found that AUD severity was linked to dACC activity during the classic Stroop. Together, these findings indicate that while alcohol words activated self-referential processing regions in adults with AUD, only participants with more severe AUD required greater recruitment of cognitive control regions to meet the task demands of the cognitive Stroop. Our findings have implications for treatment development. Specifically, while treatments that reduce the impact of alcohol cues may be beneficial for individuals across a wide range AUD severity, treatments that also improve executive function may be particularly beneficial for individuals with more severe AUD.

Keywords: Alcohol and Substance Use Disorders, Functional MRI (fMRI), Anterior Cingulate Cortex (ACC)

Disclosure: Nothing to disclose.

P770. Systemic Exposure to the HIV Protein gp120 Prevents the Inhibition of Cue-Induced Cocaine Seeking by the Novel Dopamine D3 Receptor Partial Agonist MC-25-41

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Background: Human immunodeficiency virus (HIV) and cocaine use disorders (CUDs) remain pervasive public health concerns in the United States and worldwide, which disproportionately impacts male minority populations. Exposure to HIV and its protein products (e.g.,

the HIV envelope glycoprotein gp120) enhances sensitivity to the reinforcing effects of psychostimulants, increases drug-induced dopamine transmission in the brain, induces chronic neuroinflammation within the brain, and facilitates drug-seeking behavior. Conversely, psychostimulants are known to potentiate viral replication and proliferation, which highlights the synergistic nature of this comorbidity. A barrier in combating comorbid HIV and CUDs is enhanced motivation (or "craving") for cocaine that typically grows stronger throughout protracted abstinence, an effect known as the "incubation of drug craving" effect. Importantly, the combined effects of HIV and chronic cocaine self-administration on relapse-like behavior and neuroinflammation following protracted abstinence are unclear. We addressed these critical gaps in knowledge by assessing cocaine-seeking behavior and neuroinflammation following protracted abstinence from cocaine self-administration and systemic exposure to gp120. Importantly, few studies have attempted to elucidate whether HIV disrupts the therapeutic efficacy of medications that are otherwise successful in reducing cocaine-seeking behavior. Thus, another aim of this study was to examine whether partial agonist stimulation of the dopamine D3 receptor (D3R), which we have shown inhibits cocaine (but not sucrose) self-administration, decreases cue-induced cocaine seeking in animals exposed to gp120.

Methods: We examined whether the D3R partial agonist, MC-25-41, could suppress cue-induced cocaine seeking following a period of protracted abstinence and if systemic exposure to the HIV protein gp120 modulates this effect. Male rats ($n = 6$-7/group) first received surgical implantation of intracranial guide cannulae and jugular vein catheters (for cocaine rats only). Rats were then trained to self-administer sucrose (45 mg/pellet) or intravenous cocaine (0.75 mg/kg/infusion) 2 hrs/day, first on a fixed-ratio (FR)-1 schedule of reinforcement, followed by variable ratio (VR)-2, VR-3, and VR-5 schedules of reinforcement. After a ≥ 12 self-administration sessions, rats entered a 5- or 21-day abstinence period, where they received intracerebroventricular (i.c.v.) microinfusions of gp120 (50 ng/1 µL infusion) or vehicle (1 µL PBS) once per day for days 1-5 of abstinence. The 5-day abstinence rats were euthanized for fresh brain tissue collection. The 21-day abstinence rats received a 1-hr cue reactivity test, where they received an injection of MC-25-41 (10 mg/kg, i.p.) or vehicle (20% cyclodextrin + 3% 1M HCL in saline, 1 mL/kg, i.p.) 10 mins before testing. Immediately after testing, rats were euthanized for fresh brain tissue collection for subsequent protein analyses. All animal procedures were approved by the Arizona State University 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: MC-25-41 treatment, relative to vehicle treatment, significantly attenuated cue-induced cocaine seeking in rats treated with i.c.v. PBS ($p < 0.05$). However, MC-25-41 failed to attenuate cue-induced cocaine seeking in gp120-exposed rats ($p > 0.05$). Importantly, gp120 did not suppress or potentiate cue-induced cocaine seeking on its own among vehicle-treated rats ($p > 0.05$). Ongoing analyses are being conducted to analyze protein expression of a broad panel of neuroimmune substrates (e.g., cytokines) within the nucleus accumbens (NAc) and prefrontal cortex (PFC). Interactions between reward type (cocaine vs. sucrose), i.c.v. treatment type (gp120 vs. PBS), i.*p*. treatment type (MC-25-41 vs. vehicle), and abstinence length (5 d vs. 21 d) on the expression levels of these neuroimmune substrates will be examined.

Conclusions: The present findings corroborate our previous studies demonstrating that D3R partial agonism effectively suppresses cocaine-seeking behavior. However, rats exposed to gp120 failed to respond to D3R partial agonist treatment. Taken together, these findings suggest that targeting D3Rs may not be an effective therapeutic strategy for individuals with comorbid HIV/CUD. This study highlights the need for future studies that attempt to elucidate the therapeutic value of novel medications intended for CUDs within animal models of comorbid HIV/CUD.

Keywords: Cocaine Seeking, HIV, Dopamine D3 Receptors, Abstinence, Neuroinflamation

Disclosure: Nothing to disclose.

P771. Effects of Acute Alcohol Intake on Reinforcement Learning and Sensitivity

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Background: Individual differences in the subjective rewarding and stimulatory effects of alcohol have been proposed as a risk factor for alcohol use disorder. Alcohol intoxication is also associated with risky behaviour, which may, in part, be driven by reduced sensitivity to punishment. However, few studies have directly evaluated the effects of alcohol intake on reward and punishment learning in humans or examined whether alcohol-induced behavioural changes in reinforcement learning map onto the self-reported subjective effects of alcohol. In the current study, we examined the effects of acute alcohol intake on reinforcement learning and sensitivity using a probabilistic reversal learning task, and assessed whether reward and punishment learning on this task was related to subjective feelings of alcohol intoxication.

Methods: Healthy social drinkers (university students; $n = 32$) performed a probabilistic reversal learning task (PRLT) during both alcohol (dose = 0.6 g/kg) and placebo conditions in counterbalanced order. Behavioral data were analyzed using a reinforcement learning model to provide individually fitted parameters of reinforcement (i.e., reward and punishment) learning rate and sensitivity. Self-report questionnaires were used to assess subjective mood and alcohol effects.

Results: Although overall behavioural performance on the PRLT did not differ between alcohol and placebo conditions (e.g., percent of 'correct' responses), computational modeling analysis revealed that alcohol intake was associated with both greater reward and punishment sensitivity compared to placebo. Further, punishment learning rate was greater during the alcohol condition compared to placebo, whereas reward learning rate was reduced during alcohol compared to placebo. Neither reinforcement learning rate nor sensitivity parameters were significantly associated with the self-reported rewarding (or other) effects of alcohol.

Conclusions: Alcohol intake significantly alters reinforcement-based decision making, but does so differently for reward versus punishment. Implications and future directions for understanding alcohol-induced changes in reinforcement learning as a mechanism of alcohol misuse will be discussed.

Keywords: Alcohol, Probabilistic Reversal Learning, Reward Sensitivity

Disclosure: Nothing to disclose.

P772. 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 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 exposure to drugs strongly and uniquely impacts gene expression transcriptome wide. However, aside 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 during the seeking of 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 and 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 target maladaptive drug seeking without affecting essential and biologically adaptive seeking of natural rewards.

Keywords: Cocaine Addiction, Reward, RNAseq

Disclosure: Nothing to disclose.

P773. Improving Outcomes in Patients With Co-Occurring Disorders

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Background: The Howard University Minority AIDS Network Effort (HUMANE) Program address major gaps in services by providing peer support services, case management and HIV/hepatitis prevention service in three different sites - an outpatient mental health services, a primary care clinic and an infectious disease clinic. With the overarching goal of improving the health and well-being of persons with Serious Mental Illness (SMI) or Co-occurring Disorders (COD) living with, or at risk for, HIV or hepatitis, one of the aims of the program is to deliver Integrated Dual Disorders Treatment (IDDT). The IDDT model is an evidence-based practice that improves quality of life for people with co-occurring severe mental illness and substance use disorders by combining substance abuse services with mental health services.

Methods: Center for Mental Health Services (CMHS) National Outcome Measures (NOMs) Client-Level Measures for Discretionary Programs Providing Direct Services was used during the enrollment to the program and six-month reassessment. A sample of 138 participants (18-75 years old) were enrolled in the program and avail the service of a peer support specialist trained to conduct IDDT weekly. Satisfaction with the service treatment was measured through the Consumer Perception of Care (CPC).

Results: Out of the 138 participants 76 are women and 132 are African Americans. Among the enrollees, 37 (26.81%) have a diagnosis of MDD recurrent, 22 (15.94%) with bipolar disorder, 10 (7.25%) with schizophrenia, 12 (8.70%) with anxiety disorder, 21 (15.21%) with opioid use disorder, 13 (9.42%) with alcohol use disorder. During enrollment, 125 (90.58%) participants reported having HIV test and 30 (21.74%) were HIV positive. 23 HIV + participants said they always used antiretroviral therapy (ART). 118 (85.51%) participants reported having HBV test during enrollment and 2 of them were HBV + and were connected to treatment service. Likewise, 120 (86.96%) participants had HCV test during enrollment and 8 of them were HCV + and all were connected to treatment services. During 6-month reassessment, 98.81% of participant reported of having HIV test. Likewise, 98.81% participants reported of having HBV and HCV tests during their 6 months reassessment.

Out of the 101 participants due for 6-month reassessment, eighty-four completed the NOMs survey. All received IDDT interventions provided by a trained peer support specialist. Participants were generally satisfied with the IDDT evidence-based intervention they received. During the reassessment, 77 (91.67%) participants agreed that information needed was provided to them, 73 (86.90%) agreed that they were encouraged to use consumer run programs. All participants agreed that they like the service they received from the program. Twenty-four (28.57%) strongly agreed that if given other choices they would still receive the service and recommend to others.

Conclusions: Participants' perceptions of the peer support specialist run groups using the IDDT evidence-based model within this SMI/substance abuse service program were by and large positive. General improvement of the overall health of the participants were noted during the 6-month reassessment. While this study provides support for using evidence-based intervention for dual-diagnosed, more work is needed to implement the utilization of a peer support specialist into the SMI/substance abuse service integrated program.

Keywords: Substance Use Disorders, Serious Mental Illness, Evidence-Based Approach

Disclosure: Nothing to disclose.

P774. Amplification of the Effects of Adolescent Binge Alcohol Exposure by Pretreatment With an Alpha-7 Nicotinic Receptor Positive Allosteric Modulator

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Background: Adolescent Binge Alcohol Exposure (ABAE) is a prevalent problem in our society. A recent trend in adolescent/young adult drinking is that the starting point of the incidents of binge drinking has reduced and a sharper increase in the overall rate of binge drinking during the transition from late adolescent/young adulthood into adulthood. Binge drinking during

adolescence produces numerous alterations in adolescent/adult phenotypes that have multiple deleterious consequences. Recently we have published multiple findings that ABAE produces prolonged alterations in the Alpha-7 Acetylcholine Nicotinic Receptor (a7) and that pretreatment with multiple a7 negative allosteric modulator (NAM) prevents ABAE-induced increase of adult alcohol consumption. The goal of this experiment is the exact opposite. The current study determined if pretreatment with an a7 positive allosteric modulator would enhance the effects of Adolescent Moderate Alcohol Exposure (AMAE) to promote adult alcohol consumption.

Methods: Wistar male and female rats were pretreated (2 hr) with the a7 PAM PNU (0, 3, or 5 mg/kg) prior to ABAE-like exposure (2 g/kg – half of the typical dose or water) in our standard ABAE paradigm. Adult alcohol consumption employed voluntary intake of beer to stimulate intake of pharmacological relevant levels of alcohol (>100 mg% BEC). In males, on PND 75, rats were exposed to 24 hr free-choice beer drinking for 6 weeks, beer deprived for 2 weeks, and then beer was reinstated for 2 weeks (ADE-relapse). In females, on PND 75, rats were given 1 hr operant access to beer. Daily operant access to beer was maintained for 10 weeks, followed by 1 week of extinction training, 2 weeks homecage period, 4 days of beer-seeking testing, 2 weeks of homecage period, and finally 2 weeks of beer re-exposure (relapse, ADE testing, ongoing).

Results: All male rats consumed beer excessively with no effect of AMAE or PNU (p values > 0.54). During the 6th week of beer access, male Wistar rats were consuming 115 g of beer/day (1/4 of body weight), an obvious ceiling effect (approximately 14 g/kg/day). In female rats (operant self-administration), pretreatment with 5 mg/kg PNU combined with 2 g/kg ABAE treatment significantly enhanced the acquisition of adult beer self-administration, reduced the rate of extinction responding, and enhanced beer-seeking behaviors. Overall, there was no effect of low-dose AMAE (2 g/kg) on adult alcohol consumption.

Conclusions: The data indicate that the a7 receptor is a critical target for the development of pharmacotherapeutics to treat/prevent the deleterious effects of ABAE. The fact that the data indicate that it is a bi-directional system (NAMs block, PAMS promote), provides convincing evidence for the hypothesis that the a7 receptor is critical for the persistent alterations produced by ABAE.

Keywords: Adolescence, Prevention, Adolescent Alcohol Use

Disclosure: Nothing to disclose.

P776. Modeling Polysubstance Abuse With Drug-Drug Choice Procedures in Rats

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Background: The use/co-use of multiple substances, particularly stimulants and opioids, is not a new phenomenon, and there is increasing awareness among treatment providers, policy makers, and basic scientists alike, that polysubstance abuse is the norm rather than the exception. Indeed, although the co-injection of cocaine and heroin (i.e., "speedballs") has been common for decades, recent estimates suggest that the popularity of stimulant-opioid mixtures is growing, with over 50% of treatment-seeking opioid users reporting regular stimulant use. In addition, while the co-use of stimulants and opioids has largely been one-sided (e.g., opioid users adding cocaine to their heroin), recent evidence suggests that this too may be changing, with increasing and widespread reports of opioids, primarily fentanyl, being mixed with cocaine and other stimulant products. Although valuable in its own right, the fact that the vast majority of preclinical substance use research continues to focus on the effects of individual drugs, studied in isolation, highlights the need to better understand interactions amongst abused drugs in order to more effectively treat individuals suffering from polysubstance use disorder.

Methods: Adult male Sprague-Dawley rats ($n = 33$) were surgically prepared with indwelling catheters in the left and right femoral vein. Catheters were passed under the skin and connected to a dual channel vascular access button that was exteriorized in the mid-scapular region. One group of rats ($n = 17$) was used to evaluate concurrent access to two stimulant drugs (i.e., MDPV and cocaine), whereas the other group of rats ($n = 16$) was used to evaluate concurrent access to a stimulant (methamphetamine) and an opioid (fentanyl). Initially rats had access to 0.032 mg/kg/inf MDPV and 0.71 mg/kg/inf cocaine, or 0.1 mg/kg/inf methamphetamine or 0.0032 mg/kg/inf fentanyl under concurrent FR5:TO 5-sec schedules of reinforcement. Once responding for these doses stabilized, two different sets of dose manipulations were performed: 1) the dose of the non-preferred drug was increased ½-log unit and preference was re-determined; and 2) the dose of the preferred drug was decreased ½-log unit and preference was re-determined. Finally, saline was substituted for both drugs, and the infusion-paired stimuli were omitted from the contingencies in order to extinguish responding on both levers. Once responding fell below 15% of baseline on both levers, a series of cue and cue +drug reinstatement tests were performed, including. For these tests, rats were pretreated with bolus doses of MDPV (0.32 mg/kg; IV), cocaine (3.2 mg/kg; IV), methamphetamine (1 mg/kg; IV), heroin (0.1 mg/kg; IV), or fentanyl (0.032 mg/kg; IV); tests were separated by at least 2 sessions conducted under extinction conditions.

Results: When rats had access to MDPV and cocaine, they exhibited exclusive choice of either MDPV or cocaine, however, these preferences could be reversed by increasing the dose of the less preferred, or reducing the dose of the more preferred drugs. When rats had access to methamphetamine and fentanyl, some rats responded on both levers whereas others exhibited exclusive choice of one drug (most often methamphetamine. However, just as was observed when MDPV and cocaine were available, these preferences (or lack thereof) were sensitive to dose manipulations. When rats from the MDPV and cocaine study were tested under reinstatement conditions, stimulants (i.e., MDPV, cocaine, or methamphetamine) tended to reinstate more responding on cocaine relative to MDPV lever, whereas heroin failed to reinstate responding on either lever. Although stimulants also tended to reinstate more responding on the methamphetamine relative to fentanyl levers, just as fentanyl tended to reinstate more responding on the fentanyl relative to methamphetamine lever, in rats with a history of responding for both stimulants and opioids, stimulants also reinstated responding from fentanyl and opioids also reinstated responding for methamphetamine, albeit at lower levels.

Conclusions: It is common for individuals with a substance use disorder to use multiple drugs, often from different pharmacological classes. The current studies suggest that when two drugs from the same class (i.e., MDPV and cocaine) are available, rats treat them as substitutes, choosing the functionally larger dose. However, when drugs from different pharmacological classes (i.e., methamphetamine and fentanyl) are available, some rats treat them as compliments, sampling both drugs throughout the session, whereas others display a preference for one drug or the other that is sensitive to dose manipulations as observed with MDPV and cocaine. Interestingly, reinstatement tests suggest that histories of responding for drugs from multiple pharmacological classes reduces the pharmacologic specificity of the priming drugs, which could

have implications for understanding drug-seeking behavior in individuals with a polysubstance use disorder. Future studies will investigate how these relations are impacted by opioid dependence and withdrawal.

Keywords: Polysubstance Abuse, Stimulants, Opioids

Disclosure: Nothing to disclose.

P777. Randomized Controlled Trial of Prazosin for Alcohol Use Disorder in Active Duty Service Members

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Background: Alcohol use disorder (AUD) is a major problem in the active duty military. Excessive brain noradrenergic signaling contributes to the anxiety, irritability and insomnia characteristic of alcohol withdrawal. These aversive hyperarousal symptoms are components of the "relief craving" that commonly interferes with attempted sobriety. Prazosin is a brain penetrant antagonist of noradrenaline at the alpha-1 adrenergic receptor that has been demonstrated to reduce alcohol consumption in preclinical studies1 and in randomized controlled trials (RCTs) in treatment seeking civilians2. In several recent RCTs, prazosin efficacy was restricted to participants with alcohol withdrawal symptoms and/or cardiovascular evidence of elevated noradrenergic signaling consistent with alcohol withdrawal3,4,5.

Methods: Soldier participants with AUD were recruited from a command-mandated 12-week intensive outpatient Army alcohol treatment program at Madigan Army Medical Center/Joint Base Lewis McChord in which they agreed to abstinence. 102 soldiers who were randomized and took at least one dose of study medication were included in the modified intent-to-treat (ITT) mixed models for repeated measures analysis. Participants were randomized to prazosin or equivalent placebo titrated over 3 weeks to a maximum dose of 4 mg in the morning, 6 mg in the afternoon, and 10 mg at bedtime. Following titration, participants remained on their achieved dose for an additional 10 weeks. Randomization occurred approximately 2 weeks after Army AUD treatment program entry. Primary outcome measures included diary-recorded drinks (standard drink units)/day, % days drinking, % days heavy drinking, and Pennsylvania Alcohol Craving Scale (PACS) scores. Pre-specified outcome points were 9 and 13 weeks following randomization.

Results: Prazosin was significantly superior to placebo for reducing drinks/day ($p = 0.03$). Prazosin vs. placebo changes in % days drinking, % days heavy drinking, and PACS scores were in the same direction, but did not achieve significance (all p values > than 0.105). Planned subgroup analysis with cardiovascular evidence of increased noradrenergic signaling suggestive of alcohol withdrawal (baseline standing heart rate > 90 beats per minute) demonstrated robust and significant prazosin vs. placebo reductions in drinks/day ($p = 0.010$), % days drinking ($p = 0.036$), and % days heavy drinking ($p = 0.001$). Prazosin appeared to prevent relapse during the final four weeks of the protocol in this higher heart rate subgroup analysis.

Conclusions: These findings suggest that prazosin is effective for AUD in persons who manifest increased noradrenergic signaling consistent with alcohol withdrawal, and support Sinha et al.'s5 recent demonstration in treatment-seeking civilians that prazosin is an effective drug for reducing alcohol consumption in AUD with alcohol withdrawal symptoms. Our findings also support evaluating prazosin for relapse prevention in persons with AUD who manifest alcohol withdrawal signs and symptoms.

Keywords: Prazosin, Alcohol, Brain

Disclosure: Nothing to disclose.

P778. Epigenome-Wide Association Study of Alcohol Consumption in N = 8161 Individuals and Relevance to Alcohol Use Disorder Pathophysiology: Identification of the Cystine/Glutamate Transporter SLC7A11 as a Top Target

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Background: Alcohol misuse is common in many societies world-wide and is associated with extensive morbidity and mortality, often leading to alcohol use disorders (AUD) and alcohol-related end organ damage. The underlying mechanisms contributing to the development of AUD are largely unknown; however, growing evidence suggests that alcohol consumption is strongly associated with alterations in DNA methylation. Identification of alcohol-associated methylomic variation might provide novel insights into pathophysiology and novel treatment targets for AUD.

Methods: Here we performed the largest single-cohort epigenome-wide association study (EWAS) of alcohol consumption to date ($N = 8161$) and cross validated findings in AUD populations with relevant endophenotypes, as well as alcohol-related animal models.

Results: Results showed 2504 CpGs significantly associated with alcohol consumption (Bonferroni p-value < 6.8 x 10-8) with the five leading probes located in SLC7A11 ($p = 7.75$ x 10-108), JDP2 ($p =$ 1.44 x 10-56), GAS5 ($p = 2.71$ x 10-47), TRA2B ($p = 3.54$ x 10-42) and SLC43A1 ($p = 1.18$ x 10-40). Genes annotated to associated CpG sites are implicated in liver and brain function, the cellular response to alcohol and alcohol-associated diseases, including hypertension and Alzheimer's disease. Two sample Mendelian randomization confirmed the causal relationship of consumption on AUD risk (IVW $p = 5.37$ x 10-09). A methylation-based predictor of alcohol consumption was able to discriminate AUD cases in two independent cohorts ($p = 6.32$ x 10-38 and $p = 5.41$ x 10-14). The top EWAS probe cg06690548, located in the cystine/glutamate transporter SLC7A11, was replicated in an independent cohort of AUD and control participants ($N = 615$) and showed strong hypomethylation in AUD ($p <$ 10-17). Decreased CpG methylation at this probe was consistently associated with clinical measures including increased heavy drinking days ($p <$ 10-4), increased liver function enzymes (GGT ($p = 1.03$ x 10-21), ALT ($p = 1.29$ x 10-6), and AST ($p = 1.97$ x 10-8)) in individuals with AUD. Post-mortem brain analyses documented increased SLC7A11 expression in the frontal cortex of individuals with AUD and animal models showed marked increased expression in liver, suggesting a mechanism by which alcohol leads to hypomethylation-induced overexpression of SLC7A11.

Conclusions: Taken together, our EWAS discovery sample and subsequent validation of the top probe in AUD suggest a strong role of abnormal glutamate signaling mediated by methylomic variation in SLC7A11. Our data are intriguing given the prominent role of glutamate signaling in brain and liver and might provide an important target for therapeutic intervention.

Keywords: Alcohol, Alcohol Consumption, Epigenetics, Addiction Phenotypes, DNA Methylation

Disclosure: Nothing to disclose.

P779. Release of Endogenous Dynorphin Opioids in the Prefrontal Cortex Disrupts Cognition

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Background: Opioid overdose deaths have continued to rise despite significant efforts aimed towards decreasing opioid use disorders. Drug withdrawal and abstinence can contribute to aversive states that increase the likelihood for relapse to opioid use. In the first two weeks of abstinence from opioids, there are significant deficits in working memory and executive function that can persist for months in vulnerable individuals. Disrupted cognitive function may also contribute to alterations in mood, stress reactivity, and reward processing observed in early opioid abstinence. Aversive states, including drug withdrawal, can promote the release of dynorphins, endogenous ligands for kappa opioid receptors (KORs). Although the psychopathology of substance use disorders is complex, dynorphins and KORs are expressed in the mammalian cortex and analysis of their role in mice will likely provide useful insights for developing treatments for cognitive dysfunction.

Methods: Immunohistochemical analyses using a phospho-KOR antibody determined the conditions that led to KOR activation in the prefrontal cortex (PFC) of C57BL/6 male mice. Using a genetically encoded sensor based on inert KOR (kLight1.2a), we measured the in vivo dynamics of endogenous dynorphin release in the PFC. To study the role of KORs in the PFC in working memory, we trained mice in an operant delayed alternation task to make a response on one retractable lever, wait a specified delay for reinsertion of the levers, and then respond on the alternate lever. Mice were trained until reaching stable performance with a 10s delay for reinsertion and then were injected in the PFC with either artificial cerebrospinal fluid (ACSF) or the long-lasting (~3 weeks) KOR antagonist, norBNI (1.25 µg in 0.5 µL vehicle). Following recovery from surgery, mice received either saline, KOR agonist (U50,488 5 mg/kg i.p.), or precipitated morphine withdrawal. The contribution of locally released dynorphin in the prefrontal cortex was tested using optical stimulation of prodynorphinCre neurons with Channelrhodopsin-2 prior to immunohistochemistry and behavior.

