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ABSTRACTS COLLECTION

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M1. Juvenile Social Isolation of Rats Induces Lost of Corticotropin Releasing Factor-Receptor 1 Antagonist Effect in the Nucleus Accumbens

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Background: Social isolation is a model of chronic stress widely used to study early life adversity. It is well known that early life adversity may lead to the development of psychiatric disorders in adulthood. Juvenile rats exposed to social isolation showed increased anxiety-like behavior and significant changes in Nucleus Accumbens (Nac) dopamine (DA) activity in adulthood (Lukkes et al. 2009). Interestingly, rats isolated during their juvenile stage developed an increase in anxiety-like behavior, but rats isolated during their young adult stage showed no effects upon anxiety-like behavior (Arakawa, 2003). We are interested in evaluating neuronal changes induced by isolation that could explain the difference between the expression on anxiety like behavior in juvenile and young adult rats.

Methods: We performed in vivo microdialysis to evaluate neuronal changes induced by isolation. Prolonged social isolation of juvenile rats (10 days) increases DA extracellular level in the Nac shell. Interestingly, preliminary results showed no difference between DA extracellular levels in the Nac shell of young adult isolated and no isolated rats. Next, we evaluated eventual changes in corticotropin-releasing factor (CRF) system function induced by isolation. The CRF system mediates part of the brain stress response. The CRF system is composed by CRF, urocortins, CRF-R1 and CRF-R2 receptors.

Results: Interestingly, the effect of a CRF-R1 antagonist upon DA and glutamate levels in juvenile no isolated rats is occluded when rats are isolated. These results suggest that social isolation of juvenile rats decreases CRF-R1 in Nac terminals. Supporting this hypothesis, our preliminary results showed that synaptosomes devoid of postsynaptic elements obtained from the Nac of isolated juvenile rats have lower number of dots and fluorescence intensity of CRF-R1 compared with synaptosomes obtained from control not isolated rats.

Conclusions: Further studies are needed to underlie the cellular mechanisms mediating these observed effects.

Keywords: Dopamine, Juvenile Social Isolation, Type 1 Corticotropin Releasing Factor Receptor

Disclosure: Nothing to disclose.

M2. The Contribution of the NLRP3 Inflammasome to Trauma Response in an Animal Model of PTSD

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Background: Post-traumatic stress disorder (PTSD) is a common psychiatric condition that is defined by its paradigmatic symptoms including but not limited to anxiety, avoidance, and increased arousal by environmental cues due to a severely traumatic event. Recent studies show that this condition is correlated with both peripheral and central immune dysfunction, but the relationship between inflammation and PTSD remains unclear. In a mouse model of PTSD, we found increased plasma levels of the pro-inflammatory cytokine interleukin-1β (IL-1β) after exposure to trauma. A major component in the peripheral and central inflammatory pathways is the nucleotide-binding oligomerization domain-, leucine-rich repeat- and pyrin domain containing-3 (NLRP3) inflammasome. Since the NLRP3 inflammasome is also