Results: In C57BL/6 male mice, we find that acute morphine withdrawal evokes dynorphin release in the medial prefrontal cortex (PFC) and disrupts cognitive function by activation of local kappa opioid receptors (KORs). Immunohistochemistry showed that morphine withdrawal increased dynorphin release in the prefrontal cortex. Using the KOR ligand sensor kLight1.2a, we determined the time course of endogenous dynorphin release in the prefrontal cortex in vivo following naloxone-precipitated morphine withdrawal. Local activation of KOR in PFC produced multi-phasic disruptions of memory processing in an operant delayed alternation behavioral task, which manifest as reductions in response number and accuracy during early and late phases of an operant session. Local pretreatment in PFC with the selective KOR antagonist norbinaltorphimine (norBNI) blocked the disruptive effect of systemic KOR activation during both early and late phases of the session. The early, but not late phase disruption was blocked by viral excision of PFC KORs, suggesting an anatomically dissociable contribution of pre- and postsynaptic KORs. Morphine withdrawal also disrupted delayed alternation performance and this effect was blocked by pretreatment with norBNI in the PFC. Stimulation of prodynorphinCre neurons in the prefrontal cortex increased PFC KOR activation as measured by immunohistochemistry and disrupted delayed alternation performance, suggesting a local dynorphin/KOR circuit involved in cognitive dysfunction.

Conclusions: These results suggest that novel therapeutics able to block dynorphin signaling in cortex may reduce the cognitive disruptions evident in certain psychiatric disorders including adverse responses to stress during drug abstinence.

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Keywords: Opioid Addiction, Opioid Peptides, Kappa Opioid Receptor

Disclosure: Nothing to disclose.

P780. Imaging the Component Psychological Processes of Chronic Cocaine Craving

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Background: Individuals with cocaine use disorders (CU) experience intense craving long during abstinence. Chronic cocaine craving is strongly related to the severity of recent cocaine use. A hypothesis is that the hypodopaminergic states during abstinence present a negative reward prediction error - a reward deficiency - that precipitates and sustains craving and diminishes response to natural reinforcers. Chronic cocaine craving also involves altered motivation and elevated stress and anxiety. Our previous imaging studies described how CU respond to cocaine vs. drug cues in relation to chronic cocaine craving, as evaluated by the Cocaine Craving Questionnaire (CCQ) (Wang et al. 2020 Neuropsychopharm), to both cocaine vs. food cues (Zhang et al. 2020, Intl J Neuropsychopharm), to stress cues (Zhornitsky et al. 2021, Biol Psychiatr: Global Open Sci) and to receipt of reward in a monetary incentive delay task (MIDT) (Zhornitsky et al. 2020, Intl J Neuropsychopharm). These studies implicate the hippocampal and parahippocampal gyri, hypothalamus, mid-cingulate cortex, and ventral striatum, respectively, each of the memory, motivational, limbic motor and reward circuits. These data provide an opportunity to examine 1) how these regional responses are related during exposure to drug, food and stress cues; 2) how these regional responses relate to chronic cocaine craving and physiological arousal during task challenges.

Methods: A total of 56 recently abstinent CU and 51 age- and gender-matched HC who were social drinkers participated in the study; the exact sample size varied between the behavioral tasks. CU met criteria for current cocaine dependence per SCID for DSM IV. Recent cocaine use was confirmed by urine toxicology tests. They were drug-free for about 10 days while staying in an inpatient unit prior to the current fMRI study. All participants were evaluated for drug and alcohol use, including history of use and current use. CU were also interviewed with the 18-item CSSA to assess cocaine addiction severity. Chronic cocaine craving was assessed with CCQ-Brief every 2 to 3 days during inpatient stay. The behavioral tasks, including cocaine, food, and stress cue reactivity in a block design and the MIDT in an event-related design were described in the papers listed earlier. For all experiments, skin conductance was recorded concurrently with fMRI. Imaging data pre-processing and modeling was performed following published routines and the results were evaluated at a corrected threshold.

Results: (A) Receipt of reward in the MIDT did not engage the ventral striatum (VS) differently between CU and HC, nor did the VS show reward activities in correlation with CCQ score in CU; (B) Cocaine and stress (vs. neutral) cue exposure elicited higher activity in the hippocampal and cocaine cue exposure elicited lower activation in the parahippocampal gyri (PHG). Only parahippocampal activity was correlated with CCQ score in CU -

the less PHG deactivation was, the higher chronic cocaine craving; (C) Hypothalamus showed drug and food cue reactivity in correlation with CCQ score; (D) Across tasks, hippocampal and PHG activities were correlated between food and drug cue and between food and stress cue reactivity but not between drug and stress cue reactivity; (E) Hypothalamus activation was correlated between drug and food cue reactivity but not between drug and stress or between food and stress cue reactivity; and (*F*) Stress cue reactivity engage the mid-cingulate cortex (MCC) in link with CCQ score; however, across subjects MCC activity was not correlated during drug, food and stress cue exposures.

Conclusions: These findings suggest that (1) chronic cocaine craving is reflected in PHG reactivity to drug cues, hypothalamus reactivity to both drug and food cues, and MCC reactivity to stress cues, but not in VS reward response; (2) cocaine cue exposure engages the memory, motivational and stress circuits; (3) the memory circuit is involved in a commensurate manner between food and both drug and stress cue reactivity but not between drug and stress cue reactivity; (4) the motivational circuit response is commensurate between drug and food (but not stress) cue exposure; and (5) the limbic motor circuit is unique to stress reactivity in terms of its role in reflecting chronic cocaine craving. Together, the findings highlight the distinct psychological and neural processes supporting chronic cocaine craving.

Keywords: Cocaine Addiction, Reward, Motivational, Emotional Stress, Cue

Disclosure: Nothing to disclose.

P781. Accumbens Adenosine Signaling and Microglial Activation Underlie Nicotine Seeking Behavior

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Background: Nicotine seeking behavior induces neuroimmune responses within the nucleus accumbens core (NACore), including activation of microglia. Further, adenosine 2A receptors (A2aRs) regulate the proliferation, activation, and survival of microglia. Systemically agonizing A2aRs effectively reduces ethanol self-administration, cocaine reinstatement, and changes in synaptic plasticity by controlling presynaptic glutamate release in the striatum. These neuroimmune mechanisms, however, have not been studied for their role in nicotine-motivated behaviors.

Methods: In the current study, male and female rats underwent nicotine self-administration (0.06 mg/kg/infusion) followed by extinction training and cue-induced reinstatement. A2aRs were chronically agonized (CGS 21680, 0.4 mg/kg i.p.) or antagonized (SCH 58261, 0.4 mg/kg i.p.) during extinction and immediately prior to reinstatement, and lever pressing for contingent nicotine-paired cues was then compared to vehicle-treated animals. As well, NAcore microglia morphology was quantified following nicotine self-administration in both sexes.

Results: Here we show sex-specific patterns of microglia activation whereby females displayed greater activation of NAcore microglia as compared to males ($p < 0.05$). Further, we found an increase in A2a receptor expression within the NAcore following chronic nicotine-self administration ($p < 0.05$). Finally, A2a agonism significantly decreased nicotine seeking, whereas antagonism did not change nicotine seeking compared to vehicle ($p < 0.05$).

Conclusions: These results indicate that A2a activation inhibits nicotine seeking behavior, which is likely mediated through microglia. Further, we show that females are more susceptible to microglial activation following volitional nicotine consumption. Ongoing studies are determining if accumbens A2a receptors control microglial activation and rapid, transient glutamate plasticity during nicotine seeking behavior. As well, we are currently utilizing CX3CR1-cre transgenic rats in ongoing studies to determine if chemogenetic activation or inhibition of microglia controls nicotine seeking and accumbens glutamate plasticity.

Keywords: Neuroimmune Activation, Nicotine Addiction, Nucleus Accumbens, Sex Differences, Chemogenetics

Disclosure: Nothing to disclose.

P782. Ptprd Inhibitors and Substrate-Selective Positive Allosteric Modulators for Addictions and Alzheimer's Disease

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Background: The receptor type protein tyrosine phosphatase PTPRD is highly expressed by neurons and concentrated in their presynaptic terminals. PTPRD's activation by extracellular binding partners is thought to change activity of its intracellular phosphatase in altering tyrosine phosphorylation of a number of synaptic proteins that include the tau kinases GSK3 beta and GSK3 alpha. Human PTPRD genomic variants are associated with a number of addiction-associated phenotypes and densities of neurofibrillary tangles in Alzheimer's disease (AD) postmortem brains. Mice with reduced PTPRD expression display addiction, and AD-related phenotypes. These and other results suggest that reduced PTPRD function would reduce reward from addictive drugs and that increased PTPRD function would reduce accumulation of neurofibrillary pathology.

Methods: Phosphatase assays using recombinant human PTPRD phosphatase and spectrophotometric detection of dephosphorylation products of pNPP and of orthophosphate release from phosphopeptides corresponding to candidate substrates for PTPRD's phosphatase. Candidate substrates nominated by phosphoproteomic studies of brains of wildtype vs PTPRD knockout mice. Cocaine conditioned place preference in mice. Remifentanil self-administration (FR1) in rats.

Results: We now report that selected flavanols, especially quercetin, act as substrate-selective positive allosteric modulators of the ability of PTPRD's phosphatase to dephosphorylate phosphotyrosine (pY) GSK3 alpha/beta and thus to downregulate activity of this tau hyperphosphorylator. In silico docking studies provide a novel candidate mechanism for this action. We report that NHB1109, a novel analog of our lead compound PTPRD phosphatase inhibitor 7-BIA, displays good potency and selectivity in vitro, reasonable oral bioavailability and therapeutic index and ability to reduce mouse cocaine-conditioned place preference and rat remifentanil self-administration. These results each support PTPRD as a novel target for new therapeutics that inhibit or enhance its activities in ways that should benefit these three common human disorders. Comparisons between quercetin effects on PTPRD's abilities to dephosphorylate pYGSK3 phosphopeptide vs its effects on dephosphorylation of other PTPRD substrates supports the novel concept of substrate-specific positive allosteric modulation.

Conclusions: PTPRD phosphatase inhibitors are good candidates to advance to human testing to reduce reward from stimulants and opiates. PTPRD phosphatase positive allosteric modulation and other data support trials of quercetin to slow progression from mild cognitive impairment to Alzheimer's disease. Our data with candidate substrates supports the novel concept of substrate selective positive allosteric modulation.

Keywords: Phosphatase, Psychostimulants, Opiates, Neurofibrillary Tangle, Alzheimers Disease

Disclosure: Patent: Royalties (Self)

P783. Imaging Brain Cortisol Regulation in Relation to Reward Functioning With a Neuroimaging Marker for 11-Beta Hydroxysteroid Dehydrogenase Type 1

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Background: While numerous studies have examined alterations of HPA axis activity and regulation in posttraumatic stress disorder or major depressive disorder, fewer have examined HPA axis regulation in relation to poor reward function, which can be present in both. One such study found associations of impaired reward responsiveness with a single nucleotide variant of CRHR1, which is associated with blunted HPA axis responsiveness to a CRH challenge. Many previous examinations assume that the major source of glucocorticoid signaling in the brain is peripherally-produced adrenal cortisol that subsequently enters the brain. However, peripheral cortisol enters the brain at a slow rate and may only contribute to approximately 5% of the cortisol in the brain, arguing for a local source of cortisol production in the brain as an important regulator. Using in vivo imaging of the 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1), an enzyme that generates cortisol in the brain, we aimed to examine reward functioning in relation to this putative marker of brain cortisol regulation. Furthermore, we used an objective measurement of reward function to assess possible subclinical pathology in individuals with trauma exposure but not meeting criteria for PTSD, who often have low self-reported loss symptoms (anhedonia, numbing) which are associated with reward function. We applied the well-validated Probabilistic Reward Task, designed to objectively assess an individual's propensity to respond to previously reinforced rewards. We conducted a secondary analysis to assess if, in trauma-exposed individuals, caudate 11beta-HSD1 availability is related to reward responsiveness as an objective measure of reward function and an endophenotype of loss symptoms.

Methods: In the original study, seventeen trauma-exposed individuals without PTSD (TE: 8 female; 33 +/- 8 years) and sixteen individuals with PTSD (8 female; 37 +/- 10 years) and underwent positron emission tomography (PET) imaging with [18F] AS2471907, a radioligand for 11beta-HSD1. Participants received 93 +/- 14 MBq [18F]AS2471907 as a bolus injection and were imaged for 150-240 min on the High-Resolution Research Tomograph. Of these individuals, twenty-six participated in the 25-min probabilistic reward task (TE: $n = 14$, PTSD: $n = 12$) either on the same day as the [18F]AS2471907 PET scan ($n = 21$), or within 3 months of the PET scan ($n = 5$). Participants in the TE group did not meet criteria for PTSD on the Clinician Administered PTSD Scale. 11beta-HSD1 availability was estimated as [18F] AS2471907 volume of distribution (VT), using MA1 analysis and a metabolite-corrected arterial input function. Analyses focused on the caudate brain region based on previous work that observed altered caudate functional activity in relation to impaired reward responding in MDD. The primary outcome variable from the probabilistic reward task was Response Bias, a well-validated and previously published index of systematic preference for the stimulus that is more frequently paired with a higher reward. Median split of Response Bias delineated reward responder groups. Group-wise difference in caudate 11beta-HSD1 availability was assessed using univariate ANOVA in the "Good Reward Responder" vs. "Impaired Reward Responder" group. A general linear modeling approach was used to compare caudate 11beta-HSD1 availability to Response Bias, 90-min averaged plasma cortisol, 24-hour urine cortisol.

Results: Preliminary results showed that caudate 11beta-HSD1 availability was significantly higher (approximately 25%) in the "Good Reward Responder" compared to "Impaired Reward Responder" group ($p = 0.037$). In the TE group, there was a trend level association of lower caudate 11beta-HSD1 availability with poorer reward responding or lower Response Bias ($R2 = 0.22$, $p = 0.092$). There was not a significant association of caudate 11beta-HSD1 availability with 90-min averaged plasma cortisol ($n = 13$, $p = 0.23$). Preliminary results in a much smaller subset of individuals who completed a 24-hour urine collection ($n = 7$) showed a significant negative association of caudate 11beta-HSD1 availability with urine cortisol ($R2 = 0.60$, $p = 0.041$).

Conclusions: Overall, 11beta-HSD1 availability in the caudate nucleus, a region associated with reward functioning, was higher in a group with "Good Reward Responding", with a trending positive association between caudate 11beta-HSD1 availability and higher response bias, a measure of better reward functioning. Previously presented work had found a significant association of higher prefrontal-limbic 11beta-HSD1 availability with lower PTSD loss symptoms (sum of emotional numbing and anhedonia), but there was a limited range of self-reported loss symptoms in the TE group prohibiting a similar analysis using psychometric tools. The present analysis permitted an objective assessment of reward function in the TE group. While there was no association observed with plasma cortisol levels, and the preliminary urine cortisol collection findings cannot be currently interpreted without more data, 11beta-HSD1 itself may be a useful tool in probing aspects of clinical pathophysiology along RDoC dimensions. This may help further delineate varying roles of brain and peripheral cortisol regulation in both clinical reward functioning in PTSD, MDD, as well as subclinical reward functioning in trauma-exposed individuals.

Keywords: RDoC, Reward Functioning, Traumatic Stress, HPA Axis, Neuroendocrine Responses

Disclosure: Nothing to disclose.

P784. A Novel Template-Based Ica Approach Reveals Psilocybin-Induced Changes in Thalamic Connectivity

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Background: Classic psychedelics (e.g., serotonin 2A receptor, or 5-HT2AR, agonists including psilocybin and LSD) evoke a wide array of acute alterations in perception and cognition (Nichols, 2016). The cortico-striatal-thalamo-cortical loop model of psychedelic drug action (CSTC; Geyer and Vollenweider, 2001; Vollenweider and Geyer, 2008; Vollenweider and Preller, 2020) proposed that psychedelics disrupt thalamic gating of sensory information through 5-HT2AR agonism, resulting in increased flow of sensory information to the cortex, leading to the perceptual alterations and impairments of cognition that are observed during acute psychedelic effects. Accordingly, independent groups have found increased thalamocortical functional connectivity under task-free conditions during LSD effects in humans (Tagliazucchi et al. 2016; Müller et al. 2017; Preller et al. 2018, 2019). Notably, these studies have treated the thalamus as a unitary structure, despite known differential 5-HT2AR expression, functional specificity, and anatomical connectivity of thalamic nuclei. Only a subset of thalamic nuclei (e.g. reticular, medial dorsal, ventrobasal, and pulvinar nuclei) exhibit 5-HT2AR expression or have been directly implicated in psychedelic drug effects in animal models (Inserra et al. 2021).

We used Template Independent Component Analysis (tICA) of resting-state fMRI (rsfMRI) data to identify functional subdivisions of the thalamus, and then examined psilocybin-induced changes in thalamic functional organization and thalamo-cortical connectivity. We hypothesized that psilocybin would selectively alter reticular and medial dorsal nucleus organization and cortical functional connectivity.

Methods: Baseline rsfMRI data were collected from 38 medically and psychiatrically healthy participants (22M/16F, Mage = 54.9). MRI data were minimally preprocessed using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/), and thalamic spatial components were estimated from baseline rsfMRI data using the Group ICA for fMRI toolbox (Calhoun et al. 2001). ICA was spatially constrained using a whole-thalamus inclusion mask from the Harvard-Oxford atlas (Desikan et al. 2006). The mean and between-subject variance of spatial maps for each of 7 thalamic independent components constituted the priors or "template" for template ICA. Anatomical locations of each thalamic component were determined by calculating the overlap between each thalamic component and a histologically-validated anatomical stereotactic atlas of the thalamus (Morel, Magnin, and Jeanmonod, 1997).

18 participants (11M/7F, Mage = 51.9) from the initial cohort underwent rsfMRI scans 110 minutes (consistent with peak effects of psilocybin) after single-blind oral administration of placebo and after 10 mg/70 kg psilocybin. tICA was applied to generate subject- and session- (placebo/psilocybin) specific component maps ($n = 14$ per subject) and variance estimates. Voxel-wise *t*-tests were performed for each posterior estimate map to identify voxels showing significant "engagement" (component loading greater than zero, FDRq = 0.05). Between-session changes in engagement (measured as change in the # of voxels engaged in each scan) were tested using non-parametric Wilcoxon paired *t*-tests (FDRq = 0.05). Pearson correlations were used to test the association of between-session changes in engagement for each thalamic component and between-session changes in self-reported subjective effects. Thalamocortical connectivity was compared across sessions using Fisher-transformed Pearson's correlations between the 7 thalamic component timecourses and 10 cortical ICA-derived resting-state cortical network time courses ($n = 10$; Smith et al. 2009). Dual-regression was performed on the rsfMRI data after nuisance correction (Behzadi et al. 2007; Muschelli et al. 2014) to generate component time courses for each thalamic component and cortical network.

This study was conducted as part of a registered clinical trial (NCT02145091). All procedures were approved by the Johns Hopkins Medicine Institutional Review Board.

Results: Engagement was lower during psilocybin than placebo in thalamic components corresponding to pulvinar (p[FDR] = 0.03), medial dorsal (p[FDR] = 0.032), and central lateral (p[FDR] = 0.024) nuclei. Lower functional connectivity between pulvinar and occipital pole (p[FDR] = 0.048), and between central lateral nucleus and default mode network (p[FDR] = 0.013) was observed during psilocybin. Increased subject reports of the experience of "letting go" were negatively associated with pulvinar engagement ($r = -0.47$, $p = 0.051$) and positively associated with central lateral nucleus to DMN connectivity ($r = 0.53$, $p = 0.02$). Increased central lateral nucleus engagement was positively associated with increased mystical experience ($r = 0.53$, $p = 0.024$).

Conclusions: A novel analytic approach revealed several anatomically specific psilocybin-induced changes in thalamic organization and thalamocortical connectivity that were associated with psilocybin subjective effects. These findings provide evidence for psilocybin-induced changes in specific thalamocortical circuits implicated in perceptual effects of classic psychedelics (involving pulvinar and occipital pole) as well as circuits (involving the central lateral nucleus and DMN) that may mediate subjective effects (mystical experience) shown to predict psychedelic therapeutic efficacy (Barrett and Griffiths, 2017).

Keywords: Psilocybin, Thalamus, Independent Component Analysis, Resting-State fMRI, Neuropsychopharmacology

Disclosure: WavePaths, Ltd: Advisory Board (Self)

P785. Relative Salience Signaling Within a Thalamo-Orbitofrontal Circuit Governs Learning Rate

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Background: Aberrant associative learning underlies many mental illnesses. For instance, the learning of associations between environmental cues and drug use is a strong contributor to relapse in addiction. The most well-known theoretical model for associative learning is reinforcement learning. Reinforcement learning proposes a simple, yet powerful model for learning that a cue predicts an upcoming reward: update one's reward prediction by a reward prediction error (RPE)—the difference between a received and predicted reward. Even though the bulk of experimental work into the neuronal mechanisms of reinforcement learning focus on the mesolimbic dopamine circuitry and its encoding of RPE, a rich computational literature argues that the dopamine RPE circuitry cannot operate in isolation and that the brain likely contains complementary systems for learning. For instance, reinforcement learning algorithms have parameters such as learning rate that also need to be learned. Since the net magnitude of learning due to an RPE is the product of the RPE and a learning rate for the reward, optimally tuning the learning rate (amount by which RPE updates reward prediction) can be highly beneficial to adapt learning to one's environment. Recently, multiple neural signals have been found to correlate with learning rate, including in the anterior cingulate cortex (ACC), dorsal raphe serotonergic system, dorsomedial prefrontal cortex and anterior insula.

Here, we develop an alternative approach to test learning rate control and test the hypothesis that the orbitofrontal cortex (OFC) controls the learning rate of a reward. We tested this hypothesis for a few reasons. First, OFC receives inputs from all the regions mentioned above. Second, we previously observed that the suppression of reward responses in vmOFC was sufficient to reduce the rate of behavioral learning. Third, OFC activity correlates with the attribution of stimulus salience, a key variable thought to modulate learning rate. Thus, we tested whether vmOFC neurons act as a controller of learning rate by signaling the relative salience of rewards, and whether such signaling is mediated, at least in part, by one of its major inputs from medial thalamus.

Methods: To investigate reward responses in a large number of individual ventral/medial OFC (vmOFC) neurons during reward prediction learning, we used two-photon calcium imaging during a discriminative Pavlovian trace conditioning task in head-fixed mice (Namboodiri et al. 2019). All experiments were approved by institutional IACUC.

This study involved 33 wild type C57/BL6 mice (14 female). 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 calcium sensor (AAVDJ-CaMKIIα-GCaMP6s) in vmOFC and in some cases, the injection of AAV5-CaMKIIα-eNpHR3.0-mCherry or the no-opsin control in medial thalamus, to study the effect of its inhibition on vmOFC neuronal encoding.

Trace conditioning was done exactly as before (Namboodiri et al. 2019), with an auditory tone (3kHz pulsing tone or 12kHz constant tone, 75–80 dB) lasting 2s paired with a sucrose reward (10–12.5%, ~2.5 μL) 1s after tone offset. This reward paired tone (CS +) was presented randomly interleaved with another tone (12kHz constant tone or 3kHz pulsing tone, 75–80 dB) that did not predict reward (CS −). In another experiment, random unpredictable sucrose drops were delivered along with random unpredictable drops of quinine (1.5–2.5mM) in a 3:1 ratio.

We recorded from a total of more than 7,000 vmOFC neurons across different experiments with and without the inhibition of medial thalamic inputs to vmOFC. These neurons were first clustered based on their responses in trace conditioning to reveal 9 clusters/subpopulations of neurons (Namboodiri et al. 2019). The reward response of these neurons were analyzed across different sessions early in learning, late in learning, a session in which reward probability was reduced to 50%, and a session in which random unpredicted sucrose and quinine were delivered to the animals. Data were analyzed across these conditions using standard statistical approaches.

Results: Using a novel test for neuronal control of learning rate, we show that vmOFC reward response is consistent with a learning rate controller on a trial-by-trial basis (one-tailed $p < 10\text{-}16$ for the whole population). Next, we show that consistent with learning rate signaling, some neuronal subpopulations in the vmOFC decrease their reward responses when there is low uncertainty in the environment (two-tailed $p < 10\text{-}9$ in some subpopulations). Next, we show that this vmOFC outcome response is consistent with a signaling of the relative salience of the outcome when unpredicted rewards were randomly interleaved with unpredicted presentations of a highly salient aversive stimulus (two-tailed $p < 10\text{-}9$ in some subpopulations). Lastly, we show that medial thalamic inputs to vmOFC also signal relative salience, and causally control relative salience signaling in vmOFC.

Conclusions: In conclusion, we show that vmOFC reward responses control learning rate by signaling the relative salience of a reward. These results suggest that the contribution of medial thalamus and OFC dysfunction to mental illness is, at least in part, due to disruptions in learning rate control.

Keywords: Orbitofrontal Cortex, Medial Orbitofrontal Cortex, Associative Learning, Two-Photon Calcium Imaging

Disclosure: Nothing to disclose.

P786. Predictable and Unpredictable Threat Responding Uniquely Predict Transdiagnostic Fear and Anxiety

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Background: Phasic fear and sustained anxiety may be pathophysiologically distinct dimensions that underlie and explain clinically meaningful variation in the internalizing disorders. Animal work supports this distinction, with predictable threat cues eliciting short-term fear behaviors and unpredictable threat cues eliciting sustained, anxiety-like states. NIH's Research Domain Criteria also emphasizes the distinction between acute threat and potential threat. The no-threat, predictable threat and unpredictable (NPU) threat task can be used to quantify individual differences in predictable and unpredictable threat responding. Prior work using this task in internalizing samples has focused primarily on categorical diagnoses. Knowing how predictable and unpredictable threat responding relate to transdiagnostic fear and anxiety could help refine phenotypic definitions in the internalizing disorders. Furthermore, examining predictable and unpredictable threat responding prospectively could help inform prognostic trajectories for fear and anxiety and might facilitate early intervention or prevention efforts.

Methods: Participants were 52 individuals (31 female; M age = 24.46 years, SD = 9.33) with a range of internalizing psychopathology. At Time 1, participants performed the NPU threat of shock task while electroencephalography (EEG) and startle eyeblink were recorded. Participants reported on transdiagnostic Fear (the PANAS-X Fear subscale) and Anxiety (a composite of trait anxiety/STAI, worry/PSWQ and social anxiety/SPIN) at Time 1 and at Time 2 (M = 1.68 years later; SD = 0.68). We assessed associations between psychophysiological response to predictable and unpredictable threat at Time 1 and a) concurrent (Time 1) Fear and Anxiety, as well as b) prospective (Time 2) Fear and Anxiety (controlling for Time 1 Fear and Anxiety).

Results: Greater electrocortical responding (negative-going component, the stimulus preceding negativity, SPN) to predictable threat cues was associated with increased Time 1 Fear (controlling for Time 1 Anxiety), $\beta = -.256$, $p = .04$, while larger SPNs to unpredictable threat cues uniquely predicted increased Time 2 Anxiety (controlling for Time 1 Anxiety and Fear), $\beta = -.272$, $p = .01$. Heightened startle potentiation to predictable threat cues predicted increased Time 2 Fear (controlling for Time 1 Fear and Anxiety), $\beta = .646$, $p = .04$.

Conclusions: Increased electrocortical response to predictable threat cues was associated with higher transdiagnostic fear and greater startle response to predictable threat cues predicted increased fear at Time 2. Greater electrocortical response to unpredictable threat cues predicted greater anxiety at Time 2. Results suggest a neurobiological basis to dimensional fear and anxiety and may have relevance for the development of a more data-driven nosological system.

Keywords: Anxiety and Depression, Research Domain Criteria (RDoC), EEG/ERP Electrophysiology, Startle, Threat of Shock

Disclosure: Aptinyx Inc.: Consultant (Self)

P787. Effects of Adolescent Social Isolation on Adult Value-Based Decision Making in Male and Female Mice

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Background: As a consequence of COVID-19, an increasing number of adolescents are being subjected to social isolation. While social isolation at any developmental stage can have devastating long-term consequences on physical and mental health, mounting evidence demonstrates a clear, profound impact of adolescent social isolation on a wide range of behavioral and physiological endpoints. Goal-directed behaviors and value-based decision making are integral for healthy daily functioning, and deficits are strongly linked to poor functional outcomes in individuals living with psychiatric disease. Here, we explore the effects of adolescent social isolation in male and female mice on later goal-directed behaviors and value-based decision making in adulthood.

Methods: To model adolescent social isolation, male and female C57bl6/J mice were bred in house and weaned at PND21 into single or group (3 same-sex mice/cage) housing ($n = 8\text{-}15$ per sex and housing condition). After PND60, mice were food deprived and trained in a self-initiated two-choice operant task. Trials in the task were structured in 3 discrete phases: (1) Initiation: entry into a central illuminated port initiated the trial, and two retractable levers extended, (2) choice: mice must respond on one of the two levers within 10s (no response is considered an omission), (3) outcome valuation: levers retracted and the outcome (variable volume of chocolate or vanilla liquid reward

depending on task stage, described below) was delivered. After a 1s intertrial interval, the next trial began. To test initial learning, mice were first trained to respond on one active lever, which was reinforced with 15ul liquid reward. Responding on the inactive lever resulted in 4s timeout. After task acquisition, mice were then trained for 10d in a serial reversal task, where the active lever reversed after 8/10 correct responses. We then tested how isolated and socially housed mice used information about reward value and temporal costs to guide their decisions. First, mice were presented with rewards of varying volumes (15ul vs 0, 5, or 10ul) in the serial reversal task, with 8/10 choices to the lever reinforced by the larger 15ul reward prompting contingency reversal. Each test session was separated by at least 2 sessions of 15ul vs 0ul training sessions. After 3 sessions at each reward volume contrast, varying temporal delays (1.5, 3, or 4.5s) were applied to the larger 15ul reward to test how costs were integrated into decision making.

Results: Adolescent social isolation differentially impacted operant acquisition (Two Way ANOVA interaction $p = 0.02$), flexible performance in the serial reversal task (Mixed Linear Effects Model three-way interaction $p < 0.01$), and relative value of rewards (Mixed Linear Effects Model three-way interaction $p = 0.03$) and costs (Mixed Linear Effects Model three-way interaction $p = 0.02$). Within females, mice socially isolated through adolescence learned the operant task more rapidly, but were more exploratory in choice behavior throughout the serial reversal task. In contrast, adolescent social isolation in males slowed operant learning, but after task acquisition previously isolated mice showed enhanced sensitivity to reward value, more consistently choosing the larger reward option even when the reward difference was small and the temporal cost was high.

Conclusions: Together, these data demonstrate that adolescent social isolation in mice has divergent, but long-lasting impacts on later decision-making in male and female mice. These sex differences may be a result of contrasting adaptations to social isolation, or a sex difference in the stressfulness of social isolation. Ongoing work is building neuroeconomic models to further dissect the impacts of social isolation on choice behavior, as well as explores the role of underlying corticostriatal circuitry.

Keywords: Decision Making, Juvenile Social Isolation, Social Isolation Stress, Value-Based Decision Making, Adolescent Stress

Disclosure: Nia Therapeutics: Founder (Spouse)

P788. Response Inhibition Deficit – a Transdiagnostic Risk Factor for Post-Trauma Psychopathology?

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Background: Given the high proportion of individuals experiencing trauma who develop adverse posttraumatic neuropsychiatric sequelae (APNS), identifying at risk individuals and endophenotypes associated with APNS vulnerability is of great significance. Prior neuroimaging studies have identified lower ventromedial prefrontal cortex (vmPFC) and hippocampal activation during inhibition paradigms as contributors to the development of PTSD. However, sample sizes were small and many questions regarding the effect of sex, relevance to other APNS, and the ability to identify more scalable biomarkers for neuroimaging findings remain. We aimed to address these questions in this larger-scale prospective sample.

Methods: Acutely traumatized individuals were recruited in Emergency Departments (ED) for the deep phenotyping component of the AURORA study ($N = 261$, 91M). Subjects performed a Go/NoGo fMRI task 2 weeks post-trauma. Contrast estimates for Go vs NoGo trials were extracted for vmPFC and bilateral hippocampus. APNS assessments included PTSD at 6 months (6mo), impulsivity and sleep problems at 8 weeks (8w), and depression, anxiety, and alcohol use at 6mo. Our first goal was to replicate our earlier findings of reduced inhibition-related vmPFC and hippocampal activation as risk factors for PTSD. Given sex-specific effects of stress on limbic regions, we analyzed the interaction of sex and vmPFC and hippocampal activation, in addition to main effects, using linear regression models. Second, we examined whether vmPFC or hippocampal activation was specifically related to PTSD or reflect a transdiagnostic risk factor across APNS. Finally, we explored scalable measures related to lower vmPFC and hippocampal activation, for which we used Test My Brain for neurocognitive assessment of response inhibition, i.e., Gradual Onset Continuous Performance Task (CPT) d', a measure of hits corrected for false alarms.

Results: Neuroimaging and clinical data for $N = 261$ subjects (91M/170F; 46 Hispanic/89 white/114 Black/11 other race; mean age 33.6y, SD 12.6) were included in the analyses. Linear regression analyses showed a significant model for the vmPFC ($F(2,260) = 4.33$, $p = .014$), with female sex ($B = -.132$, $p = .032$) and lower vmPFC activation ($B = -.123$, $p = .046$) predicting 6mo PTSD symptoms. A significant model for the hippocampus ($F(1,260) = 4.61$, $p = .033$) showed that hippocampal activation and sex interacted in predicting 6mo PTSD symptoms ($B = -.132$, $p = .033$). Specifically, lower hippocampal activation in men was related to greater 6mo PTSD symptoms ($r = -.234$, $p = .025$). Next, lower vmPFC activation was related to greater impulsivity ($r = -.157$, $p = .011$) and sleep impairment ($r = -.131$, $p = .049$) at 8w, but not alcohol use, depression or anxiety symptoms at 6mo. In fact, lower vmPFC activation was related to lower levels of alcohol use at 6mo ($r = .149$, $p = .016$). Lower hippocampal activation did not correlate with other post-trauma sequelae. Finally, a significant correlation between CPT d' and bilateral hippocampal activation was observed in men ($N = 85$, $r = .29$, $p = .007$).

Conclusions: This large ED study partly replicates and extends our earlier findings; lower hippocampal activation was specifically related to later PTSD symptoms in men, whereas vmPFC activation predicted transdiagnostic APNS, particularly those in the inhibitory domain, i.e., PTSD, impulsivity, and sleep problems, suggesting a potential transdiagnostic mechanism of interest for early interventions. For the hippocampus, a correlation with the mobile neurocognitive assessments of response inhibition was observed, again only in men. This measure is much easier to collect than an fMRI scan and could be further explored as a method to identify individuals at risk or employ as a potential target for early interventions.

Keywords: Emergency Department, Functional MRI (fMRI), Trauma Exposure, Ventromedial Prefrontal Cortex, Hippocampus

Disclosure: Nothing to disclose.

P789. Cingulate Activation and IL-6 Covary With Psychiatric Symptom Changes Across Psychotherapy in a Transdiagnostic Sample With Clinically-Elevated Anhedonia

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Background: Anhedonia—core deficits in motivation and pleasure—remains one of the most difficult psychiatric symptoms to treat. Given the centrality of anhedonia to a large number of

psychiatric disorders, improved interventions and validated biomarkers of anhedonia treatment response are critical public health needs. The current study addresses a gap in our understanding of the relations between treatment-related changes in anhedonia (and related symptoms), brain function, and inflammation.

Methods: A transdiagnostic outpatient sample of 73 adults (18-49 years; 51 female), recruited for clinically significant anhedonia, completed pre- and post-treatment 7T fMRI scans that included a face-matching task known to elicit robust responses in neural circuits implicated in threat-processing and emotional regulation (Hariri et al. 2002). Participants were randomized to 15 weeks of Behavioral Activation Therapy for Anhedonia (BATA) or Mindfulness-Based Cognitive Therapy (MBCT) and reported regularly on symptoms of anhedonia (SHAPS), depression (BDI) and perceived stress (PSS) during treatment. Additionally, prior to each scan, whole blood was collected to assess peripheral inflammation (Interleukin-6; IL-6). A subset of participants (~25) completed mid-treatment assessments, such that some participants received up to four scans and blood draws. The permutation analysis of linear models toolbox (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM/) was used to generate whole-brain task-based functional activation maps for the primary fMRI task contrast (i.e., Faces > Shapes). A 2x2 mixed-effect ANOVA examined the effect of Time (Post vs. Pre) and the interaction of treatment (BATA vs. MBCT) by Time; significant effects were identified through threshold-free cluster enhancement method (TFCE) controlling for family-wise error (FWE) rate of $P < 0.05$. BOLD percent-signal change values were calculated and extracted from significant clusters for each participant and time point for use in Hierarchical Linear Models (HLMs). HLMs examined treatment and time effects on self-reported symptoms, task-based brain activation, and IL-6. Additional HLM models examined how within-person fluctuations in task-related brain activation and IL-6 were associated with symptoms to understand how these variables covary together over time.

Results: There were no treatment by time effects on any outcome of interest (i.e., symptoms, task-fMRI, IL-6). Both treatment groups showed significant improvements in self-reported anhedonia, depression, and perceived stress over time (p's < .0001). Further, IL-6 showed a trend towards reduction over time across all participants ($p = 0.08$). At post-treatment, relative to pre-treatment, increased activation in a large cluster (k = 723) spanning the midline posterior cingulate, dorsal anterior cingulate, and supplementary motor cortex was observed for the primary fMRI task contrast (Faces > Shapes; $p < 0.05$, FWE-corrected). A HLM using all time points confirmed percent-signal change in this cluster significantly increased over time across the entire sample ($\gamma = 0.01039$, $p < 0.01$). HLMs evaluating within-person associations demonstrated that higher than usual cingulate activation was associated with lower symptoms at a given timepoint for anhedonia ($\gamma = -4.0945$, $p < 0.01$), depression ($\gamma = -5.5513$, $p < 0.05$), and stress ($\gamma = -5.6443$, $p < 0.001$). Further, lower than usual IL-6 was associated with lower anhedonia at a given time point ($\gamma = 17.8700$, $p = 0.05$), although it was not associated with depression or stress (p's > .05). IL-6 and cingulate activation did not significantly covary within-person ($p > 0.05$).

Conclusions: BATA and MBCT were equally effective in reducing self-reported anhedonia, depression, and perceived stress in a transdiagnostic anhedonic sample. Additionally, both treatments showed a trend towards reducing IL-6. Across the sample, participants demonstrated increased cingulate activation over time in a task commonly used to probe neural circuits implicated in threat- and emotion-processing. The cingulate is involved in mediating complex behaviors and connected with a range of intrinsic brain networks (including cognitive control and attentional networks). Results are consistent with previous research showing cognitive-behavioral interventions reduce symptoms and inflammation, and increase activity in brain regions associated with emotion regulation and cognitive control. Finally, within-person associations suggest potential pathways by which psychotherapy may alter brain and immune function to reduce psychiatric symptoms in patients with anhedonia.

Keywords: 7T fMRI, Inflammation, Anhedonia

Disclosure: Nothing to disclose.

P790. Comparison Between the 2020 CPDD Membership Survey and Meeting Attendance: Transition From Meeting Attendance to Membership

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Background: Efforts to advance equity, inclusion, and diversity within CPDD are vital to increasing innovation and excellence in addiction science and relevance to societal and public health needs. In line with these objectives, a survey was developed to support and accelerate such efforts with a more flexible approach to characterize diversity and measure of inclusion. This project examines associations between our membership survey and attendance at the 2020 annual meeting.

Methods: For the membership survey, individuals on a CPDD listserv were contacted via email to participate in an online survey of 10 items ($N = 657$). Demographic questions used an expanded range of options related to ancestry/ethnicity/race, gender identity, and sexual orientation. In addition, and option was included for self-identification ("None of these describe me. I describe myself as _____.") and an option to select if they preferred not to answer. Respondents reported membership status, time since terminal degree completion, perceptions of welcomeness within the organization, and given an opportunity to provide feedback on efforts to enhance diversity and inclusion within CPDD. The meeting attendance survey ($N = 1189$) was more limited and included membership status, country of origin, ethnicity (but not race), gender, and whether they were attending the meeting for the first time.

Results: Earlier survey approaches only allowed selection of one of six ethnic/racial options and male vs. female or other. Most in the membership survey self-identified along the lines of conventional surveys employing 6 ethnicity/race, and as either male or female. However, many chose a variety of other options related to their Hispanic ethnicities (13.8%), non-White identification (24.4%), nonbinary and/or transgender identities (1.2%). A substantial portion of minority members provided their own preferences for self-identification. Responses to the inclusion proxy ("How welcome do you feel at CPDD?") suggest an association with some demographic factors, such as ancestry and sexual orientation. Individuals who identify as Hispanic and those who identify as women were less likely to be members than those who identified and non-Hispanic or male. The majority of meeting attendees were non-members and the percentages were higher for those who identified as female and Hispanic.

Conclusions: Our findings suggest using expanded response options can more comprehensively characterize diversity of CPDD members and the need to use these for meeting attendance as well. They also suggest that efforts should focus on transitioning from meeting attendance to membership for those who identify as Hispanic and/or female. We aim to use these findings to inform a data-driven approach that will guide efforts to foster a more inclusive and equitable scientific community.

Keywords: Diversity, Inclusion and Exclusion, Membership

Disclosure: Nothing to disclose.

P791. A Transdiagnostic Study of Morphometric Similarity Networks in Apathy and Disinhibition in Dementia

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Background: Apathy and disinhibition are two neuropsychiatric symptoms (NPS) that are common in dementia, particularly behavioral variant frontotemporal dementia (bvFTD) but also dementia of the Alzheimer's type (DAT). They are highly distressing, predict institutionalization and are extremely difficult to treat. The present transdiagnostic study examined the relationships between these symptoms in bvFTD and DAT and brain structure measured by T1-weighted MRI in three major functional networks, the salience (SN), cognitive control (CCN) and default mode networks (DMN). Given previous neuroimaging findings, we hypothesized apathy would be associated with abnormalities in the SN and disinhibition would be associated with abnormalities in the SN and CCN.

Methods: Participants were 157 individuals with a clinical diagnosis of DAT ($n = 94$; 36 female), or bvFTD ($n = 63$; 22 female) from the Alzheimer's Disease Neuroimaging Initiative and the Frontotemporal Lobar Degeneration Neuroimaging Initiative, respectively. All participants had a 3T T1-weighted MRI and data from the Neuropsychiatric Inventory Questionnaire (NPI-Q), a caregiver-rated measure of NPS in dementia. We used Freesurfer 6.0 and the Glasser atlas (Glasser et al. 2016) to parcellate each MRI into 360 parcels and calculated 7 statistics per parcel: gray matter volume, surface area, cortical thickness, intrinsic curvature, mean curvature, curved index and folding index. Using a functional network atlas (Ji et al. 2019) we then identified parcels representing nodes of the SN, CCN and DMN. The statistics for every pair of parcels within each network were correlated to produce within-subject morphometric similarity networks (MSNs, Seidlitz et al. 2019) for each of the SN, CCN and DMN. We removed self- and negative correlations and performed density thresholding at 0.40 (King and Wood, 2020) on each weighted, undirected MSN. Following normalization of MSNs, we calculated the following network measures using Brain Connectivity Toolbox: Transitivity for network segregation, reflecting how much nodes cluster together (the normalized mean clustering coefficient), Global efficiency for network integration, (the average inverse shortest path length). For each NPS, we calculated a 2 (present vs absent) x 2 (diagnosis) ANCOVA, controlling for age, sex, estimated total intracranial volume (eTIV), days between MRI and NPI-Q (days_MRI-NPI), and Clinical Dementia Rating scale Sum of Boxes (CDR-SB). We also regressed global efficiency and transitivity of each subnetwork (SN, CCN, DMN) on age and eTIV. The residuals of these regressions were correlated with severity of apathy and disinhibition (0=absent, 1=mild, 2=moderate, 3=severe) using Spearman correlations, controlling for age, days_MRI-NPI and CDR-SB.

Results: ANCOVAs found presence of apathy was associated with SN transitivity ($F = 3.95$, $p = .049$) and SN global efficiency ($F = 5.17$, $p = .024$), with both metrics lower in those with apathy. SN transitivity was also lower in DAT than bvFTD ($F = 5.17$, $p = .024$). Individuals with disinhibition had lower CCN global efficiency than those without ($F = 6.31$, $p = .013$). There was an interaction of disinhibition and diagnosis for SN global efficiency ($F = 4.88$, $p = .029$), with higher SN efficiency in bvFTD without disinhibition. After Bonferroni correction ($p < .016$) the effect of disinhibition on CCN global efficiency remained significant. Spearman correlations of severity of apathy with network metrics were n.s. However, Spearman correlations with disinhibition severity were significant for SN global efficiency (rho = -.163, $p = .044$), CCN transitivity (rho = -.210, $p = .009$) and CCN global efficiency (rho = -.228, $p = .005$). Both CCN correlations survived Bonferroni correction. Sensitivity analyses found that removing CDR-SB as a covariate did not affect the results.

Conclusions: This is the first study to examine MSNs as they relate to NPS in dementia. Our findings are consistent with neuroimaging evidence that both apathy and disinhibition are associated with damage to SN regions, particularly medial prefrontal and orbitofrontal cortices, and that disinhibition can also occur following damage to the dorsolateral prefrontal cortex. It should be noted that while MSNs have been shown to capture known cortical cytoarchitecture and axonal connectivity, they are only a proxy for anatomical connectivity. Importantly, our transdiagnostic study found that the presence and severity of symptoms were associated with structural network similarity, independently of clinical diagnosis. This finding has implications for treatment, particularly those focused on modifications of brain networks, as these results suggest that there are common underlying structural changes associated with these symptoms, regardless of clinical diagnosis of dementia.

Keywords: Neuropsychiatric Symptoms (NPS), Transdiagnostic, Alzheimer's dementia, Behavioral Variant Frontotemporal Dementia, Network Neuroscience

Disclosure: Nothing to disclose.

P792. Pupil Size Anticipates Exploration and Predicts Disorganization in Prefrontal Cortex

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Background: In uncertain environments, we balance exploitation and exploration: we mostly exploit opportunities that we expect to be rewarding, but also sometimes explore uncertain alternatives that could be even better. Exploration is associated with a disorganization of choice-predictive activity in the prefrontal cortex, which could be a powerful way to randomize choice in order to promote exploratory discovery and learning. Although the mechanisms behind this disorganization remain unknown, exploration is also associated with changes in pupil size under constant luminance—a peripheral index of neuromodulatory mechanisms that could, in theory, cause disorganization in choice-predictive activity. Alternatively, pupil size may simply track variables like uncertainty and volatility that make exploration more likely without predicting the neural correlates of exploration per se.

Methods: Here, we examined how pupil size under constant luminance (1) changed with exploration and exploitation, and (2) predicted prefrontal neuronal activity within and between goal states. We analyzed pupil data from a large neuronal dataset (574 units, 21,793 trials, 28 sessions, 2 male monkeys). (NB we have an ethical mandate to minimize the number of animals, so cannot consider sex in individual primate experiments.) Parts of this dataset were analyzed previously (Ebitz, Albarran and Moore, 2018), though all analyses here are new and the pupil data has not been reported before. The monkeys performed a classic explore-exploit task known as a restless k-armed bandit: they chose between 3 options, whose reward value changed randomly, unpredictably, and independently over time. This encouraged monkeys to exploit rewarding options when they were found, but also to explore uncertain alternatives that could become the best at any time. To determine when the monkeys were exploring (versus exploiting) we modeled these as the latent goal states underlying behavior in a hidden Markov model. We previously reported that these model-inferred goal state labels explain more

variance in neural activity than rewards, subjective value, switch or stay choices, and other related variables.

Results: Consistent with previous studies, we found that pupil size under constant luminance was larger during exploratory choices, compared to exploitative choices (both monkeys: paired *t*-test: $p < 0.0001$, $t(27) = 4.95$, mean offset = 0.23, 95% CI = 0.13 to 0.32; monkey 1: $p < 0.0005$, $t(9) = 5.50$, mean = 0.4, 95% CI = 0.24 to 0.57; monkey 2: $p < 0.02$, $t(17) = 2.85$, mean = 0.13, 95% CI = 0.03 to 0.23). In fact, trial-by-trial variability in pupil size predicted the likelihood that the trial was exploratory (logistic GLM, beta = 0.009, $p < 0.0001$). However, this effect was not confined to the explore trials. Over the 10 trials before monkeys started to explore, pupil size slowly ramped up (both monkeys: GLM, beta = 0.016, $p < 0.003$, $n = 28$; monkey 1: beta = 0.020, $p < 0.02$, $n = 10$; monkey 2: beta = 0.013, $p < 0.05$, $n = 18$). This was not an artifact of some misalignment of the goal state labels: there was no ramping in other behavioral and neural measures that differed between exploration and exploitation, like movement velocity or the neural signatures of exploration.

Next, we asked whether pupil size predicted choice-predictive activity in the prefrontal cortex. We trained decoders to predict choice from the firing rates of the units that were simultaneously recorded in each session. Choice decoding accuracy was decreased during explore choices, compared to exploit choices (paired *t*-test, $p < 0.0001$, $t(27) = 8.65$), and increasing pupil size predicted worse choice decoding accuracy (GLM: beta = -0.032, $p < 0.0001$, $n = 28$). This was not a trivial consequence of averaging over explore trials (where pupil size was larger and decoding accuracy lower) and exploit trials (where pupil size was smaller and decoding accuracy higher) because it was also true and of a similar magnitude solely within exploit trials (beta = -0.037, $p < 0.002$, $n = 28$). We previously reported that exploration also increases the "scatter" between trials (i.e. the trial-to-trial variability in patterns of prefrontal activity) and increasing pupil size also predicted an increase in scatter, both across trials (beta = 0.04, $p < 0.0001$, $n = 28$) and again within the exploit trials alone (beta = 0.03, $p < 0.0005$, $n = 28$). In sum, pupil size predicted trial-by-trial variability in choice predictive activity in the prefrontal cortex, even though it ramped up in advance of exploration and these neural measures did not.

Conclusions: The fact that pupil size ramped up before exploration could suggest that the pupil tracks the variables that make exploration more likely, rather than predicting exploratory brain states per se. However, the pupil also predicted trial-to-trial fluctuations in exploratory neural signatures within exploitation, in the absence of any overt changes in behavior. These results are difficult to reconcile if we imagine the brain as a linear system, but perhaps make more sense if exploration represents a critical "tipping point" for the prefrontal cortex—a shift into a new dynamic regime, where all choices are equally likely to occur. In this nonlinear model, pupil-linked mechanisms could drive the brain towards a regime where tipping points are more likely, but the ultimate trigger for the onset of exploration and the disorganization of prefrontal activity is a complex and nonlinear positive feedback loop that only partially depends on pupil-linked mechanisms.

Keywords: Explore-Exploit Dilemma, Value-based Decision-Making, Pupillometry, Neurophysiology, Exploration

Disclosure: Nothing to disclose.

P793. Focused Ultrasound (FUS) Modulation of Amygdala Blood Oxygenation Level Dependent (BOLD) fMRI Signal in Humans: Preliminary Findings From a Concurrent FUS/fMRI Pilot Study

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Background: First-line treatments for mood, anxiety, and traumatic stress disorders include pharmacological and psychotherapeutic interventions, but many individuals do not respond adequately and maintain significant residual symptoms and functional impairment. Non-invasive neuromodulatory device-based interventions, including repetitive transcranial magnetic stimulation (rTMS) and transcranial electrical stimulation, are second-line approaches that have demonstrated some efficacy in treatment-resistant populations. However, these neuromodulation approaches are critically limited by their capacity to only modulate brain function in cortical areas due to the dispersion of the electromagnetic force more than a few centimeters past the skull. The amygdala, a collection of gray matter nuclei located within the medial temporal lobe, has repeatedly demonstrated abnormally heightened activity in numerous clinical populations with mood and anxiety disorders, demonstrating brain-behavior relationships with fear-based and negative affect symptoms such as anxiety, anger, and worry. These observations are consistent with a voluminous scientific literature demonstrating the centrality of the amygdala to the detection of threatening environmental stimuli, fear-based learning, and the generation of a subjective fear response. Indeed, neuromodulatory interventions such as rTMS have been theorized to augment the function of subcortical circuitry such as the amygdala (and an extended limbic circuit) as part of their mechanistic action to alleviate mood, anxiety, and traumatic stress disorder symptoms. Thus, it is reasonable to surmise that a non-invasive intervention approach that could directly target and modulate the function of subcortical brain structures may be particularly useful as a device-based neuromodulatory intervention approach. Low-intensity focused ultrasound (FUS) has been demonstrated in animals and in a small number of human studies to reversibly alter the function of both cortical and subcortical substrates. Importantly, as it utilizes a different fundamental physical force than existing electromagnetic approaches, it has the capacity to more deeply penetrate and target deep brain structures, such as the amygdala, that are currently not directly reachable with current clinical neuromodulation approaches. Here, utilizing a FUS device specifically designed to target subcortical brain structures in humans, we conducted a small pilot study to examine two sets of FUS parameters administered during functional magnetic resonance imaging (fMRI) for the potential to attenuate amygdala blood oxygenation-level dependent (BOLD) signal in humans. Our goal was to provide initial evidence of target engagement of a clinically-relevant brain structure, thereby setting the stage for future studies to more thoroughly examine FUS as a potential neuromodulatory probe and intervention tool.

Methods: Six mentally healthy individuals ($N = 6$) underwent concurrent FUS and fMRI in a within-subject, repeated measures design. On separate days (counterbalanced for order), MRI-guided targeting was utilized to establish placement of a 65 or 55 mm focal depth (depending on individual anatomy) single-element focused ultrasound transducer, part of the Brainsonix Pulsar 1002 neuromodulation system. Operating at a fundamental frequency of 650 kHz, the transducer was placed on the side of the head and positioned to deliver FUS through the temporal bone window. Iterative MRI anatomical scouts were utilized to refine placement and identify the optimal position such that the focus of the ultrasound beam would be centered in the individuals' left amygdala. Individuals underwent concurrent FUS and resting state fMRI with two sets of parameters (both: 5% duty cycle, temporal average intensity at the spatial peak: 720 mw/cm squared) using either a 10 Hz or 100 Hz pulse repetition frequency (PRF) delivered in 30 second blocks with 30 second rest periods for 10 cycles (5 min FUS in total). fMRI analyses modeled regressors specifying

FUS on vs. off periods convolved with the hemodynamic response function, with individual average beta-weights from an anatomical amygdala mask carried to a second level linear mixed model (random intercept, fixed effect of session) to examine the effect of 10 vs. 100 Hz PRF on amygdala BOLD signal.

Results: 10 Hz vs. 100 Hz PRF FUS resulted in a significantly greater attenuation of left amygdala BOLD signal ($F = 6.182$, $p = 0.047$) with a large effect size ($d = 0.92$). This effect was driven entirely by BOLD signal attenuation with the 10 Hz PRF (one sample *t*-test: $t = -3.6$, $p = 0.015$) with no effect of the 100 Hz PRF (one sample *t*-test: $t = -0.07$, $p = 0.95$).

Conclusions: To our knowledge, this is the first demonstration of amygdala fMRI target engagement with FUS in humans. Though this is a small pilot study that requires replication in larger samples, results suggest FUS demonstrates promise as a potential non-invasive subcortical neuromodulation tool. Concurrent FUS/fMRI may be a promising methodology to test target engagement and optimize parameter settings, and future work with this methodology is currently underway.

Keywords: Amygdala, Focused Ultrasound, BOLD fMRI Signal

Disclosure: Alto Neuroscience: Stock / Equity (Self)

P794. Transcriptional Diversity and Spatial Variability of Glutamatergic Neurons in the Basal and Lateral Nuclei of the Primate Amygdala

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Background: In rodents (O'Leary et al. 2020. eLife), it was recently shown that different gene expression patterns for glutamatergic neurons can be used to define discrete boundaries within the basolateral amygdala. Moreover, within each nucleus, the lateral and basal, there exists graded transcriptomic variability among glutamatergic neurons. Understanding the functional implications of this heterogeneity in glutamatergic function is vital for translating basic research into the clinic. But to do so we must first understand if similar gene expression boundaries and functional heterogeneity exists in non-human primates. The goal of this project is to build off recent work in rodents to determine if similar gene-markers can be used to dissociate the lateral and basal nuclei in non-human primates and use state-of-the art transcriptomic approaches to identify the functional heterogeneity of glutamatergic neurons in each nucleus.

Methods: Immunohistochemistry assessing protein expression under the control of two candidate gene markers complexin 1 (CPLX1) and neuronal growth regulator 1 (NEGR1) was completed for tissue spanning the entire amygdala in 5 (3 female) rhesus macaques. A double-labeling immunofluorescence experiment was performed in a subset of these animals to confirm that CPLX1 and NEGR1 protein expression within amygdala neurons overlapped with the expression of CaMKII or PSD-95. In both experiments, histological sections were imaged and then quantified using the open-source algorithm Cellpose. Statistical analyses then focused on identified differences between the lateral and basal amygdala in terms of the spatial distribution of cells expressing each marker in adjacent sections. In a third experiment, we used the Chromium Next GEM Single Cell 3′ kit v3.1 (10X Genomics) to generate single nuclei RNAseq libraries. Nuclei were isolated from fresh tissue brain punches collected from dorsal and ventral subdivisions of the lateral and basal nucleus of the amygdala in 2 rhesus macaques, following the recommended protocol with minor modifications. Briefly, a gradient centrifugation was used to obtain a clean single nuclei suspension from each brain's region homogenate. Then, ~16k nuclei per brain region were used to generate RNAseq libraries following manufacturer's instructions. Libraries were sequenced in a NovaSeq6000 at the Massively Parallel Sequencing Shared Resource (OHSU). A custom bioinformatics pipeline was used to generate and compare transcriptomic markers for each nuclear subdivision.

Results: The spatial distribution of cells expressing CPLX1 and NEGR1 in the lateral versus basal amygdala did not explicitly differ when assessed along either the mediolateral or dorsoventral axes of the histological sections. Yet, transcriptomic analyses comparing gene expression patterns for tissue punches taken from the lateral versus basal nucleus similarly did not reveal discrete expression of CPLX1 versus NEGR1 in the basal versus lateral nucleus of the amygdala. However, against a non-specific background of expression multivariate analyses of gene dependent protein expression in the immunohistochemistry datasets did indicate that there were distinct clusters of expression within each nucleus. Pockets of enriched expression of CPLX1 were observed in the basal nucleus and NEGR1 in the lateral nucleus of the amygdala. Ongoing analysis of the transcriptomic data is focused on identifying whether discrete boundaries can be identified using other candidate gene markers and the extent to which gene expression in glutamatergic neurons maps onto the lateral and basal nuclei in a discrete or continuous manner.

Conclusions: Discrete heterogeneity in the spatial expression of cell-type specific marker identified previously in rodents is not as evident in non-human primates. Instead, there appears to differential pattern of gene expression among glutamatergic neurons found within the lateral and basal nuclei of the amygdala that are consistent with the evolutionary expansion of these nuclei in primates. Transcriptomic approaches that account for spatial variability in the expression of cell-type specific markers of glutamatergic neurons will likely be important in developing novel markers for clinically relevant circuit specific manipulations of amygdala function in non-human primates

Keywords: Amygdala, Transcriptomics, Macaque, RNAseq

Disclosure: Nothing to disclose.

P795. Impact of Human Infant Gut Microbiota on Murine Behavior and Dendritic Complexity in Prefrontal Cortex

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Background: Recently, the microbiome has emerged as a key player in neurodevelopment, potentially influencing risk for psychiatric conditions, including anxiety, depression, and autism spectrum disorder. This includes work by our group showing that certain features of the human infant gut microbiome are associated with fear reactivity, cognitive performance, and brain structure and connectivity. Given the observational nature of our human infant studies, it remains unclear whether the human infant gut microbiome causes individual differences. To help address this gap, we conducted a pilot study in which we transplanted microbial communities from human infants into germ-free pregnant mice and evaluated effects on offspring behavior and brain structure.

Methods: Pregnant Swiss-Webster germ-free mice were divided into four groups. Group 1 (BIF group) was inoculated with a mixed human fecal slurry derived from infants with relatively high levels of Bifidobacterium. Group 2 (BAC group), was inoculated with a mixed human fecal slurry derived from infants with relatively high levels of Bacteroides. Group 3 (SPF group) received fecal slurry from specific pathogen free mice (SPF group) and group 4 (GF group) received autoclaved fecal slurry from SPF mice. Offspring

were assessed in the elevated plus maze (EPM), novel object recognition, and three-chamber sociability tests between 6-8 weeks of age and dendritic length and volumes were evaluated in the medial prefrontal cortex and amygdala using Golgi-Cox staining. Both sexes were studied and sample size ranged from 2 to 13 per group.

Results: In sex stratified analyses, SPF male mice spent more time with the familiar mouse in phase 2 of the social interaction tests than BIF male mice (*$p < 0.05$) or BAC male mice (**$p < 0.01$), but all 4 groups spent a similar amount of time with the novel mouse. Germ free males appeared more similar to the SPF males, but did not differ significantly from any other group. Dendritic length and volume were significantly greater in the prefrontal cortex of humanized Bif mice ($N = 4$) compared to humanized Bac mice ($N = 2$), $p < 0.05$, suggesting that microbiomes with a high relative abundance of Bifidobacterium may promote dendritogenesis in this region.

Conclusions: This was a pilot study to assess feasibility and inform statistically relevant sample size calculations for future studies evaluating the impact of human infant microbiomes on murine behavior and brain structure. Preliminary findings suggest that that male mice carrying humanised infant gut microbiomes may differ in their social behavior compared with SPF and GF mice and that differences in the composition of the human gut microbiome may influence development of the prefrontal cortex, which is involved in social information processing and emotional regulation. However, results must be considered preliminary given the small sample size.

Keywords: Gut Microbiome, Medial Prefrontal Cortex, Social Behavior, Dendritic Arborization

Disclosure: Nothing to disclose.

P796. Evidence of Neurovascular Coupling Changes After Psychedelics From Task and Resting fMRI

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Background: Classic psychedelics have become a topic of great interest in psychiatry because of large antidepressant responses sustained after a single dose. Neuroimaging offers a non-invasive tool to measure neurobiological correlates of distinctly human response to psychedelic drug (psychological, entheogenic, and psychiatric). Functional MRI (fMRI) studies of acute effects of psychedelics have observed dramatic changes (primarily decreases) in functional connectivity (FC). But these studies have also reported reduction in fMRI signal variance. The 5-HT2A receptor is expressed on neurovasculature and has known vasoactive effects in the central nervous system. Thus, it is not clear to what extent FC changes caused by psychedelics reflect changes at the neuronal, neurovascular, or vascular level.

Methods: We test the hypothesis that changes in FC during psychedelic exposure are dependent on 5-HT2A-related changes in neurovascular coupling. Using human neuroimaging datasets with LSD ($N = 17$ after motion censoring) and Psilocybin ($N = 9$ after motion censoring) from Imperial College London, we investigate the cause of FC changes. Using resting fMRI data, we measure blood-oxygenation-level-dependent (BOLD) spectral power and functional connectivity. Using retinotopy fMRI data, we measure stimulus-evoked response in V1 (an area with high 5-HT2A receptor expression). Finally, we use RNA Seq data from the Allen Mouse Brain Atlas and Allen Human Brain Atlas to measure 5-HT2A receptor expression differences across cell types (mouse) and across brain areas (human).

Results: We observed that psychedelics cause significant decreases in spectral power of the BOLD signal in the infraslow range ($f < 0.1$hz, corresponding to neuronally-driven BOLD fluctuations). Using data from the Allen Brain Atlas, we show that the 5-HT2A receptor is expressed on GABAergic interneurons as well as astrocytes and we show that spatial differences in spectral power change are proportional to 5-HT2A receptor expression. Spatial differences in spectral power also correlate with global brain connectivity changes ($r = 0.46$, $p < 0.01$). Finally, using retinotopy data, we observe a significant decrease (31%) in the peak magnitude of the evoked hemodynamic response.

Conclusions: These results suggest that psychedelics may be acutely altering or uncoupling the link between neural activity and hemodynamic response. Animal studies are needed to confirm neurovascular uncoupling and to clarify the effects of psychedelics on large-scale brain network using methods that do not depend on neurovascular response. However, fMRI-based precision functional imaging approaches still have utility in understanding persisting effects of psychedelics on the human brain.

Keywords: Psychedelics, BOLD fMRI Signal, LSD, Psilocybin, Resting State Functional Connectivity

Disclosure: Nothing to disclose.

P797. Neural Activation During Response Inhibition, but Not Error Processing, Differentiated Distinct Symptom Profiles of Irritability and Attention-Deficit/Hyperactivity Disorder in the ABCD Study

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Background: Irritability, characterized by developmentally inappropriate temper outbursts and low frustration tolerance (Leibenluft, 2017), is a hallmark symptom of disruptive mood dysregulation disorder (DMDD) in the DSM-5. Irritability commonly co-occurs with attention-deficit/hyperactivity disorder (ADHD; Shaw et al. 2014). It is estimated that >35% of youths in the community samples, and >70% in the clinical samples, have both chronic irritability/DMDD and ADHD (Shaw et al. 2014; Leibenluft and Kircanski, 2021). While individual differences exist in the symptom manifestations of irritability and ADHD, the shared and nonshared neurobiological pathways underlying these differential symptom profiles are not well-understood. Here, we used a latent phenotyping approach to identify youths with distinct phenotypic profiles of irritability and ADHD symptoms. Next, we examined differences between these phenotypic profiles in behavioral performance and neural responses during a Stop Signal Task that probed response inhibition and error processing—two neurocognitive processes central to both irritability and ADHD (Kessel et al. 2016; Shaw et al. 2014; Walcott and Landau, 2004).

Methods: Data were from the Adolescent Brain and Cognitive Development (ABCD) study at baseline ($N = 11{,}875$, mean age = 9.9 years, female = 47.8%). Irritability symptoms were assessed using 4 items from the parent reports of the computerized Kiddie-Structure Assessment of Affective Disorders and Schizophrenia (KSADS). These included 1 item from the DMDD module (i.e., temper outbursts ≥ 3 times/week), 2 items from the oppositional defiant disorder module (i.e., often touchy or easily annoyed; often loses temper), and 1 item from the major depressive disorder module (i.e., irritability). ADHD symptoms were assessed using 18 items from the ADHD module of the parent-reported KSADS. Behavioral responses and neural activations during the Stop Signal fMRI Task (Casey et al. 2018) were measured. The main behavioral measure was the Stop Signal reaction time. Neural measures were the beta coefficients representing BOLD (blood-oxygen-level-dependent) signal changes during two contrasts

of interest: Correct Stop vs. Correct Go (response inhibition) and Incorrect Stop vs. Correct Go (error processing). We focused on 29 brain regions that showed good test-retest reliability (i.e., intra-class correlation [ICC] > .60; Korucuoglu et al. 2021) during this task.

Taking a latent modeling approach, we first conducted latent class analysis (LCA) to identify unique irritability and ADHD symptom profiles. We then conducted exploratory factor analysis and then confirmatory factor analysis across the 29 brain regions to derive networks of regions with coherent coactivation patterns. Next, we examined whether the task behavioral measure (i.e., Stop Signal reaction time) and neural activations differed across the LCA-derived groups with distinct symptom profiles.

Results: Using LCA, we identified four groups of youths based on irritability and ADHD symptoms; Akaike Information Criterion (AIC) = 56111.24, Bayesian Information Criterion (BIC) = 56782.04, sample-size adjusted BIC (ABIC) = 56492.85, Entropy = .97, bootstrapped parametric likelihood ratio test (BLRT) < .001, smallest class proportion = 2.4%. These four groups are: (1) high irritability and low ADHD (n = 279, 2.4%); (2) high ADHD and low irritability (n = 901, 7.7%); (3) high ADHD and high irritability (n = 787, 6.7%); (4) and low irritability and low ADHD symptoms (n = 9,781, 83.3%).

Exploratory factor analysis and subsequent confirmatory factor analysis revealed two distinct yet correlated networks — a "response inhibition" network that included the left supramarginal gyrus, left inferior parietal cortex, and left pars orbitalis and an "error processing" network that included the left and right lateral orbital-frontal cortex and left pars orbitalis.

The four LCA groups did not differ in Stop Signal reaction time ($F3,\ 5246 = 1.08,\ p > .05$). The four groups differed in neural activations during response inhibition but not error processing. Specifically, during response inhibition, relative to those with low irritability and low ADHD (Intercept = 0), those with high ADHD and high irritability showed increased activation (Intercept = .21), whereas those with high irritability and low ADHD (Intercept = -.54) and those with high ADHD and low irritability (Intercept = -.15) both showed decreased activation (especially so for those with high irritability and low ADHD). Results adjusted for potential confounds such as age, sex, sociodemographic variables (e.g., caregiver education, marital status, and total household income), and scanning sites.

Conclusions: Using latent modeling, this study showed that neural activation during response inhibition, but not error processing, differentiated distinct symptom profiles of irritability and ADHD in youths. Specifically, the co-occurrence of irritability and ADHD symptoms was characterized by heightened activation in the response inhibition network, whereas the presence of irritability or ADHD alone was characterized by decreased activation in the same network. Dissociable neurobiological pathways across distinct symptom profiles of irritability and ADHD may provide novel insights into the potential neural mechanisms for change that are specific to unique symptom profile, paving the way for transdiagnostic interventions and precision medicine.

Keywords: Pediatric Irritability, ADHD, Response Inhibition, Error Processing, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P798. Dissecting Biological Pathways of Psychopathology Using Cognitive Genomics

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Background: Cognitive deficits are known to be related to most forms of psychopathology. Recent work in the psychiatric and cognitive genomics literature indicated that pleiotropic associations between cognitive dimensions and psychiatric traits could further illuminate putative underlying biological mechanisms.

Methods: Here, we perform local genetic correlations as a means of identifying independent segments of the genome that might show biologically interpretable pleiotropic associations between cognitive dimensions and psychopathology. We utilized GWAS summary statistics for two major cognitive dimensions: General Cognitive Ability (GCA) and Non-Cognitive Skills (NCS); the former indexes "g" while the latter is derived from Educational Attainment after removing variance related to "g". We then carried out a series of local genetic correlation analyses with the GWAS summary statistics of 17 psychopathological traits that were previously published. We developed a novel approach to pleiotropy analyses, in which genomic segments with similar patterns of local genetic correlations are grouped into biologically interpretable units which we termed "meta-loci." These meta-loci offer higher resolution and greater interpretability of the shared genetic architecture between cognitive dimensions and psychopathology as compared to global genetic correlations.

Results: Differential pleiotropic patterns for GCA and NCS against psychopathological traits were found across meta-loci. Functional transcriptomic annotation and gene set analyses revealed a broad distinction between meta-loci associating psychopathology to GCA as compared to NCS. Statistically prioritized genes within GCA meta-loci tend to be expressed during prenatal and early childhood periods, whereas genes implicated with NCS were expressed in later childhood, young adulthood, and beyond. Most notably, we found that many genes whose protein products were identified as presently druggable for psychiatric and nootropic indications were found within NCS meta-loci. These genes were predominantly within the GABA-ergic receptor, glutamate, and cholinergic family of genes.

Conclusions: The results in the current study extended our prior work in schizophrenia to psychopathology more broadly: neurodevelopmental pathways predominated in GCA-relevant meta-loci, while synaptic pathways were more involved in NCS-relevant meta-loci. It is notable that further dissecting the pleiotropy underlying the cognitive-psychopathology continuum via the meta-loci approach allowed resolution for gene prioritization beyond loci-based or genome-wide approaches. The findings underscore the need for novel strategies to exploit the rapidly accumulating evidence of widespread pleiotropy across neuropsychiatric traits. The results are particularly relevant for specifying biological mechanisms that might be targeted for nootropic purposes within psychopathology.

Keywords: Cognition, Psychopathology, Genomics

Disclosure: Nothing to disclose.

P799. Frustration-Induced Reconfigurations of Brain Networks: Implications for Irritability

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Background: In youth, the transdiagnostic symptom of irritability is among the most frequent reasons for psychiatric consultation. Aberrant responses to frustration are posited to be an important mechanism of irritability, but neural responses to frustration have not been well-studied. Here, we examine brain network dynamics during and after frustration and test whether network metrics can

predict irritability and the course of co-occurring depressive symptoms.

Methods: Using fMRI, we investigated changes in neural networks from a baseline resting-state, through a task that included frustrative non-reward (FNR) and anticipation of new feedback after frustration (FNR + 1), to a post-task resting-state in a transdiagnostic sample of 66 youth (33 female, mean age 14 years). Using a train/test/held-out procedure, we aimed to predict past-week irritability from the global efficiency (i.e., Eglob, capacity for parallel information processing) of brain networks before, during, and after frustration. We also examined whether Eglob improves the prediction of the course of depressive symptoms using linear mixed effects models.

Results: Compared to baseline resting-state, FNR + 1 and the post-task resting-state were uniquely associated with a more segregated brain network organization. Nodes that were originally affiliated with default mode, fronto-temporal-limbic, and fronto-parietal modules contributed most to reconfiguration during and after frustration. Predictions of irritability were detected only during post-task resting-state, in modules that had emerged after frustration. Child-reported irritability was predicted by Eglob in a fronto-temporal-limbic module, while parent-rated irritability was predicted by Eglob in motor-parietal and ventral-prefrontal-subcortical modules. Moreover, Eglob in the fronto-temporal-limbic module that emerged after frustration enhanced the prediction of the course of depressive symptoms over one year.

Conclusions: These findings elucidate the clinical importance of the post-frustration recovery period and, if replicated, suggest specific intervention targets for irritability.

Keywords: Pediatric Irritability, Frustration, Neural Networks, Brain-Based Predictor

Disclosure: Nothing to disclose.

P800. Neural and Behavioural Evidence From Reinforcement Learning Converge in Support of Relaxed Beliefs Under LSD

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Background: The REBUS (RElaxed Beliefs Under pSychedelics) and the anarchic brain model of psychedelic drug action proposes that confidence in beliefs – and thus expectations (priors) – are relaxed under these drugs. The brain makes inferences to minimize surprise, or discrepant expectations and outcomes (prediction error [PE]), and better model the world. We recently showed that the non-specific 5-HT2A receptor agonist LSD heightened sensitivity to PEs, reflected by speeded updating of value representations following better and worse than expected outcomes. Indeed, events that are surprising are inherently less expected. There is evidence that LSD produces ego dissolution that is positively correlated with disintegration of the default mode network (DMN), assessed using resting state functional connectivity (RSFC), and is correlated inversely with alpha oscillatory power. The DMN has been proposed to be a seat at the ego's table and decreased alpha power has been posited to reflect relaxation of the cognitive hierarchy, mediated by 5-HT2A receptors on deep layer V pyramidal neurons in cortex. Here we aim to demonstrate neural correlates of an objective, behaviorally derived marker of REBUS.

Methods: Greater speed at which choice value increased following a reward PE – higher reward learning rates (RLRs) – was operationalized as enhanced sensitivity to surprise. We tested whether medial prefrontal cortex (mPFC) RSFC within the DMN – a region often associated with value – and diminished alpha power correlated significantly with the RLR. Alpha power was derived from magnetoencephalography (MEG). The functional magnetic resonance imaging (fMRI) resting state functional connectivity (RSFC) data of interest were extracted from mPFC regions of the DMN. To assess anatomical specificity, we also predicted that static RSFC of primary visual cortex (V1) would not be correlated with the RLR. We additionally extended our analysis to the brain's temporal organization, parsing the data into dynamic states where network integration or segregation predominate, again focusing on mPFC regions of the DMN.

Results: The RLR was inversely correlated with MEG alpha power ($r(13) = -.618$, $p = .024$), and positively correlated with the extent of disintegration of the DMN as assessed using static RSFC ($r(15) = .641$, $p = .010$). V1 static RSFC did not correlate with the RLR, as hypothesized ($r(15) = .204$, $p = .465$). The RLR was positively correlated with dynamic RSFC in a mPFC a priori region of interest during a predominantly integrated sub-state of brain dynamics ($r(15) = .624$, $p = .013$).

Conclusions: A behaviorally derived computational marker of surprise was correlated with neural signatures of relaxed beliefs under psychedelics (REBUS).

Keywords: Psychedelics, Computational Reinforcement Learning Model, Probabilistic Reversal Learning, Serotonin 5-HT2A Receptor

Disclosure: Nothing to disclose.

P801. Prefrontal Cortex Involvement in Emotional and Non-Emotional Inhibitory Control in Adolescents

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Background: Inhibitory control by the prefrontal cortex (PFC) has broadly been associated with a wide array of cognitive processes that may interact with psychopathology. Aberrant PFC function has been associated with heightened emotional interference and with non-emotional motor inhibition. Studies often examine neural activation associated with these processes separately or in parallel, but few studies have specifically interrogated the overlap in patterns of activation related to both emotional and non-emotional inhibitory control. The present study aimed to examine shared and distinct patterns of PFC function across tasks that probe emotional and non-emotional inhibitory control using data from early adolescents in the Adolescent Brain Cognitive Development (ABCD) dataset.

Methods: Participants ($n = 2266$; Mage = 10.02; SDage = 0.62; 56% Female) completed the Emotional N-Back (EN-Back) Task as a probe of emotional interference and the Stop Signal Task (SST) as a probe of non-emotional motor inhibition. Patterns of activation were identified for the fearful vs. neutral face contrast on the EN-Back Task and on the incorrect stop trials vs. incorrect go trials contrast on the SST. A conjunction analysis was then conducted to identify overlapping patterns of activation in PFC between the EN-Back Task and SST, controlling for motion, age, sex, race, and household income.

Results: Conjunction maps were created for all participants on both tasks at a threshold of $p < 0.001$. Preliminary findings revealed common activation between tasks in the rostral anterior cingulate cortex and dorsolateral prefrontal cortex.

Conclusions: These preliminary results demonstrate overlap in patterns of PFC activation associated with emotional and non-emotional inhibitory processes. Further examination of PFC activation in a large, well powered dataset of adolescents may

provide critical insight into patterns of activation that could convey risk for the future development of psychopathology.

Keywords: Adolescent Brain Cognitive Development Study, Inhibitory Control, Imaging

Disclosure: Nothing to disclose.

P802. Social Behavior Deficits Following 5-HT2aR Constitutive Deletion

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Background: Social behavior is defined as interactions among individuals, mostly from the same species that offer mutual benefits. Social behaviors comprise different actions which are based on social interaction. Deficits in social interaction (SI) are a hallmark of different psychiatric disorders, including but not limited to autism spectrum disorders. Serotonin (5-HT), as a neurotransmitter, is involved in a wide variety of brain functions, from early development and throughout the lifespan of the individual that includes emotional and social behavior. Changes in 5-HT levels, as well as some activity of key molecules within the system, have been associated with deficits in SI. Serotonin 2A receptors (5-HT2aR) are one of the main excitatory serotonergic receptors. Social deficits in humans were associated with 5-HT2aR hypofunction. Interestingly, 5-HT2aR agonists appear to increase social interaction in humans and animal models suggesting that 5-HT2aR might modulate SI. We started to investigate the role of 5-HT2aR in SI using a genetic mouse model. We are presenting preliminary data regarding the role of 5-HT2aR in social interaction.

Methods: We used genetically modified male and female mice (htr2a-/-) and their littermates controls (htr2a + /+). P90 or older animals were exposed to different behavioral paradigms: three-chambers social interaction test. Animals were exposed to a three-compartment black rectangular maze that contained a holed Plexiglass cylinder that would enclose a juvenile same-sex animal or an object. Mice were habituated to the chamber for 20 minutes. 24hs later, the subjects were placed in the central compartment for 5 minutes before allowing them to explore the maze. Social interaction was evaluated during a 10-min period. For the pharmacological experiment, htr2a + /+ male mice were cannulated using a stereotaxic frame (mPFC; AP = + 3.20 mm, LL = ± 0.75 mm, DV = − 3.50 mm) under anesthesia (ketamine/xylazine) and led to recover for 5 days. Fifteen minutes before the experiment, mice were infused with MDL 11,939 (300 ng/µl) or vehicle (5% DMSO). Dominance tube: Mice were habituated to a plexiglass 30 cm long cylinder for 5 minutes during 5 consecutive days. Tests are conducted among cagemates using a round-robin design in which two mice were placed, each at one of the entries until one retreated. Rank is assessed by the number of times a particular mouse wins. Olfactory habituation test: Mice were exposed to cotton swabs from the top of a clean homecage embedded in different odors. We used water, a non-social odorant (almond extract), and two social odorants (same sex and opposite sex). Each odor was presented three times consecutively for 2 minutes with an ITI of 1 minute. Analysis: All behaviors were recorded using a webcam. Offline analysis was conducted using Stopwatch + (Emory University). Discrimination index (DI) was calculated for the SI test. Statistical analysis: Data were analyzed using R 4.1.0. For all analyzes, the normality used in the Shapiro-Wilk test was evaluated. Homoscedasticity was tested by the Levenne test. If appropriate, we choose parametric statistics. For multiple comparisons, the ANOVA test was used. Posthocs were performed using the Tukey's test. When only two groups were compared, Welch's *t*-test for independent samples or a *t*-test for dependent samples was used. For the dominance tube test, we used a mixed linear model with genotype as a between-subject variable and day of testing as a within-subject variable.

Results: htr2a-/- male and female mice show decrease social interaction in the social interaction test compared with same sex htr2 + /+ mice (Males: t (16.37) = 3.8, $p = 0.0015$. $d = 1.54$, power = 0.99. Females: t (23.76) = -2.99, $p = 0.0062$. $d = 1.17$, power = 0.98). Though there were no differences between sex (F (1, 45) = 0.2323, $p = 0.6322$), the deficit observed in females appeared to be stronger, since only for females htr2a-/- the DI was not different from zero (t (11) = 1.298, $p = 0,2208$). The deficit appeared to be due to the lack of 5-HT2aR expression throughout their lifespan, since local infusion of MDL 11,939 into the medial prefrontal cortex of male htr2a + /+ mice before the social interaction test did not generate any difference compared with the response observed in vehicle treated mice (t (6) = -0.33, $p = 0.75$). The deficit observed appeared to depend on 5-HT2aR forebrain population, since restoring 5-HT2aR expression in htr2a-/-; Emx1-Cre-/+ mice rescued the deficit observed in the social interaction task (F (3, 33) = 6.754, $p = 0,0011$). Hierarchy is the result of social interaction among multiple individuals. Preliminary studies of hierarchy using the dominance tube test showed no significant differences between htr2a-/- and htr2a + /+ males ($\chi2(1) = 0.51$, $p = 0.47$) and females ($\chi2(1) = 0.28$,$p = 0.6$) mice .Odor is a key cue for SI, that could underlie the effects observed in the social interaction test. In an odor habituation test, we found that htr2a-/- and htr2a + /+ mice solve the task similarly.

Conclusions: Our preliminary results suggested that 5-HT2aR is required for social interaction in mice. This deficit is observed in male as well as female htr2a-/- mice, with a small tendency to a stronger deficit in females. The reduced social interaction observed in the three-chamber task appeared to be due to a developmental or chronic role of 5-HT2aR and is dependent on the excitatory forebrain 5-HT2aR population. Taken together, these results suggest the importance of 5-HT2aR as a major modulator of social behavior

Keywords: 5-HT2A Receptors, Social Interaction, Behavioral Tasks, Medial Prefrontal Cortex

Disclosure: Nothing to disclose.

P803. White Matter Microstructure Associated with Anhedonia Among Individuals With Bipolar Disorders and High-Risk for Bipolar Disorders

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Background: Anhedonia - a key symptom of depression - is highly associated with poorer outcomes and suicidal behavior. Alterations in the circuitry of reward-related brain regions have been robustly associated with anhedonia in unipolar depression, but not bipolar disorder (BD). We investigated white matter microstructures associated with anhedonia in participants with BD types I and II and first-degree relatives of patients with BD (BD-siblings).

Methods: Eighty participants (BD types I and II: 56 [70%], and BD-siblings: 24 [30%]) underwent diffusion tensor imaging (DTI); Fractional anisotropy (FA) of different tracts were computed. Anhedonia was assessed using item 8, ("inability to feel") of the MADRS scale. General linear models were used to compare the FA

of different tracts in participants with and without anhedonia controlling for several clinical and demographic variables.

Results: The mean age of the sample was 37 (±11) years old, and 68.8% were female. Participants with anhedonia (32.5%) presented lower mean FA in the left uncinate fasciculus (UF) ($p = 0.005$), right temporal endings of the superior longitudinal fasciculus (SLFT) ($p = 0.04$), and in the left and right parietal endings of the superior longitudinal fasciculus (SLFP) ($p = 0.003$, and $p = 0.04$, respectively). Similar comparisons between participants with or without current depressive episodes and between participants with or without inner tension according to the MADRS did not show significant differences, specificity of the findings for anhedonia.

Conclusions: Lower FA in the left UF and SLF are potential neuroimaging markers of anhedonia in individuals with BD and high-risk for BD.

Keywords: Schizophrenia and Bipolar Disorders, Neuroimaging Biomarkers, Anhedonia

Disclosure: Nothing to disclose.

P804. Gambling Disorder is Associated With Reduced Functional Connectivity in a Frontoparietal Network During Stroop Performance

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Background: Impairments in cognitive control have been implicated in gambling disorder (GD) and cocaine use disorder (CUD), although neural correlates of cognitive control have not been simultaneously examined across the disorders. Decreased resting-state functional connectivity within a frontoparietal executive control network has been observed in individuals with a behavioral addiction (internet gaming disorder) and linked to poorer Stroop performance. To investigate further in behavioral and substance addictions, we examined frontoparietal connectivity during fMRI Stroop task performance in 3 groups (GD, CUD and healthy control (HC)). We hypothesized that relative to the HC group, the GD and CUD groups would show decreased functional connectivity within the frontoparietal network and that, transdiagnostically, functional connectivity within the frontoparietal network would relate to Stroop performance.

Methods: Event-related fMRI color-word Stroop data were collected in 78 participants (40 men, 38 women; ages 19-57 years; 39 Black and 39 White) who met criteria for GD ($N = 26$) or CUD ($N = 27$) or neither disorder ($N = 25$ HC). Functional connectivity was assessed in a frontoparietal network based on the Shen atlas and that included 8 brain parcellation nodes including the left/right temporal, left/right frontal, left/right parietal, cerebellum, and left Brodmann area 6 regions. ANCOVAs were conducted controlling for age, gender, and race to examine between-group differences in functional connectivity. ANOVAs followed by Tukey's multiple comparisons tests were performed to determine task performance Stroop effect differences between groups (GD, CUD, and HC). Linear regression models were used to assess correlations between Stroop effect and functional connectivity across groups.

Results: Relative to HC subjects, GD subjects demonstrated reduced overall functional connectivity in the frontoparietal network ($p < 0.05$), with reduced left frontal to right temporal functional connectivity also observed ($p < 0.05$). Between the cerebellum and right temporal cortex, GD participants displayed reduced functional connectivity relative to CUD participants ($p < 0.05$). Task performance (Stroop effect) did not significantly differ between groups ($F = 1.43$; n.s.). Additionally, there was no correlation between Stroop effect and total frontoparietal, left frontal to right temporal, or cerebellum to right temporal functional connectivity for any diagnostic group ($R2 = 0.00\text{-}0.02$).

Conclusions: GD participants show relatively reduced frontoparietal connectivity during fMRI Stroop performance in the absence of differences in task performance. The extent to which individuals with GD utilize alternate brain pathways to exert cognitive control warrants additional investigation, as does the extent to which strengthening frontoparietal functional connectivity in GD may help advance treatment development.

Funding: This work was supported by NIH grant(s): T32 DA022975; R01DA019039; R01DA039136.

Keywords: Gambling Disorder, Functional MRI (fMRI), Transdiagnostic, Cocaine Use Disorder, Cognitive Control Network

Disclosure: Nothing to disclose.

P805. Safety and Pharmacokinetics of Varying Doses of Cannabidiol (CBD) and CBD/Tetrahydrocannabinol (THC) Combination Oral Tablet Formulation in Recreational Users: A Randomized, Double-Blind, Cross-Over Design Pilot Study

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Background: Medical marijuana use has increased with more states legalizing marijuana to treat various medical and neurological disorders, including chronic pain-related illnesses, Post-traumatic Stress Disorder (PTSD) as well as epilepsy, multiple sclerosis, and cancer. Recent data show increased use of Cannabidiol (CBD)-derived products to treat a myriad of diseases and conditions due to its non-psychoactive nature. However, there is heterogeneity in local and regionally produced cannabis composition, dose, route, and overall drug formulations, and more importantly, significant ambiguity regarding the specific pharmacokinetics and pharmacodynamics as well as information on specific therapeutic doses for specific conditions. In this Phase I, randomized, double-blind, cross-over study we assessed the safety and PK/PD profile of acute 5 single doses of natural oral tablets of two doses of cannabidiol (CBD) (40 mg, 100 mg), and three CBD/delta-9-tetrahydrocannabinol (THC) combination doses (CBD40mg/THC10mg, CBD40mg /THC20mg and CBD100mg/THC30 mg) and matching placebo (PBO) in six separate sessions, conducted one week apart in healthy adult men and women who are current recreational cannabis users, with a focus on pain and anxiety symptoms and alteration in stress responses.

Methods: Eight subjects (5 male, 3 female; mean age 28.2 years) were randomized to receive a single dose of 40 mg CBD alone (CBD40), 100 mg CBD alone (CBD100), or combination 40 mg CBD/10 mg THC (CBD40/THC10), 40 mg CBD/20mg THC (CBD40/THC20), and 100 CBD/ 30 mg THC (CBD100/THC30), or placebo on separate sessions one week apart over six weeks. Repeated serum samples were collected and analyzed by liquid chromatography/mass spectrometry (LC-MS/MS) for THC and CBD. Linear Mixed Effect (LME) models were utilized to assess repeated Within Subject Dose effects across repeated sampling over time, as well as peak (Tmax) levels.

Results: A significant main effect of Dose was observed for THC ($F = 6.02$; $p = <.0001$) and for CBD ($F = 4.76$; $p = .0004$) rises relative to baseline over time. THC serum concentration were significantly increased relative to baseline compared to placebo for CBD40/THC20 ($p = .01$) and CBD100/THC30 ($p = .0001$) but not at the CBD40/THC10 ($p = .14$) dose. Statistically significant increases were also observed at CBD40/THC20 ($p = .01$) and CBD100/THC30 ($p = .0001$) compared to CBD 40 mg alone dose. Compared to CBD100 alone serum THC was increased at CBD40/THC20 ($p = .03$) and for CBD100/THC30 ($p = .0001$). Also, the

CBD100/THC30 dose showed an increase in serum THC compared to CBD40/THC10 ($p = .01$) and but trended for CBD40/THC20 ($p = .08$) respectively. CBD serum levels were significantly higher compared to placebo at CBD100 alone ($p = .001$) and at CBD100/THC30 ($p = .0001$). Higher serum CBD concentrations were seen at CBD100 ($p = .01$) and CBD100/THC30 ($p = .002$) compared to CBD40 alone. Lastly, higher CBD serum levels was observed at 100mg/30mg ($p = .02$) compared to 40mg/20mg. There was no significant effect observed for CBD40 alone compared to CBD40/THC10 for serum THC ($p = .11$) or serum CBD ($p = .12$). Peak serum THC concentrations for each dose were as followed, placebo (.228ng/mL; $p = .73$), CBD 40mg (.11ng/mL; $p = .87$), CBD 100mg (.49ng/mL; $p = .46$), CBD40/THC10 (1.52ng/mL; $p = .03$) CBD40/THC20 (2.42ng/mL; $p = .0006$), and CBD100/THC30 (3.97ng/mL; $p = <.0001$). CBD peak serum concentration for each dose were as followed, placebo (.90ng/mL; $p = .56$), CBD 40mg (2.55ng/mL; $p = .11$), CBD 100mg (7.07ng/mL; $p = .0001$), 40mg/10mg (5.31ng/mL; $p = .002$) 40mg/20mg (4.04ng/mL; $p = .01$), and 100mg/30mg (8.0ng/mL; $p = <.0001$). All doses were well-tolerated, and no serious adverse events were reported.

Conclusions: The 40mg CBD /20mg THC and 100mg CBD/30mg THC doses elicited the greatest serum levels for THC, while the 100mg CBD alone and 100mg CBD/30mg THC doses produced the highest CBD response. Implications for pain and anxiety symptoms and stress responses will also be discussed.

Keywords: delta9-tetrahydrocannabinol, Cannabidiol, Pharmacokinetics, Pain Therapeutics

Disclosure: CT Pharma: Grant (Self)

P807. Social Place Representation in the Human Hippocampus

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Background: Humans' ability to navigate social situations is akin to physical navigation. This capacity may depend upon social mapping: the hippocampus organizing information about social relationships into a multidimensional map, like that of physical space. In this framework, other people occupy social places based on their locations along abstract social dimensions of power and affiliation. While other work has suggested the hippocampus represents social information that depends on a place representation, such as distance, no work to date has shown that it represents abstract social place.

Methods: To test for such a brain representation, functional magnetic resonance imaging (fMRI) was collected in two independent samples of healthy individuals ($n = 27$, 12 female and $n = 16$, 6 female) while they completed a "choose-your-own-adventure" game involving interactions with fictional characters. Participants' relationships (i.e., series of decisions) with each character were modeled as sequences of locations in an abstract two-dimensional social space of power and affiliation, which were then compared to the brain using multivariate pattern analyses.

Results: Hippocampal fMRI patterns consistent with a social place representation were observed in a region-of-interest (ROI) representational similarity regression in both the left and right hippocampus for both samples (1 sided *t*-tests Ps < .01, 1 sample Hedges' g = [0.55-1.02]). A whole-brain searchlight corroborated the hippocampus finding with bilateral anterior clusters (mean Z-scores = 3.31, 3.07), which further overlapped with results from an automated meta-analysis of 189 studies reporting "place" effects. A decoding probability analysis in the right hippocampus also supported these results: the 2D place distances between characters related to decoding probabilities assigned to them (1 sided *t*-tests Ps < .05, Hedges' g = 0.41, 0.77). These effects were present in both samples, and were not explained by other measures of task behavior or other kinds of categorical or continuous task-based social information (e.g., character identity or familiarity), demographic variables (i.e., age and sex) or other 1D and 2D control models.

Conclusions: Hippocampal fMRI signals consistent with a social place representation were observed in a representational similarity regression with both region-of-interest (ROI) and whole-brain searchlight approaches, as well as an ROI-based decoding probability analysis. Our findings are the first to show that other people's social places in an abstract, multidimensional social space are represented in the human hippocampus – similarly to how physical places are represented. Future work will ask how social place representations are combined into maps and used for tracking behavior – so-called "social navigation" – and how alterations in these processes may be implicated in disorders that feature both hippocampal and social dysfunction.

Keywords: Hippocampus, Computational Neuroscience, Social Cognition

Disclosure: Nothing to disclose.

P808. Preventing Adult Sociability Deficits by Adolescent Modulation of Prefrontal Parvalbumin Interneurons Following Juvenile Social Isolation in Mice

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Background: Experience that occurs during sensitive periods can permanently alter the brain and behavior, leading to deficits that can be difficult to correct once they are instantiated. Recent research has made huge strides in understanding timing and mechanisms for these sensitive windows for social experience, however, leveraging these windows to achieve permanent rescue of deficits after experiences have already taken place has not been widely studied. Uncovering novel windows for intervention has the potential to provide a vast improvement to treatment strategies in psychiatric disorders. Juvenile social isolation (jSI) during a 2-week sensitive window immediately following weaning is known to induce sociability deficits in adult mice. Here we examine the timing of deviations from a normal developmental trajectory in dorsal-medial prefrontal cortex parvalbumin positive interneurons (dmPFC-PVIs), a circuit previously shown to be impacted by jSI.

Methods: To examine the mechanistic underpinnings of jSI-induced changes of dmPFC-PVIs, we sorted fluorescently labeled PV nuclei (Fluorescence Activated Nuclei Sorting, FANS) from mouse frontal cortex for transcriptomic analyses of both juvenile (immediately following isolation, p35) and adult (> p60) male mice (p35 $n = 2$ jSI, $n = 3$ group housed control (GH); p60 $n = 3$ jSI $n = 3$ GH, Significance cutoff for differential expression: $p = 0.05$). Only male mice were used as female jSI mice did not show sociability deficits. To modulate activity of dmPFC-PVIs through GPCR-mediated signaling, male mice were injected with either Excitatory Designer Receptors Activated by Designer Drugs (eDREADD) or mCherry at p14, isolated for 2 weeks from p21-35 and given CNO, the activating ligand of the Gq-coupled, modified muscarinic receptor, in their drinking water for 2 weeks during a late adolescent period (p35-49). After 2 weeks of eDREADD-mediated dmPFC-PVI activation, mice were returned to regular drinking water for 2 weeks followed by social behavior testing (3 chamber sociability test), open field, elevated plus maze, or light dark box in adulthood (>p60) ($n = 9$ eDREADD, 10 mCherry mice).

Results: dmPFC-PVIs show a delayed response to the effects of jSI, with transcriptional alterations in G-Protein Coupled Receptor (GPCR) signaling emerging during the month-long re-housing period, and not during the social isolation itself, mirroring the delayed emergence of electrophysiological deficits of dmPFC-PVIs (Ingenuity Pathway Analysis > -log10(pvalue) of 2). Since jSI mice show changes in the RNA of several GPCR related canonical pathways, while also showing largely normal transcriptome profiles immediately following isolation at p35, we hypothesized that the effects of isolation may be largely taking place following isolation, due to failed maturational increases in neuronal activity of dmPFC-PVIs following re-housing. We thus employed a GPCR-based chemogenetic strategy to increase activity of dmPFC-PVIs for a brief 2-week adolescent period (p35-49) at the start of re-housing. We found that this adolescent intervention leads to a long-term rescue of social behavior deficits (Mixed-effects ANOVA, interaction effect: $F(1,17) = 6.15$, $p = 0.024$. Bonferroni corrected post hoc tests: eDREADD social vs object: $p = 0.0016$, mCherry social vs. object: $p = 0.087$, eDREADD vs. mCherry social: $p = 0.045$). This manipulation did not alter anxiety or locomotor behaviors examined in the open field, elevated plus maze, or light dark box.

Conclusions: Our findings show that adolescent intervention by modulating dmPFC-PVIs following the post-weaning juvenile social isolation period can stably rescue social deficits. Adolescent intervention and subsequent adult rescue of the behavioral phenotype implicates a key role of the expression of GPCR genes, disrupted in adult but not adolescent jSI mice. Activity-based intervention strategies during a novel adolescent sensitive window could prevent the emergence of social behavioral deficits in adulthood even if previously exposed to juvenile social isolation.

Supported by grants from the National Institutes of Health, SFARI/Simons and BSF (US/Israel)

Keywords: Sociability, Parvalbumin Interneurons, Prevention, Medial Prefrontal Cortex, Chemogenetics

Disclosure: Nothing to disclose.

P809. Emergent Intra-Pair Sex Differences and Neural Synchrony in Pair Bonded Prairie Voles

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Background: In pair bonding animals, coordinated behavior between partners is required for the pair to accomplish shared goals, such as raising young. Despite this, experimental designs rarely assess the behavior or neural activity of both partners within a bonded pair. Thus, we lack an understanding of the interdependent behavioral and neural dynamics between partners that likely facilitate relationship success.

Methods: To identify intra-pair behavioral and neural correlates of pair bonding, we used socially monogamous prairie voles, a species in which females and males exhibit both overlapping and distinct pair bond behaviors. We tested both partners using social choice and non-choice tests at short- and long-term pairing timepoints ($n = 16$ pairs). In a separate cohort, we examined the similarity of aggregate neural Ca2+ activity in the prefrontal cortex of interacting voles detected via GCaMP-mediated fluorescence and fiber photometry ($n = 9$ pairs).

Results: Females developed a preference for their partner more rapidly than males, with preference driven by different behaviors in each sex. Further, as bonds matured, intra-pair behavioral sex differences and coordinated behavior emerged -- females consistently huddled more with their partner than males did (RM-ANOVA with paired *t*-tests, $t15 = 6.6$ $p = 3.2x10\text{-}5$), and partner huddle time became correlated between partners (Spearman's Rho $= 0.53$, $p = 0.035$). When animals were allowed to freely interact with a partner or a novel in sequential free interaction tests, pairs spent more time interacting together than either animal did with a novel (RM-ANOVA with paired *t*-tests, females: $t15 = 3.9$, $p = 0.013$; males: $t15 = 4.4$, $p = 0.0047$). Pair interaction was correlated with female, but not male, behavior (Females: Rho $= 0.60$, $p = 0.013$, Males: Rho $= 0.015$, $p = 0.96$).

When examining bulk prefrontal cortical Ca2+ signals in voles, we observed an increase in correlation of inter-individual Ca2+ activity traces during social interaction indicative of interbrain synchrony (paired *t*-test, $t8 = 2.87$, $p = 0.02$). Synchrony likewise scaled with the level of active, dyadic engagement during periods of social access (ANOVA, $F49 = 5.2$, $p = 0.003$), and correlated interbrain activity was reliably stronger with the bonded partner compared to a novel vole ($t2 = 3.07$, $p = 0.008$).

Conclusions: Together, our data indicate that as pair bonds mature, sex differences and coordinated behavior emerge, and that these intra-pair behavioral changes are likely organized and driven by the female animal. Prairie voles also exhibit interbrain synchrony during social interaction, which is stronger with a pair bonded partner than a novel vole - perhaps reflecting or contributing to the organization behavior within the pair.

Keywords: Prairie Voles, Pair Bond, Interbrain Synchrony, Sex Differences

Disclosure: Nothing to disclose.

P810. The Relationships Between ECT-Induced Electric Field, Dose, and Volume Increases in the Brain

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Background: Due to historical reasons, most experts considered the seizure as the necessary and sufficient component of the Electroconvulsive treatment (ECT) to achieve a clinical effect and electric aspects of the treatment as nuisance variables. There is new evidence however suggesting that the strength of the electric field has also biological implications, and with or without interaction with the seizure effect has a direct effect on brain structures and potential outcomes.

Recent developments in the electric field (EF) modeling provided a novel way to separate - at least partly - the electric aspects of the treatment from the induced seizure (Lee et al. 2012; Bikson et al. 2012; Huang et al. 2017; Bai et al. 2017). In a recent large-scale study with the GEMRIC (The Global ECT-MRI Research Collaboration, (Oltedal et al. 2017)) consortium we showed that ECT-induced EF strongly correlated with volume increase across almost the entire brain (Argyelan et al. 2019). While the relationships found in that study were strong on a population level ($N = 151$), they were insufficient to predict individual changes with meaningful precision.

Our current study was set out to shed light on these individual differences.

Methods: Four independent cohorts of patients ($N = 76$, 51, 15, 14, altogether 156 subjects) who underwent ECT treatment and longitudinal imaging were analyzed. Longitudinal structural neuroimaging analysis and baseline EF modeling were conducted as per our previous report (Argyelan et al. 2019 eLife). We then estimated the individual relationship between EF and volume change with Bayesian multilevel modeling where the individual levels of slopes (correlation across 84 regions of EF and volume change) were estimated from a higher level average prior. The

slopes (the strength of intra-subject relationships) then were regressed against several parameters (number of ECT treatments, the number of stimulations, frequency, pulse width, etc.) with multiple regression. The goal of this analysis was to find the parameters that can account for the strength of the relationship between EF and the volume change.

Results: We found a wide range of inter-subject variance in the slope (min: -0.19, max: 0.58). Meaning that the relationship between EF and volume change varied significantly across the subjects. However, a considerable amount of this variance was accounted for by a single metric: the number of total pulses (this is adding up all the pulses across all the treatments) ($r = 0.29$, 0.30, 0.60, 0.58. This result was highly significant across all 4 different cohorts ($p = 0.01$, 0.009, 0.02, 0.03, respectively). The treatment numbers in themselves showed correlations but this was due only to the fact that higher treatment numbers tended to deliver a higher number of pulses.

Conclusions: The effect of ECT-induced EF on volume changes in the brain is therefore similar to a hammer that hits a nail. Using this analogy, the magnitude of the hit (the magnitude of the EF) matters but how many times we hit it matters equally. We only can expect a strong relationship between volume change and EF after a couple of thousand pulses, usually at least 10-12 ECT treatments. This model significantly improves our understanding of the relationship between ECT and volume changes and provides the next necessary step toward precision medicine in ECT.

Keywords: Precision Medicine for Depression, Electroconvulsive Therapy, Longitudinal MRI, Electric Field Modeling

Disclosure: Nothing to disclose.

P811. Stability and Plasticity of Steady-State Visual-Evoked Potential Contrast-Response Functions

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Background: A repeated measure of neural activity that is stable over time when unperturbed is needed to be able to meaningfully measure neuroplastic changes, e.g. following a plasticity induction protocol with transcranial magnetic or ultrasound stimulation. We assessed the repeated-measure across-day and within-day stability of the steady-state visual-evoked potential (ssVEP), an exceptionally high signal-to-noise electrophysiological readout of neural activity in human visual cortex that is retinotopically localizable, in preparation for studies of visual cortical neuroplasticity.

Methods: We re-analyzed data from Dmochowski et al. 2015, "Maximally reliable spatial filtering of steady state visual evoked potentials", in which ssVEP contrast-sweep responses (90 trials, 22 subjects) were measured daily for 4 days using a 128-channel EGI hydrocel EEG system and custom xDIVA software. Reliable Components Analysis was used for electrode dimensionality reduction.

Results: High signal-to-noise (SNR > 1) responses were acquired from 20/22 subjects (mean 2.6, s.d. 1.8, range 0.4-8.9 above 20% contrast). The across-day coefficient of variation (CV = S.D./ Mean) of the response was 0.22 ± 0.13 (mean±s.d.) and did not significantly vary with stimulus contrast ($p = 0.57$, 1-way ANOVA). Response amplitude CV varied across subjects, from 0.09 to 0.42. Contrast-sweep ssVEP responses were fit well by the Naka-Rushton equation $R = \text{Rmax} / (1 + (\text{C50} / \text{contrast}).\text{^exponent}) + \text{baseline}$ (r-squared goodness-of-fit $= 0.24 \pm 0.18$). Naka-Rushton parameter fits were also mostly stable across days, with across-day CVs of 0.26 ± 0.2 (C50), 0.18 ± 0.14 (baseline), 0.25 ± 0.17 Rmax), and 0.27 ± 0.25 (exponent). We calculated the sample size needed to detect changes in response amplitude or contrast-response function parameters with a power of 0.8 given the measured across-day variability. This ssVEP protocol could detect a 10% change with 40 subjects, a 20% change with 12 subjects, and a 40% change with 6 subjects. Restricting the analysis to the 1/3 most reliable subjects ($n = 8$, CV $= 0.11 \pm 0.04$) the contrast-sweep ssVEP was powered to detect a 5% change with 40 subjects, a 10% change with 12 subjects, a 20% change with 5 subjects, and a 40% change with 3 subjects.

Conclusions: We conclude that steady-state visual evoked potential contrast-response functions demonstrate sufficient within-subject stability and functional retinotopic localization for robust measurement of plasticity in the human brain. We are currently working to partition the sources of response variability including brain state, learning/plasticity effects, physiological noise, and measurement inconsistencies. Future work will employ ssVEP contrast-response functions to measure and optimize the neuroplasticity induced with repetitive transcranial ultrasound and magnetic stimulation.

Keywords: Neuroplasticity, Event Related Potentials, Focused Ultrasound

Disclosure: Nothing to disclose.

P812. Neural Correlates of Social Exclusion in a Transdiagnostic Sample: A Meta-Analysis

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Background: The adverse consequences of social exclusion on mental health transcend diagnosis. While transdiagnostic approaches to psychological treatment have gained wider recognition in recent years, this perspective is less common in clinical neuroscience. Identifying neural correlates of social exclusion is essential for developing new, transdiagnostic, biological treatment targets. Findings from a recent meta-analysis of functional magnetic resonance imaging (fMRI) studies in healthy individuals suggest that brain areas involved in social cognition, mental state, theory mind and autobiographical processes are reliably active during exclusion. However, the extent to which these patterns are representative of social exclusion in clinical populations is unclear. Here, we conducted a quantitative meta-analysis of fMRI studies evaluating social exclusion in both clinical and healthy populations.

Methods: We reviewed studies of social exclusion using functional magnetic resonance imaging published from January 1, 2002 to March 19th, 2021 cataloged in the PubMed/MEDLINE and PsychINFO databases. Studies were included if they used the Cyberball Task, evaluated group-level main effects of exclusion versus inclusion, and reported whole-brain imaging results in Montreal Neurologic Institute (MNI) or Talairach coordinates. Reviews, studies not in English, and those with only passive exclusion conditions were excluded from analyses. Coordinates from the final sample were extracted and submitted to Activation Likelihood Estimation (ALE) meta-analysis using the GingerALE 3.0.2. BrainMap application. ALE was conducted for two groups of studies: 1) those with a diagnosis-defined clinical population ($n = 18$), and 2) healthy controls ($n = 38$). We then performed a group-level ALE contrast analysis to identify brain regions where exclusion evokes different activation foci between populations, and regions sensitive to exclusion across populations. Meta-analytic data were dual-threshold corrected for multiple comparisons at the voxel wise $p < .005$ and cluster-level family-wise error

$p < .05$. We then applied the Neurosynth meta-analytic decoding algorithm to ALE maps to explore the elemental cognitive terms associated with significant clusters.

Results: We found that significant clusters observed only in the clinical group were located in the inferior parietal lobe (BA40; $p < 0.05$). Clusters unique to the healthy individuals were in the anterior cingulate (BA32; $p < 0.05$), inferior frontal gyrus (BA47; $p < 0.05$) and medial frontal gyrus (BA8; $p < 0.05$). Significant clusters in the mid to posterior ventral insula (BA13; $p < 0.05$) emerged in both clinical and control groups. The pattern of ALE activation in clinical populations was associated with self-referential (e.g., negative feedback, autobiographical memory,) and reward-related (e.g., gambling, incentive) terms. The overlapping ALE activation was associated with cognitive perseveration and behavioral urges (e.g., obsessive-compulsive, invasive) and sensory (visual information, taste) terms.

Conclusions: Pending replication, these preliminary findings suggest that there are both unique and overlapping activation patterns during the exclusion trials of the Cyberball task. Notably, the association of clinical populations with reward-related cognitive phenomena potentially implicate positive valence circuits in elevated response to social exclusion.

Keywords: Cyberball, Human Neuroimaging, Meta-Analysis

Disclosure: Nothing to disclose.

P813. Eliminating System Xc- Signaling Between Astrocytes and Neurons Selectively Impairs Complex Cognition

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Background: A fundamental barrier in developing CNS pharmacotherapeutics is the inability to produce highly precise changes in brain function. Neuronal transporters and receptors are often targeted despite being involved in many neural processes. As a result, there is often a need to compromise intrinsic efficacy to achieve suitable safety and tolerability. The need to balance efficacy and safety has been especially problematic in developing glutamatergic therapeutics. As a result, there are very few CNS medications that directly target the most ubiquitous and powerful excitatory signaling network in the brain. Fortunately, the signaling network for glutamate extends beyond the synapse and is comprised of transporters, receptors, and/or release mechanisms that are expressed by neurons, astrocytes, and potentially every other type of cell in the brain. The purpose of this study was to determine whether non-neuronal, non-synaptic glutamate mechanisms could be targeted to produce a highly specific, narrow change in brain function that would benefit CNS disorders. To do this, we investigated functional changes after manipulating the activity of the astrocytic glutamate release mechanism system xc- (Sxc).

Methods: Sxc activity was eliminated by mutating the gene Slc7a11 through pronuclear injection of zinc-finger nucleases into Sprague Dawley rat embryos to create a line of rats lacking Sxc (MSxc rats). To confirm a lack of Sxc activity, we verified that tissue from MSxc rats had a complete lack of xCT, which is the primary subunit of Sxc that is encoded by Slc7a11. We also verified that astrocyte cultures generated from MSxc tissue lacked cystine-evoked glutamate release. Next, we measured development (body weight), CNS regulation of metabolism, and other indicators of generalized, non-specific brain function as well as behaviors that are reliant on executive function, such as cognitive flexibility, attentional gating, and decision-making.

Results: Eliminating Sxc was not lethal and did not impair development or produce widespread changes in brain function as is commonly observed when deleting other glutamate mechanisms. MSxc rats did not differ from litter-mate wildtype (WT) in growth rate ($N = 25$-28/genotype; effect of genotype $F1,51 = 0.33$, $p > 0.05$), central regulation of metabolism as reflected by absolute or diurnal changes to core body temperature ($N = 7$-8/genotype; genotype x time $F120,1560 = 0.952$, $p > 0.05$; genotype $F1,13 = 0.113$, $p > 0.05$; time $F120,1560 = 51.133$, $p < 0.001$), locomotor activity in a familiar or novel environment (Familiar: $N = 12$/genotype; genotype x day $F7,91 = 0.458$, $p > 0.05$; genotype $F1,13 = 0.331$, $p > 0.05$; day $F7,91 = 2.759$, $p < 0.05$; Novel: $N = 12$/genotype; total distance $t22 = 1.60$, $p > 0.05$; center time $t22 = 0.306$, $p > 0.05$) or simple forms of cognition such as novel object recognition ($N = 9$/genotype; $t16 = 0.216$, $p > 0.05$) or operant responding (Food-reinforced: $N = 19$-31/genotype; $t48 = 0.84$, $p > 0.05$; Cocaine-reinforced: $N = 13$/genotype; $t24 = 0.903$, $p > 0.05$). In contrast, behaviors that rely on executive function were impaired. MSxc rats displayed deficits in attentional set-shifting ($N = 6$-7/genotype; Trials to criterion: Extradimensional Shift: $t11 = 0.3.83$, $p < 0.05$ * relative to WT; Interdimensional Shift: $t11 = 2.96$, $p < 0.05$ * relative to WT; Perseverative Errors: $t11 = 3.39$, $p < 0.01$), Kamin Fear-Blocking ($N = 8$/genotype; $F2,14 = 6.94$, $p < 0.01$; * versus baseline (BL), Tukey HSD, $p < 0.05$), cocaine reinstatement ($N = 13$/genotype, $t11 = 3.26$, $p < 0.05$ * relative to WT), and the rodent gambling task ($N = 7$-9/genotype, $t14 = 2.213$, $p < 0.05$ * relative to WT).

Conclusions: Eliminating Sxc activity in rats produce deficits in behaviors reliant on executive function without impacting development or simple brain function. MSxc rats displayed enhanced drug-seeking when exposed to a subthreshold dose of cocaine, as well as impairments in behavioral flexibility (attentional set-shifting), gating of memory/attentional resources (Kamin Fear-Blocking), and decision making (rodent gambling task). A testable conclusion supported by these data is that Sxc is a glutamatergic mechanism that can be targeted to enhance cognition without generating therapeutically limiting adverse effects resulting from generalized or non-specific changes in brain function.

Keywords: Glutamate, Astrocyte, Cognition, Cocaine

Disclosure: Promentis Pharmaceuticals: Advisory Board, Founder, Honoraria, Other Financial Support (Self)

P814. The Claustrum Synaptically Connects Cortical Cognitive Network Motifs in Mouse

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Background: Spatially distant areas of cerebral cortex coordinate their activity into networks that are integral to cognitive processing. However, the neural circuit mechanisms underlying such widespread inter-areal cortical coordination – and therefore its mechanisms of dysfunction in neuropsychiatric disorders - are lacking. By virtue of its high interconnectivity with the cortex and functional connectivity with cortical networks as assessed by whole brain imaging, the claustrum is hypothesized to support this function.

Methods: As task-positive and task-negative cortical networks are initiated by frontal cortical regions, we herein utilized a channelrhodopsin circuit-assisted long-range neural circuit mapping approach to assess the strength of synaptic connectivity of 1,050 claustrum neurons from 35 unique frontal cortico-claustral-cortical connections in mice ex vivo.

Results: Cluster analysis reveals two significantly distinct large-scale network motifs: one emanating from dorsal prefrontal cortices and the other from ventromedial prefrontal cortex.

Conclusions: These data suggest a possible neural circuit mechanism for the emergence of anti-correlated cortical networks supporting cognition.

Keywords: Cognition, Circuit Optogenetics, Default Mode Network (DMN), Frontoparietal Network, Cognitive Dysfunction

Disclosure: Nothing to disclose.

P815. Chronic Nicotine Improved Reinforcement Learning in a Mouse Model Relevant to HIV Associated Neurocognitive Disorders

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Background: Despite the advent of antiretroviral therapy (ART), resulting in the movement of HIV from a fatal to a chronic disease, people living with HIV (PLH) continue to exhibit cognitive deficits, known as HIV Associated Neurological Disorders (HAND). Typical HAND symptoms include deficits in attention, motivation, reinforcement learning, and memory. In addition, despite zero virologic levels resulting from ART administration, PLH also continue to exhibit neuroinflammation, which may contribute to these cognitive deficits. Observations that PLH smoke cigarettes at higher rates (40%) than the general population (16%), with evidence that nicotine induces pro-cognitive and anti-inflammatory effects, the directional impact of long-term nicotine treatment on such behaviors in animal models relevant to HAND and neuroinflammation needs to be determined. Since the envelope glycoprotein (gp)120 drives entry of HIV into neurons and can induce inflammation, we determined the impact of chronic nicotine administration on mice over-expressing gp120 (tg) and their wildtype (WT) littermates, on motivation, reinforcement learning, and neuroinflammation.

Methods: Male and female gp120 tg and WT littermate mice ($n = 120$), were bred from heterozygous breeding pairs and trained to respond in 5-hole operant chambers. After training, mice were baseline tested in the progressive ratio breakpoint task (PRBT) to match into 3 groups (by sex and gene), to be implanted with osmotic minipumps containing vehicle or one of two doses of nicotine (14 or 40 mg/kg/day). The mice were then tested in the PRBT on days 24/25, then in the probabilistic reversal learning task (PRLT) on days 26/27, after which brains were removed for inflammatory histopathology.

Results: Baseline: No gene effect or sex*gene interaction [Fs<1, ns], was observed on breakpoint (motivation from PRBT), although a trend sex effect was observed [$F(1,118) = 3.9$, $p = 0.051$]. During nicotine: In the PRBT, a sex effect was observed [$F(1,117) = 7.2$, $p = 0.009$], revealing that male mice exhibited a higher breakpoint than female mice. No gene, nicotine, gene*nicotine, or sex*-gene*nicotine interactions (Fs<1, ns) were observed. In the PRLT, on % target responses, a sex effect [$F(1,118) = 4.8$, $p = 0.031$], was observed, though no gene effect [$F(1,118) = 2.41$, $p = 0.123$], or drug [$F(2,118) = 1.49$, $p = 0.230$], was observed. Importantly, a gene*drug interaction was observed [$F(2,188) = 4.0$, $p = 0.022$]. Post hoc analyses revealed that nicotine did not affect WT mice ([$F < 1$, ns), but did increase %target responses in gp120 mice [$F(2,58) = 4.9$, $p = 0.011$], at both 14 and 40 mg/kg/day vs. vehicle ($p = 0.008$ and 0.013 respectively). No other sex*gene interactions were observed in any other measure except shifting responses after being punished (lose-shift), in the non-target side [$F(1,118) = 3.2$, $p = 0.046$]. Nicotine increased the lose-shift ratio of gp120 tg mice when responding at the non-target [$F(2,58) = 4.08$, $p = 0.022$], at both 14 and 40 mg/kg/day vs. vehicle ($p < 0.015$ and $p = 0.026$ respectively). Histopathology: While brains continue to be processed, a trend toward a nicotine effect was observed [$F(2,21) = 3.3$, $p = 0.056$], revealing that nicotine at the highest dose reduced Iba1 levels. Although no interaction was seen with genotype, only half the data have been processed, gp120 transgenic mice exhibited higher levels of Iba1 than their WT littermates, with nicotine exerting a greater effect in these mice, consistent with tobacco-induced reduction in PET-measured neuroinflammation in humans.

Conclusions: These data demonstrate that chronic nicotine treatment improves reinforcement learning in the gp120 tg model relevant to HAND, driven by increased punish-sensitivity. Furthermore, this mechanism may relate to nicotine-induced lowering of neuroinflammation as measured by Iba1 staining. Studies are ongoing to determine whether PWH that smoke exhibit normalized reinforcement learning using the PRLT, and neuroinflammation using positron emission tomography.

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Keywords: Reinforcement Learning, HIV Associated Neurocognitive Disorder, Nicotine, Neuroinflammation, Mice

Disclosure: Nothing to disclose.

P816. Changes in Personal Space During the COVID-19 Pandemic: A Virtual Reality Study

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Background: Personal space, i.e., the physical distance that people prefer to maintain from others, is moderately influenced by multiple situational, social and psychological factors, including cultural differences and symptoms of psychiatric disorders. However, when controlling for these factors, personal space preferences remain fairly stable over time; "trait-like" personal space characteristics (which vary substantially across individuals) become established during late adolescence. Since early 2020, personal space preferences have been affected by social distancing recommendations that have been widely adopted to reduce transmission of the COVID-19 virus. These consciously adopted interpersonal distances (e.g., 6 feet in the US) are much larger than those generated by the intrinsic brain mechanisms involved in personal space regulation (which are typically 50-100 cm). However, it is unknown whether the practice of social distancing during the pandemic has influenced personal space in a pervasive manner, even in virus-free contexts. To examine this question, we compared the size of personal space and the discomfort elicited by personal space intrusions in the same individuals at two time points, before and during the COVID-19 pandemic, using both conventional methods and a virtual reality (VR)-based approach.

Methods: Two versions of a well-validated, highly reliable (kappa ~.8) experimental paradigm, the Stop Distance Procedure (SDP), were used to measure personal space size at the two time points. The SDP measures the distance at which subjects first become uncomfortable (the personal space boundary) when another person approaches them (passive trials) or when the subject approaches another person (active trials). To control additional variables that could potentially influence personal space size (including concerns about risk of COVID-19 exposure during the second assessment), we also collected personal space measurements at both time points using an immersive virtual reality (VR) version of the SDP using digital simulations of humans ("avatars"). VR-based measurements of personal space to avatars have been shown to correspond closely to those measured to real humans. Responses to personal space intrusions (discomfort ratings) were also measured at different distances within (as well as outside of) personal space boundaries, to both real and virtual

humans. These measurements were first collected before the COVID-19 pandemic (September 2019 - early March 2020) in 19 subjects (47 % female, mean age = 30.6 ± 11.3 years) and were then repeated in a subset of this group ($n = 12$) after March 13th, 2020, when social distancing measures and other restrictions related to the COVID-19 pandemic were instituted in Boston, MA, where this study was conducted. During the second session, beliefs and experiences related to the COVID-19 pandemic and risk of COVID-19 infection (local case rates for each subject) were also measured. Lastly, prior work has shown that human "intruders" within a subject's personal space increase the discomfort of subjects at progressively closer distances, following a power law function. To test whether such intrusion-driven discomfort levels changed during the pandemic, subjects were asked to rate their discomfort in response to real and virtual humans presented at various distances (25%, 50%, 100%, 200%, 400% of each subject's personal space size), both before and during the pandemic.

Results: As expected, the size of personal space in response to real humans was highly correlated with the size of personal space measured to avatars (all $p < .001$). The SDP data also revealed that personal space size, measured with respect to both real humans and avatars, was significantly larger during (compared to before) the COVID-19 pandemic (to humans: passive trials: $t(11) = 5.732$, $p < .001$; active trials: $t(11) = 3.863$, $p = .003$; to avatars: passive trials: $t(11) = 2.918$, $p = .014$; active trials: $t(11) = 3.082$, $p = .01$). In contrast, control measurements collected at two timepoints spanning a similar length of time before the COVID-19 pandemic in a prior sample showed no significant changes in personal space over time ($p = .646$). In addition, the increase in personal space size during the pandemic, in response to both humans and avatars, was significantly correlated with the perceived risk of being infected with the COVID-19 virus (all $r > .676$; all $p < .016$) but not with actual infection risk (all $p > .337$) or pandemic-related anxiety and distress (all $p > .148$). Lastly, during the pandemic, discomfort during personal space intrusions increased significantly compared to pre-pandemic levels in response to both humans and avatars, and both response functions followed a power law (humans: $p < 0.0001$, KS statistic 0.53; avatars: $p < 0.0001$; KS statistic 0.21).

Conclusions: The COVID-19 pandemic has led to a consistent enlargement of personal space and correspondingly greater discomfort within personal space. Crucially, this personal space enlargement was evident even when there was no infection risk, in a virtual setting. These findings suggest that the social distancing practiced during the COVID-19 pandemic altered the function of the sensorimotor circuits of the human brain involved in regulating personal space and maintaining the safety of the body. These data also suggest that for some individuals, active interventions may be needed to address the persistent discomfort with the physical proximity of others that this altered behavior reflects.

Keywords: the COVID-19 Pandemic, Personal Space, Anxiety, Parietal Cortex, Virtual Reality

Disclosure: Nothing to disclose.

P817. A Multi-Modal Assessment of Self-Knowledge in Adolescents With Non-Suicidal Self-Injury

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Background: Adolescence is a critical period for brain development, formation of identity, and consolidation of self-understanding. It is also notable for the onset of non-suicidal self-injury (NSSI), a risk factor for future suicide attempts. Multimodal assessment of brain systems implicated in self-processing in adolescents with NSSI may shed light on mechanisms underlying NSSI and suicide risk and advance the Research Domains Criteria (RDoC) initiative.

Methods: We utilized a multimethod (self-report, behavior, brain) approach to study brain systems underlying self-knowledge in 168 adolescents assigned female at birth ages 12-16 years with and without a history of NSSI. NSSI frequency and severity was evaluated using the Self-Injurious Thoughts and Behaviors Interview. Adolescents completed the Self Perception Profile for Adolescents and underwent brain imaging including a structural scan, a resting-state fMRI scan, and the Self Versus Change (SVC) task. In SVC, adolescents viewed positively and negatively valenced adjectives and evaluated whether each adjective described themselves or whether those characteristics could be changed in a person as the control condition. The relationship between global self-worth as measured by the SPPA was assessed using Pearson Correlation. For SVC fMRI analysis, we conducted a whole-brain linear regression examining the relationship between NSSI Lifetime Episodes and brain activation at each voxel for the Self versus Change and the Negative versus Positive contrasts (z threshold 3.1, cluster correction $p < 0.05$). For SVC behavioral analysis, we conducted a linear regression to evaluate the relationship between NSSI severity and frequency of and reaction times towards negative and positive self-evaluations. Remaining analyses were conducted on an a-priori defined Self network, which consist of brain regions (using the Glasser 2016) implicated in self-referential processing: anterior cingulate cortex, orbitofrontal cortex, posterior cingulate cortex, precuneus. For the resting-state data, we calculated a Self Network Connectivity measure by averaging resting-state functional connectivity (RSFC) values among brain regions within the Self network. We similarly calculated a Self Network Thickness measure by averaging the cortical thickness values of Self Network regions. We then used Pearson correlations to examine the relationship between NSSI severity and Self Network RSFC and Self Network Thickness.

Results: The number of participants with usable data per measure was as follows: SITBI $n = 158$; SPPA, $n = 96$; Structural MRI, $n = 130$; SVC task behavioral data, $n = 132$; SVC fMRI data, $n = 122$, resting-state fMRI, $n = 124$. Lifetime NSSI episodes correlated with lower self-reported global self-worth, $r(94) = -.47$, $p < .001$. Behaviorally, higher lifetime NSSI episodes correlated with more frequent negative evaluations ($r = 0.421$, $p < 0.001$), less frequent positive evaluations ($r = -.421$, $p < 0.001$), longer reaction times for positive evaluations ($r = 0.242$, $p = 0.006$) and shorter reaction times to negative evaluations ($r = 0.242$, $p = 0.006$). Whole-brain analysis of SVC fMRI task data revealed significant clusters in the Negative > Positive valence contrast located in posterior Self network regions (posterior cingulate, precuneus). Self network RSFC correlated inversely with NSSI severity $r(123) = -.23$, $p = .01$. NSSI severity was not significantly associated with Self Network cortical thickness.

Conclusions: Across multiple levels of analysis, we found that NSSI severity in adolescents is associated with disruptions in different aspects of self-processing. Those with higher NSSI severity viewed themselves more negatively as assessed via both self-report and a behavioral task. Brain correlates of NSSI severity included lower RSFC within the Self network paired with lower deactivation of Self Network regions (which overlap with the default mode network, a set of brain regions known to deactivate during tasks) in response to negatively valenced words (regardless of self or change condition). Although the cross-sectional design can not support causality inference, one possible interpretation of this set of results is that impaired synchrony within the Self Network may disrupt efficiency of the system, leading to persistent activation (or less deactivation) of Self Network regions,

where the normative response is deactivation, especially in negatively valenced situations. Since this is happening in regions known to underlie self-referential thinking, this dysfunction could underlie the tendency to persistently and inflexibly view oneself in a negative light. These findings suggest potential neurobiological targets for treatment for adolescents with NSSI, with a focus on enhancing neuroplasticity to allow increased efficiency of the Self Network in tandem with greater flexibility (more frequently positive) of self-views.

Keywords: Adolescent, Self-Injury, Self-Referential, RDoC

Disclosure: Nothing to disclose.

P818. Repeated Microdoses of LSD in Healthy Adults: A Placebo-Controlled Study

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Background: The resurgence of interest in therapeutic use of psychedelic drugs has raised interest in microdosing of lysergic acid diethylamide (LSD). There are numerous media reports that doses of LSD about 1/10th of typical tripping doses, taken at 3–4-day intervals, improve mood and cognition. However, these effects have been difficult to demonstrate under placebo-controlled conditions.

Methods: We conducted a double-blind controlled study examining effects of repeated doses of LSD (13 or 26 µg) or placebo, in healthy adults. Participants were randomly assigned to receive placebo ($N = 18$), 13 µg LSD ($N = 19$), or 26 µg LSD($N = 19$) during four 5-hour laboratory sessions, separated by 3-4 days. They also attended by a drug-free followup session 3-4 days after their last session. We assessed mood and subjective states, cognitive and emotional function, and cardiovascular function during the drug sessions and at followup.

Results: LSD produced small increases in ratings of 'feeling a drug effect', including both stimulant-like and LSD-like effects, especially during the first administration of the drug. The drug produced few effects on emotion or psychomotor tasks or measures of heart rate or pressure, and no residual effects were detected on the drug-free followup session.

Conclusions: We conclude that repeated low doses of LSD are safe and that the repeated dosing design is feasible for future studies, but under these conditions the drug produces negligible changes in mood or cognition. Future studies are needed with larger sample sizes, longer duration of dosing, additional measures of cognitive or affective function, and with participants who exhibit clinical symptoms of anxiety and depression.

Keywords: Psychedelics, LSD Microdosing, Healthy Individuals

Disclosures: PharmAla: Board Member, (Self)
Springer-Nature: Honoraria (Self)
Awakn, Gilgamesh, Schedule I Therapeutics: Advisory Board (Self)

P819. Associations Between Endogenous Opioid System Functioning and Polygenic Risk for Major Depression and Opioid Use Disorder

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Background: Common genetic factors may contribute to the high rate at which c major depressive disorder (MDD) and opioid use disorder (OUD) co-occur. However, the mechanism by which genetic factors influence the development of these disorders is not well understood. Based on substantial evidence that variation in endogenous opioid system functioning contributes to the risk of both disorders, we explored the association of polygenic risk for MDD and OUD with endogenous opioid system functioning.

Methods: 144 participants (88 females, 56 males aged 18-58 years) underwent [C11]-carfentanil positron emission tomography (PET) imaging during performance of a pain-stress challenge, which has been shown to stimulate endogenous opioid system activity; and provided DNA samples for genotyping. We explored endogenous opioid neurotransmission within five regions of interest: subgenual anterior cingulate, ventral pallidum, amygdala, nucleus accumbens, and dorsal striatum. Polygenic risk scores (PRS) for MDD and OUD were calculated using summary statistics from genome-wide association studies of depression (Howard et al. 2019) and opioid use disorder (Zhou et al. 2020) and correlated with mu-opioid activation based on the ratio of receptor binding potential measured before and during the pain-stress challenge.

Results: Both MDD and OUD PRS taken individually were significantly associated with endogenous opioid system activation across the entire sample, with the MDD PRS accounting for 14.5% and the OUD PRS 5.3% of the variance in stress-induced opioid system activity. The associations between MDD and OUD PRS and stress-induced mu-opioid activity were most robust within the female subsample, accounting for up to 18.5% and 16.6%, respectively, of the variance in opioid responses to the pain-stress challenge. Among males, there were no significant associations between MDD or OUD PRS and mu-opioid activity. When modeled jointly, the MDD and OUD PRS accounted for up to 3.2% of the variance in opioidergic activation across the entire sample and up to 10.1% of the variance in females.

Conclusions: Both MDD and OUD PRS accounted for substantial variance in stress-induced changes in mu-opioid mediated neurotransmission, with the associations principally seen among female participants. These findings are consistent with the sex-specific risk factors and clinical manifestations of MDD and OUD. Further, the findings suggest that the overlap of effects on endogenous opioid system activity associated with MDD and OUD PRS may help to explain the high co-occurrence of MDD and OUD.

Keywords: Mu-Opioid Receptors, Positron Emission Tomography, Polygenic Risk Score, Opioid Use Disorder, Major Depressive Disorder

Disclosure: Nothing to disclose.

P820. Investigation of Progesterone and Emotion Regulation Neural Correlates in Midlife Women

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Background: Emotion regulation, or the ability to dynamically respond to affectively valenced stimuli in the context of goal-oriented behavior, is disrupted in multiple mental disorders including mood disorders. Connectivity within and between the default mode network (DMN), salience network (SN) and central executive network (CEN) has been proposed to underlie emotion regulation and abnormal neurometabolic levels in core nodes of these networks have been found in multiple psychiatric disorders characterized by difficulty with emotion regulation. Reproductive hormones may impact on emotion regulation function; allopregnanolone (a metabolite of progesterone) has been shown to correlate with DMN network function and its exogenous

administration to improve depressive symptoms. The goal of this study was to examine the relationship of progesterone with behavioral and neurocircuitry measures of emotion regulation, along with markers of neuronal function in core emotion regulation network nodes, in midlife women.

Methods: 14 women ages 35-58 were recruited at Brigham and Women's Hospital, including both healthy participants and participants with a mood disorder in order to enrich the sample for a range of emotion regulation function. An affective go/no-go task with positive, neutral, and negative valenced stimuli was administered and attentional bias toward negative stimuli was measured by comparing number of commission and omission errors and reaction time to negative stimuli against responses to positive and neutral stimuli. Resting state fMRI (rfMRI) was obtained at 3 Tesla and functional connectivity within and between nodes of the DMN, SN and CEN was measured using the CONN Toolbox. MR spectroscopy, using STEAM at 7 Tesla, was used to measure markers of neuronal function (N-acetylaspartate [NAA], glutamate [Glu]) in core nodes of the DMN (medial prefrontal cortex [mPFC]), SN (anterior cingulate cortex [ACC]) and CEN (dorsolateral prefrontal cortex [dlPFC]). LCModel was used for neurometabolite quantification. Spectroscopy results were filtered to exclude results with a CRLB > 20. We conducted correlation analyses of ln(progesterone) with each emotion regulation measure.

Results: We observed higher progesterone levels in women correlated with reduced attentional bias towards negative stimuli at a trend level (reaction time $r = 0.45$, $p = 0.16$). In rfMRI analyses, we found higher progesterone levels were associated with lower functional connectivity within the DMN (mPFC-PCC; $r = 0.78$, $p = 0.008$). Based on this finding, we carried forward the mPFC as a region of interest for analysis of neurometabolites. We observed progesterone levels correlated with NAA levels in the mPFC ($r = 0.65$, $p = 0.029$). Progesterone did not significantly correlate with Glu levels.

Conclusions: Our results indicate that higher progesterone levels are related to neural markers consistent with better emotion regulation function in midlife women. This line of research will advance understanding of underlying neurobiological mechanisms of abnormal emotion regulation in women. These insights will help identify novel treatment intervention strategies for psychiatric disorders involving deficits in emotion regulation, such as depression.

Keywords: Emotion Regulation, Progesterone, Neural Circuitry, Neurometabolism, Women's Mental Health

Disclosure: Nothing to disclose.

P821. Differential Impact of Motivational Deficits Across the Psychosis Spectrum

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Background: Studies examining negative symptoms in patients with schizophrenia spectrum disorders (SSDs) have shifted away from viewing these symptoms as a unitary construct and towards a dimensional approach that distinguishes between motivational and expressivity deficits. Work examining these dimensions in patients with SSDs have generally demonstrated that motivational deficits are the primary driver of poor outcomes, with little additional contribution from expressivity deficits. Although motivational deficits are thought to be driven by aberrant reward processing and reward circuitry in patients with SSDs, such processes may also be affected by other illness-related factors and/or antipsychotic medication. To date, little work has been done to examine the impact of motivational deficits on reward-related behavior and circuitry in healthy, unmedicated adults.

Methods: Study 1 evaluated the impact of diagnostic status (SSDs vs. Healthy controls (HCs)) and high vs. low deficits in motivation (dichotomized within each group using scores derived from the Clinical Assessment Interview for Negative Symptoms (CAINS)) on responding during the Effort Expenditures for Rewards Task (EEfRT) using a univariate ANOVA controlling for age, sex and expressivity deficits. In study 2, SSDs ($N = 58$) and HCs ($N = 90$) underwent a resting-state fMRI exam on a GE Signa HDx 3T scanner and we used a fine grained parcellation of the orbitofrontal cortex (OFC), a core component of reward circuitry, to compared OFC resting state functional connectivity (RSFC) across the whole brain in both SSDs and HCs dichotomized by high vs. low motivational deficits.

Results: Study 1 found a significant difference between SSDs ($N = 38$) and HCs ($N = 33$) on the proportion of high effort task selections ($F(1,64) = 4.40$, $p = .04$) with SSDs showing a lower proportion of high effort task selections than HCs. Surprisingly, although we did not find a main effect of motivational deficits, we did find a significant Diagnosis x Motivation interaction ($F(1,64) = 4.95$, $p = .03$). Specifically, we found that while SSDs with higher motivational deficits had a lower proportion of high effort task selection relative to SSDs with less motivational deficits, the HCs evidenced an opposite pattern in which those with higher motivational deficits had a higher proportion of high effort task selection relative to HCs with less motivational deficits. In Study 2, we found that in the HCs, those with higher motivational deficits ($N = 42$) evidenced significantly greater RSFC between the Right medial OFC and a region spanning the Right supramarginal gyrus and superior parietal lobe (cluster threshold: $Z > 3.29$, FWE corrected) than those with less motivational deficits ($N = 48$). No significant differences in OFC RSFC were observed in SSDs that were high ($N = 26$) vs. low ($N = 32$) in motivational deficits.

Conclusions: Although these results provide support for the involvement of reward-related processes and circuitry in motivational deficits, they suggest that the relationship may be dependent on diagnostic status. It is unknown, however, whether these differences reflect a progression in impairment across the spectrum of motivational deficits or a mechanism of resilience associated with the expression of these deficits in those not diagnosed with a psychiatric disorder.

Keywords: Avolition, Negative Symptoms, Orbitofrontal Cortex

Disclosure: Nothing to disclose.

P822. Head Motion During Functional MRI is Higher in Children With Externalizing Disorders and Lower in Children With Internalizing Disorders

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Background: Head motion during magnetic resonance imaging (MRI) acquisition is ubiquitous and can introduce large, non-linear artifacts into resting-state functional connectivity. Head motion can be particularly problematic in pediatric and mental health research, leading to false conclusions when confounded by effects of interest, e.g., age, or psychiatric diagnosis or severity. Though head motion has been shown to be increased in children and adults with certain clinical disorders (e.g. attention deficit hyperactivity disorder [ADHD]), no study has systematically documented head motion in youth across a wide range of psychiatric diagnoses and across functional MRI (fMRI) tasks and rest. This study used data from a large community sample of children to examine differences in in-scanner head motion across different clinical diagnoses and multiple fMRI sequences.

Methods: Clinical diagnoses and motion quality assurance data for all functional MRI series were used from the Healthy Brain Network (HBN) study, data releases 1-7 ($N = 1,235$, ages = 5-21 years, both sexes). In-scanner head motion was quantified as the percentage of frames flagged as outliers based on a framewise displacement cutoff of 0.2 mm. Diagnoses were divided into externalizing (ADHD and disruptive), internalizing (anxiety, depressive, obsessive, and trauma and stressor related), and neurodevelopmental (ASD, specific learning, language, and motor) disorders. The relationship between diagnosis and head motion was modeled in two ways. First, a linear mixed effects model was fitted predicting head motion with fixed effects for the participant's primary diagnosis as a factor, functional sequence (e.g., rest, task, movie), age, and sex, and a random effect for participant. Second, a linear mixed effects model was fitted predicting head motion using binary flags for whether the participant was given a diagnosis in each given category, regardless of primary diagnosis; the other fixed and random effects were the same as before.

Results: Primary diagnosis was a significant predictor of head motion, with children with externalizing disorders displaying a higher percentage of high-motion frames (beta = 0.0069, $t = 2.54$, $p = .011$). Functional sequence was also a significant predictor, with less motion during task compared to rest (beta = -0.0042, $t = -4.09$, $p < 10\text{-}4$). Female sex was associated with less head motion (beta = -0.0047, $t = -2.34$, $p = .019$). As expected, older children displayed less head motion (beta = -0.0045, $t = -16.32$, $p < 10\text{-}16$). No significant diagnosis-by-functional sequence interactions emerged.

Data were then modeled accounting for all of each child's diagnoses. Children in the HBN sample exhibited 2 diagnoses on average (range 0 - 10). Head motion was significantly lower in children with internalizing disorders (beta = -0.0057, $t = -2.81$, $p = .005$) and higher in children with externalizing disorders (beta = 0.0085, $t = 4.30$, $p < 10\text{-}4$). Consistent with the primary diagnosis model, task data showed less motion than rest (beta = -0.0042, $t = -4.07$, $p < 10\text{-}4$) and older children displayed less head motion (beta = -0.0044, $t = -16.07$, $p < 10\text{-}16$). No significant sex effects or diagnosis-by-functional sequence interactions were detected.

Conclusions: In this large community sample, large head movements during fMRI scanning were more frequent in children with externalizing disorders, in line with prior research. Children with internalizing disorders showed significantly lower head motion. These results held even when the disorder was not necessarily a child's primary diagnosis, underscoring the importance of carefully controlling for comorbid illnesses in mental health research. Diagnosis-related differences in head motion persisted across different functional sequences, including task, movie, and rest. Across diagnostic categories, however, less head motion was detected during task than rest. Thus, for hard-to-image groups, such as very young children, introducing a task rather than relying on rest may yield more usable data. This study builds upon prior work by examining a wider range of diagnoses and functional sequence types in a community sample, rather than in a case-control design focused on a specific disorder. Furthermore, this study uses a readily interpretable measurement of head motion (percentage of outliers). These findings have potential implications for study design and sample size estimates for future research that crosses diagnostic categories. Furthermore, they underscore the importance of rigorously controlling for head motion in fMRI studies and considering potential confounding of findings by diagnosis. Future work should examine replicability of these results in the remaining portion of the HBN dataset (data collection ongoing), attempt to quantify how much any identified group differences across diagnostic categories may be due to head motion, and explore which specific diagnoses (e.g. subtypes of ADHD) are associated with the most differences in motion.

Keywords: Functional MRI (fMRI), Externalizing Disorders, Internalizing Disorders

Disclosure: Nothing to disclose.

P823. Distinct Roles of Dopamine D1 and D2 Receptor-Expressing Neurons in the Nucleus Accumbens for a Strategy Dependent Decision Making

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Background: To optimize decision making, animals need to execute not only a strategy to choose a good option but sometimes also one to avoid a bad option. A psychological study indicates that positive and negative information is processed in a different manner in the brain. The nucleus accumbens (NAc) contains two different types of neurons, dopamine D1 and D2 receptor-expressing neurons which are implicated in reward-based decision making and aversive learning. However, little is known about the neural mechanisms by which D1 or D2 receptor-expressing neurons in the NAc contribute to the execution of the strategy to choose a good option or one to avoid a bad option under decision making. Here, we have developed two novel visual discrimination tasks for mice to assess the strategy to choose a good option and one to avoid a bad option.

Methods: In this study, we have developed a pair of visual discrimination learning tasks to assess the strategy dependent decision making in mice. While in the visual discrimination-based cue-guided attendance learning (VD-Attend), mice need to acquire the strategy to choose a good option. In contrast, in the visual discrimination-based cue-guided avoidance learning (VD-Avoid), mice need to acquire the strategy to avoid a bad option. To establish the contribution of these two subpopulations in these different tasks, we chemogenetically manipulated the neuronal activities of D1-MSN or D2-MSN in the NAc while male heterozygous D1-cre ($n = 11$) or D2-cre ($n = 11$) mice performing the VD-Attend or VD-Avoid tasks. Moreover, we performed optogenetic manipulation to test whether the NAc contributes to the execution of the strategy in a timing-specific manner ($n = 19$). We further performed in vivo calcium imaging with a miniature microscope to investigate the underlying neural representations of D1-MSN and D2-MSN (460 neurons).

Results: We showed that chemogenetic suppression of D2-MSN in the NAc selectively decreased the performance of the VD-Avoid but not VD-Attend task. We also found that chemogenetic suppression of D1-MSN in the NAc decreased the number of earned rewards and increased reward latency, both of which indicated decreased motivation. On the other hand, chemogenetic suppression of D2-MSN in the NAc did not affect the motivation indices. Our optogenetic experiments showed that optogenetic inhibition of D2-MSN in the NAc during Outcome period selectively decreased the performance but not in ITI or Cue period stimulation trials. Furthermore, a decrease in performance by optogenetic inhibition during Outcome period was observed on the next trial after making an error choice on the previous trial but not after making a correct choice. In addition, our calcium imaging data showed that the proportion of responsive cells statistically increased in Outcome period compared to ITI and Cue period. Moreover, the proportion of Error activation Type neurons in D2-MSN was more dominant than D1-MSN.

Conclusions: Our data indicate that D1-MSN and D2-MSN in the NAc contribute to the choice behavior differently; D1-MSN contributes to both the VD-Attend and VD-Avoid tasks, and D2-MSN selectively involved in the VD-avoid task. We provide the evidence in which D2-MSN in the NAc selectively contributes to

the strategy to avoid a bad option. Moreover, activation of D2-MSN in the NAc by error choices plays an important role in acquisition of the strategy to avoid a bad option. On the other hand, the majority of D1-MSNs in the NAc was activated by correct choice. Given that inhibition of D1-MSN in the NAc decreased the performance on the next trial after making a correct choice, it could be that neuronal activation of D1-MSN in the NAc contributes to keep same strategy with confidence.

Keywords: Decision Making, Nucleus Accumbens, Dopamine, Basal Ganglia, Calcium Imaging

Disclosure: Nothing to disclose.

P824. Creative Processes Under the Influence of a Low Dose of LSD: A Placebo-Controlled, Double-Blind Trial in Healthy Volunteers

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Background: Previously scientific studies have shown the potential of psychedelics in full hallucinogenic doses to change creativity, with enhancing effects on divergent thinking specifically while under the influence of the substance. This effect might be beneficial in a therapeutic context, in patients suffering from rigid thinking patterns, like depression. Anecdotal evidence has suggested that psychedelics in small, sub-hallucinogenic doses (microdosing) might improve creativity, and some users self-medicate with it to find relief for their depressive symptoms. While scientific research with small doses of psychedelics is scarce, we aimed to quantify the cognitive effects of single small LSD doses on creative processes in healthy volunteers in a dose-finding study.

Methods: The study was conducted according to a double-blind, randomized, placebo-controlled, within-subject design with four oral LSD doses (0, 5, 10, and 20 µg, LSD base) given on four separate test days, with minimally five days of washout in between. LSD was formulated as a solution of 25 µg LSD base in 1mL ethanol (96%) which resulted in 0.2, 0.4, and 0.8 mL LSD, for 5, 10, and 20 µg LSD. Doses were administered sublingually with a 1 mL syringe. To mask the treatment condition, ethanol was added so that all syringes contained the same volume. Placebo was 1 mL ethanol only (96%).

Participants were 24 healthy males ($N = 12$) and females ($N = 12$) aged 22.75 on average (SD = 2.97). All consumed alcohol and had experience with other recreational drugs. Task-based creativity was assessed three hours post-treatment with the Alternate Uses Task (AUT) and five hours post-treatment with the Story Writing task. In the latter participants were asked to write a (non)-fictive story around two given words. To assess the level of divergent thinking or creativity of the story, two independent raters assigned a creativity score between zero to five to the stories and their scores were averaged to a final creativity score. The AUT is a measure of divergent thinking. Responses are scored afterward by two independent raters. Dependent outcome variables are Fluency, Originality, the ratio of Fluency and Originality, Flexibility, and Elaboration. Parallel versions of tasks were used on separate test days. In addition, the participant self-rated how creative s/he felt on a visual analogue scale (VAS). This was done eleven times during the test day, and daily, up to four days after treatment administration. Blood samples were taken during the test day to determine LSD concentrations.

The study was conducted according to the declaration of Helsinki and its amendments, which is the international convention governing drug studies in human volunteers (World-Medical-Association, 2013). A permit for obtaining, storing, and administering LSD was obtained from the Dutch Drug Enforcement Administration. The study was registered in the Netherlands Trial Register (NTR7102).

All data entered SPSS version 24.0, where a General Linear Model Repeated Measures (GLM RM) Analysis of Variance (ANOVA) was run on data of the AUT, the Story Writing Task, and the VAS. LSD (four levels) was included as a within-subject factor; additional within-subject factors were Time (ten levels) or Day (four levels) for the VASs. In the case of a main LSD or Time effect, Bonferroni-corrected pairwise comparisons and contrast analyses for that particular measure were conducted with the placebo or the baseline as a reference category. Partial eta squared (partial η2) is reported to demonstrate the effect magnitude, and it is based on Cohen's *f*, which defines small, medium, and large as respectively 0.10, 0.25, and 0.50, which corresponds to partial η2 values of 0.01, 0.06, and 0.14.

Results: GLM RM ANOVA did not show main effects of LSD on AUT parameters Fluency ($F3,69 = 0.65$, $p = 0.59$, partial $\eta2 = 0.03$), Elaboration ($F3,69 = 0.09$, $p = 0.99$, partial $\eta2 = 0.004$), Flexibility ($F3,69 = 2.30$, $p = 0.08$, partial $\eta2 = 0.09$), Originality ($F3,69 = 0.28$, $p = 0.84$, partial $\eta2 = 0.01$), and Ratio ($F3,66 = 0.17$, $p = 0.92$, partial $\eta2 = 0.01$). RM GLM ANOVA revealed no main LSD effect ($F3,69 = 1.31$, $p = 0.28$, partial $\eta2 = 0.05$) on rater-scored Creativity which was 2.76 (0.22) on average (SEM) on a scale from zero to five.

GLM RM ANOVA did not reveal main effects of LSD ($F3,57 = 1.22$, $p = 0.31$, partial $\eta2 = 0.06$) or Day ($F3,57 = 0.14$, $p = 0.93$, partial $\eta2 = 0.008$), or their interaction ($F9,171 = 1.03$, $p = 0.42$, partial $\eta2 = 0.05$) on the self-rated levels of Creativity experienced up to four days after treatment administration.

At 3 hours post-administration the AUT was performed the predicted mean (SD) LSD plasma concentrations were 113 (32), 212 (70), and 391 (146) pg/mL after LSD 5, 10, and 20 µg, respectively. Predicted LSD concentrations at 5 hours after drug administration, when the Story Writing Task was performed, were 69 (21), 137 (57), and 258 (112) pg/mL after the 5, 10, and 20 µg dose, respectively.

Conclusions: Despite anecdotal reports of positive effects of psychedelic microdoses on creative cognitive processes in users, the present study did not demonstrate LSD effects on task-based or self-rated creative thinking. Nonetheless, findings stimulate further research in a placebo-controlled setting, and eventually in patient populations, to test whether LSD microdoses change potentially creative processes earlier after LSD administration, closer to LSD plasma peak concentrations, or after repeated administrations.

Keywords: LSD Microdosing, Creativity, Placebo-Controlled Trial

Disclosure: Nothing to disclose.

P825. Links Between Childhood Adversity and Extended Amygdala Stressor-Evoked Activity and Connectivity in a Transdiagnostic Sample

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Background: Childhood adversity dysregulates stress reactivity and shapes trajectories of risk for affective disorders throughout the lifespan. Despite the associations between childhood adversity, affective disorders and stress reactivity, neural mechanisms underlying these associations are unclear. The extended amygdala (amygdala and bed nucleus of the stria terminalis (BNST)) and associated structures, including the subgenual anterior cingulate cortex (sgACC) and paraventricular nucleus of the hypothalamus

(PVN), are key regions in controlling physiological stress responses and are associated with emotional processing and affective symptoms. We hypothesize that these regions contribute to adversity-related differences in stress reactivity and affective symptoms. To this end, we examine the relationships between childhood adversity and stressor-evoked activity and connectivity among these extended amygdala and related structures in a transdiagnostic sample.

Methods: Participants were young adults ($n = 100$) with a full distribution of maltreatment history and affective symptom severity. Childhood maltreatment and childhood socioeconomic status (chSES) were the primary measures of childhood adversity. Neural stress reactivity outcomes were evaluated by administering the Multisource Interference Task (MSIT), a mild cognitive stress task, during a functional MRI scan. Parameter estimates from the contrast between control and stress conditions were extracted from each region of interest (ROI). Generalized psychophysiologic interaction (gPPI) analyses were performed to examine MSIT-evoked ROI-to-ROI connectivity. Curvilinear/quadratic and linear regression analyses were performed covarying for age, gender and race.

Results: Childhood maltreatment and chSES were differentially associated with stress reactivity ($n = 97$) within the ROIs. An inverse quadratic relationship was found between childhood maltreatment and amygdala stress reactivity ($\beta = -1.402$, $p = 0.010$). A quadratic relationship was also found between chSES and sgACC stress reactivity ($\beta = 1.209$, $p = 0.025$). Relationships between childhood adversity and gPPI outcomes were only found with chSES, demonstrating that greater chSES was associated with more anti-correlated connectivity among amygdala-to-BNST ($\beta = -0.253$, $p = 0.028$), PVN-to-BNST ($\beta = -0.261$, $p = 0.023$), BNST-to-sgACC ($\beta = -0.290$, $p = 0.011$), and sgACC-to-BNST ($\beta = -0.255$, $p = 0.026$) pathways.

Conclusions: The observed quadratic relationships between childhood adversity and stressor-evoked activity may indicate thresholds at which greater childhood maltreatment or lower SES may contribute to dysregulated stress reactivity. Taken together, observed relationships between chSES and stressor-evoked sgACC activity and BNST-sgACC connectivity suggest that individuals with lower SES may have diminished capacity to mount an appropriate neural stress response, which may have downstream consequences for physiological stress reactivity, emotional processing and affective symptoms.

Keywords: Childhood Adversity, Extended Amygdala, Bed Nucleus of the Stria Terminalis, Stress Reactivity, Functional Connectivity

Disclosure: Nothing to disclose.

P826. Cognitive Reappraisal Tendency Determines Both Laboratory and Real-World Stress-Related Negativity Bias

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Background: A key marker of psychological resilience is the ability to cope with stressful events by regulating one's emotions. This capacity has been especially relevant during the COVID-19 pandemic, which has led to global increases in stress. One consequence of stress is that it renders evaluations of others more negative, particularly under conditions of ambiguity. This is significant because social stimuli, such as faces, are among the most pervasive and biologically relevant sources of information in our environment. However, it remains unknown whether tendencies to use emotion regulation strategies—such as cognitive reappraisal, which involves altering the meaning or relevance of affective stimuli — can shape individual differences regarding how stress affects perceptions of ambiguity. Here, across two independent studies, we examined whether increased reappraisal use is one factor that can determine whether stress exposure induces negativity bias.

Methods: In Study 1, healthy participants ($n = 43$; 21 female) rated the valence of emotionally ambiguous (surprised) facial expressions selected from the NimStim2 and Karolinska Directed Emotional Faces3 stimulus set. Valence ratings were measured before and after an acute stress manipulation (cold-pressor task; water 0-4°C) or matched control task (water ~37°C). In Study 2, we extended this investigation to a larger M-Turk sample. Participants ($n = 97$; 53 female) rated the valence of emotionally surprised faces before vs. after the onset of the COVID-19 pandemic. Reappraisal habits in both studies were measured using the Emotion Regulation Questionnaire and perceived stress were measured using the Perceived Stress Scale5 (Study 2).

Results: In Study 1, regression analysis on valence bias change scores (valence ratings before vs. after stress/control task) revealed that greater reappraisal scores were negatively associated with change scores only for the stress group ($p = .01$), but not controls ($p = .69$). Thus, participants in the stress group who reported more frequent use of reappraisal showed less of a stress-related increase in negative perceptions of ambiguity. In Study 2, we sought to replicate and extend our findings in an independent sample experiencing stress during the onset of the COVID-19 pandemic. We directly tested whether reappraisal tendency moderates the effect of perceived stress during the COVID-19 pandemic on change in negativity bias. A moderation model revealed that reappraisal tendency significantly moderated the relationship between negativity bias change score and perceived stress ($p = .02$). A regions of significance analysis demonstrating the conditional effects of perceived stress on change in negativity bias revealed that more highly stressed participants that also reported lower reappraisal use showed more of a stress-related increase in negative perceptions of ambiguity.

Conclusions: Collectively, these findings suggest that the propensity to use cognitive emotion regulation attenuates stress-induced negativity bias when evaluating ambiguous facial expressions. Our findings point to meaningful individual differences in ambiguity processing and social evaluations under uncertainty that are modulated by real-world perceived stress and emotional resilience.

Keywords: Acute Stress, Ambiguity, COVID-19, Decision-Making

Disclosure: Nothing to disclose.

P827. Expanding the Scope of Auditory-Based Targeted Cognitive Training for Transdiagnostic Applications in VA Settings: A Pilot Study

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Background: Auditory-based targeted cognitive training (TCT) programs are computerized "bottom-up" interventions recently being tested for use in the treatment of schizophrenia (SZ). Since TCT engages neural mechanisms of learning that are intact in SZ, it is conceivable that it may also benefit individuals living with other neuropsychiatric conditions. In order to understand whether TCT may attenuate cognitive impairment in a transdiagnostic manner, we are conducting a TCT study for Veterans with heterogeneous mental health histories. The goal of the study is to identify biomarkers which predict performance gains after a one hour "test dose" of TCT, understand Veteran attitudes towards TCT, and

clarify how TCT may be implemented in VA mental health rehabilitation programming. Here, we present pilot data from this ongoing study from an initial recruitment cohort.

Methods: Veteran participants ($n = 10$) with a range of psychiatric diagnoses engaged in VA-based mental health rehabilitation were recruited. Symptoms were measured via PHQ-9, GAD-7, PCL-5, and PANSS as relevant. Cognitive functioning was assessed via the MATRICS Consensus Cognitive Battery (MCCB), and ratings of disability and quality of life were assessed via WHODAS 2.0 and WHOQOL-BREF. The TCT exercise consisted of 1 hour of Sound Sweeps, an auditory frequency discrimination task, in sets of 32 stimuli presentations. Change in performance on 1 hour of TCT was measured by subtracting participants' baseline score from the best score that they received within the hour. Attitudes towards TCT were assessed using a 7-point Likert questionnaire.

Results: Veteran participants had a primary diagnosis of a chronic psychotic disorder (5/10) or MDD/PTSD (5/10) with at least mild-to-moderate illness severity (average [standard deviation]; PHQ-9 = 5.80[7.55]; GAD-7 = 3.30[4.08]; PCL-5 = 51.00[11.36]; PANSS total = 62.75[17.69]). MCCB scores across all domains revealed significant cognitive impairment (composite *t*-score = 23.60[14.72]). The hour of TCT was well tolerated and associated with high scores on enjoyment, perceived competence on task, effort/importance of completing the task, and value/usefulness to participants (average rating = 5.1 [1.82]). Participants completed an average of 8.6 stimuli sets. 6/10 demonstrated improvement ("responders") in auditory discrimination metrics after 1 hour of TCT. Compared to non-responders, responders demonstrated lower PHQ-9 ratings (12.75 [7.63] vs.1.17 [1.84], $p = 0.006$), lower GAD-7 ratings (6.50[3.70] vs. 1.17[2.86], $p = 0.032$), lower WHODAS 2.0 ratings (28.75[10.08] vs. 16.67[5.50], $p = 0.038$), and higher WHOQOL-BREF Physiological domain ratings (10.57[3.78] vs.16.48 [2.54], $p = 0.017$).There were no differences in age, education, marital status, or VA-rated disability between responders and non-responders.

Conclusions: Auditory-based TCT is well tolerated among Veterans with a range of psychiatric diagnoses and co-morbidities. Participants who self-report lower depression/anxiety symptoms, lower ratings of disability and higher quality of life may demonstrate greater initial learning on the initial "test dose" of TCT across diagnostic conditions. Future analyses of the full study dataset will help establish early predictors of TCT response across different psychiatric conditions for deployment across a variety of mental health recovery milieus.

Keywords: Targeted Cognitive Training, Cognitive Dysfunction, Transdiagnostic, Mental Health Service Use

Disclosure: Nothing to disclose.

P828. Toward Precision Characterization of Dimensions of Threat Response Pathology in Depression and Anxiety: Testing a Theoretical Model That Integrates Circuits, Symptoms and Quality of Life

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Background: Exaggerated threat responses are characteristic of anxiety disorders and depression and implicate underlying dysregulation of amygdala-anterior cingulate cortex (ACC) circuitry and an accompanying impact on symptoms and functioning. Comorbidity between diagnostic entities may be partly explained by shared dimensional features such as threat responses. Thus, a comprehensive model including precise representations of neural circuitry, symptoms, and quality of life may provide the basis for characterizing the pathology of anxiety and depression. We use the Research Domain Criteria (RDoC) as an organizing framework to integrate these units of analysis and clinically relevant outcomes. RDoC differentiates between "acute threat'" and "potential threat" (Cuthbert, 2014). At the symptom level, acute threat and potential threat are thought to manifest as "fear" and "anxiety", respectively. At the neural circuit level, the nuances of threat responsivity are captured by the condition-dependent ways in which the amygdala-ACC circuit is dysregulated. Given this context, we set out to accomplish the following three aims: 1) assess whether functional connectivity between the amygdala and dACC during acute and potential threat conditions is associated with the severity of fear and anxiety, respectively; 2) assess whether fear and anxiety are associated with quality of life; 3) assess the empirical support for a theoretical model linking precise representations of threat circuitry, symptoms, and quality of life using structural equation modeling.

Methods: We leveraged data from a NIMH funded R01 project that used an RDoC approach to study the neural correlates of phenotypic heterogeneity in depression and anxiety disorders (Williams et al. 2016). Of the 284 subjects with data on at least one measure, 168 had data on all measures and the degrees of freedom represent these subject numbers. Power was estimated for generalized linear models (GLMs) assuming a small effect size of Cohen's $f2 = 0.10$. With 168 subjects we have well over 80% power to detect effect with family-wise corrected alpha level of $p < .05$ (Cohen et al. 2003).

To operationalize acute threat and potential threat at the symptom level, we calculated composite measures of fear and anxiety from self-reports based on empirically derived symptom phenotypes (Goldstein-Piekarski et al. 2021). At the circuit level, we used a functional magnetic resonance imaging paradigm involving the viewing of emotional faces consciously and nonconsciously (Williams et al. 2016). Based on prior work, we operationalized acute threat as the amygdala-dACC connectivity during consciously viewing threatening vs. happy faces, and operationalized neural response to potential threat as amygdala-dACC connectivity during nonconsciously viewing threatening vs. happy faces (Etkin et al. 2011; Robinson et al. 2019). To calculate these connectivity values, we used the psychophysiological interaction analysis seeded in amygdala and calculated the connectivity of amygdala to dACC (Goldstein-Piekarski et al. 2021). As a measure of quality of life, we used scores on the psychological domain of the World Health Organization Quality of Life measure (WHOQOL Group, 1994).

We tested four bivariate GLMs to examine the relationships between symptoms and circuits (Aim 1) and between symptoms and quality of life (Aim 2). To assess the degree of empirical support for representing threat responses with precision at circuit and symptom levels in the context of predicting quality of life (Aim 3), we tested four structural equation models (SEMs). The precision with which circuitry and symptoms were represented varied across models. We first tested a model in which fear and anxiety were included as separate terms at the symptom level and conscious and nonconscious amygdala-dACC connectivity were included as separate terms at the circuit level. We specified links between each measure of neural circuitry and the respective symptom phenotype, and between each symptom phenotypes and quality of life. We compared the fit of this model to models in which 1) amygdala-dACC connectivity was averaged across the two tasks, 2) symptoms of fear and anxiety were averaged, and 3) both connectivity and symptoms were averaged.

Results: Fear symptoms were significantly associated with nonconscious amygdala-dACC connectivity (std. est. =.213, $t1,167 = 2.82$, $p = .0054$). Anxiety symptoms were not significantly associated with conscious amygdala-dACC connectivity (std. est. = -.135, $t1,177 = -1.824$, $p = .069$). Quality of life was associated with the fear (std. est. = -.189, $t1,282 = -3.23$, $p = .0014$) and

anxiety symptoms (std. est. = -.131, t1,282 = -2.221, $p = .027$). The SEM in which threat responses were represented separately at both the symptom and the circuit levels demonstrated good fit and theoretical interpretability relative to the models in which threat response or symptoms were merged across units of analysis.

Conclusions: These results provide empirical validation for a theoretical model linking neural circuit dysregulation, symptom phenotypes, and quality of life. By leveraging a modeling technique that allows us to test these relationships in the context of a theoretical model, our results suggest that these linkages may only be apparent given optimally precise representations of symptoms, circuits, and functional outcomes. Novel classification approaches that rely on precise representations of symptoms and circuits may inform personalized treatment.

Keywords: Research Domain Criteria (RDoC), Anxiety, Functional Connectivity

Disclosure: Nothing to disclose.

P829. A New High-Throughput Method to Induce Robust Psychological Stress

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Background: Psychological stress is a significant risk factor for several psychiatric disorders, including depression, anxiety, panic disorder, post-traumatic stress disorder, schizophrenia, and bipolar disorder. Stress can have physiological impacts by activating cortisol release and downstream effects through the hypothalamic-pituitary-adrenal (HPA) axis and sympatho-adrenomedulary (SAM) axis to direct the physical and emotional responses. But the underlying genetic, genomic, and epigenomic regulators of stress responses remain largely elusive. To study the genetics of stress responses, high-throughput phenotyping method is needed. The Trier Social Stress Test (TSST) is a laboratory procedure used to induce psychological stress, but it is a time-consuming protocol. We developed a new substitute procedure that can induce a similar level of stress. It can be administered to many participants in parallel.

Methods: We used a modified version of the N-back test, which is typically used for evaluating working memory functions, coupled with a monetary penalty to create a demanding, stressful experience. We used the computerized 2 to 5-back tests with varying numbers and positions displaying on the computer screen for the participants to memorize. Errors in the responses will lead to the reduction of monetary rewards. Additional psychological stress was introduced by reporting inflated error rates and manipulated ranking of performance among peers. The participants were measured for blood pressures, heart rate, and visual analogue scale (VAS) at the rest time (t1), before (t2), and after the test (t3). The complete procedure takes 30 minutes, including 20 minutes resting and preparation, and 10 minutes test. We call this method N-back stress test (NBST).

We recruited 141 people into the study. Thirty-six of them had the NBST tests first. After 12.77 ± 0.93 (Mean ±SEM) days, the same participants had the TSST. Another 105 people had TSST first and followed by NBST with a gap of 249.09 ± 8.79 days. Twelve people had NBST twice with an interval of 23.33 ± 1.32 days. Paired *t*-test was used to evaluate the correlation of stress induced by NBST and TSST.

Results: The NBST induced the same level of stress according to the measures of systolic, diastolic pressures, heart rate and VAS. The blood pressures, heart rate and VAS score increased right before the tests, and after the tests comparing to the rest time (SBP: d(t2-t1) = 0.222, p(t2-t1) = .049, d(t3-t1) = 0.151, p(t3-t1) = .177; DBP: d(t2-t1) = 0.262, p(t2-t1) = .021, d(t3-t1) = 0.195, p(t3-t1) = .083; Pulse: d(t2-t1) = 0.276, p(t2-t1) = .015, d(t3-t1) = 0.362, p(t3-t1) = .002; VAS: d(t2-t1) = 1.656, p(t2-t1) < .001, d(t3-t1) = 1.717, p(t2-t1) < .001), which is similar to the effects induced by TSST (SBP: d(t2-t1) = 0.349, p(t2-t1) < .001, d(t3-t1) = 0.249, p(t3-t1) = .008; DBP: d(t2-t1) = 0.300, p(t2-t1) = .001, d(t3-t1) = 2.031, p(t3-t1) = .001; Pulse: d(t2-t1) = 0.419, p(t2-t1) < .001, d(t3-t1) = 0.199, p(t3-t1) = .033; VAS: d(t2-t1) = 1.240, p(t2-t1) < .001, d(t3-t1) = 1.232, p(t3-t1) < .001). The response using the area-under-curve with respect to ground (AUCG) induced by NBST is correlated with that of TSST on the same participants (SBP: d = 0.547, r = 0.054, p < .001; DBP: d = 0.284, r = 0.279, p = .041; Pulse: d = 0.130, r = -.239, p = .081; VAS: d = 0.013, r = 0.489, p < .001). The NBST also has high test-retest correlation (SBP: d = 1.038, r = 0.890, p < .001; DBP: d = 0.398, r = 0.726, p = .007; Pulse: d = 0.385, r = 0.121, p = .709; VAS: d = 0.426, r = 0.772, p = .003). In contrast, the measures from unrelated participants have no correlation.

Conclusions: We created a new laboratory procedure that can be performed in parallel on many participants in a short time to induce robust psychological stress that is comparable with TSST, particularly based on the measures of VAS. It will be a useful tool for studying the genetic and genomics of stress-related biology.

Keywords: Acute Stress, Computational Methods, High-Throughput

Disclosure: Nothing to disclose.

P830. Circulating Extracellular Vesicles are Required for Social Behaviors in Mice

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Background: Extracellular vesicles (EVs) are small lipid bilayer membrane vesicles secreted by various cells in the body under both physiological and pathological conditions. EVs contain cellular components, such as microRNAs (miRNAs), proteins, and metabolites, and transfer them between cells in a paracrine and endocrine manner. As neuron- and glia-derived EVs have been reported to circulate in the blood, extensive efforts are being made to identify distinct EV molecular signatures as biomarkers for neurological and psychiatric disorders. In contrast, few studies have been conducted to address if circulating EVs affect brain function and behavior. Lymphocytes, including *T* and B cells, have been shown to communicate with the healthy brain, modulate its function, and influence behaviors. Mouse studies so far have revealed that the lack of adaptive immune cells impairs learning and memory, anxiety-related behaviors, and social behaviors. Nevertheless, the underlying mechanisms are not fully understood. In this study, we have discovered that circulating EVs mediate adaptive immune cell-dependent modulation of social behaviors by directly communicating with neurons and microglia.

Methods: Mice deficient for adaptive immune cells (immunodeficient mice, e.g., scid, Rag1-/- and Rag2-/- mice) were used to study the effects of circulating EVs on brain cellular phenotypes and behaviors. Circulating EVs in the peripheral blood were enriched by precipitation or ultracentrifugation, and analyzed with electron microscopy, nanoparticle tracking assay, and Western blots. Mouse behavioral phenotypes were also examined in the open field test, three-chamber social interaction test, and the buried food-seeking test. Changes in brain cellular and molecular phenotypes were assessed by immunohistochemistry and RNA-seq experiments. EV distribution in the brain was analyzed by injecting chemically or genetically labelled EVs. Chemogenetic manipulation of neuronal activities by a DREADD system was

conducted to determine the causal role of neuronal changes in the observed behaviors.

Results: Both Rag1-/- and Rag2-/- immunodeficient mice showed impaired sociability and social novelty preference in the three-chamber social interaction test ($n = 5$-15 mice per group; $p < 0.01$, one-way ANOVA with post hoc Dunnett's test). As expected, sociability deficits were rescued by adoptive transfer of splenocytes (containing both *T* and B cells) or isolated *T* cells from wild-type (WT) mice ($n = 5$-15 mice per group; $p < 0.01$, one-way ANOVA with post hoc Dunnett's test). Unexpectedly, similar rescue effects were observed with the injection of WT mouse-derived sera into these mice ($n = 5$-8 mice per group; $p < 0.05$, one-way ANOVA with post hoc Dunnett's test). Although cytokines such as IFN-g have been implicated in the modulation of sociability behavior, their levels were below the detection limit in the WT sera. Notably, we found that multiple miRNAs in serum EVs of Rag1-/- mice were significantly increased by adoptive transfer of *T* cells ($n = 6$ mice per group; *p*-adj <0.05). These miRNAs were enriched with those related to social behaviors and their putative target mRNAs were categorically overlapped with those related to synaptic functions. Thus, we speculated that changes in circulating EVs mediated immunodeficiency-induced social behavioral changes. Indeed, intravenous injection of serum EVs from WT mice, but not Rag1-/- mice, rescued the sociability deficits in Rag1-/- mice ($n = 4$-9 mice per group; $p < 0.01$, one-way ANOVA with post hoc Dunnett's test). In contrast, social novelty preference, which was also impaired in Rag1-/- mice, was not rescued by the transfer of either serum EVs or *T* cells. Chemical and genetic labeling of serum EVs allowed us to detect their co-localization with neurons and microglia, but not with astrocytes and oligodendrocytes, in the medial prefrontal cortex (mPFC), hippocampus, and cerebellum. To gain insight into the underlying mechanisms, we conducted RNA-seq experiments to compare the gene expression profiles of prefrontal cortices (PFC) of Rag1-/- mice with or without serum EV injection ($n = 3$ mice per group; *p*-adj <0.05). Genes whose expression were modified by EV injection were associated with dendrite morphogenesis, regulation of postsynaptic membrane, neurotransmitter receptor levels, and regulation of neuronal synaptic plasticity (gene ontology analysis; $p < 1$x10-7). Consistent with these findings, neuronal c-Fos protein expression in the mPFC was enhanced in Rag1-/- mice and normalized by the transfer of WT serum EVs ($n = 7$ mice per group; $p < 0.05$, *t*-test). We also confirmed that the enhanced activities of excitatory neurons in the mPFC was responsible for sociability deficits in Rag1-/- mice by a DREADD experiment using AAV-CaMKIIa-hM4D(Gi) vector ($n = 3$ mice per group; $p < 0.005$, *t*-test).

Conclusions: Our data demonstrate that EVs in the blood mediate immune modulation of neuronal gene expression in the medial prefrontal cortex. This study provides a novel biological insight into the mechanisms underlying peripheral-to-brain immune communications and may have a broad impact on psychiatric disorders and other brain diseases.

Keywords: Social Behavior, Extracellular Vesicles, Immune System, Medial Prefrontal Cortex

Disclosure: Nothing to disclose.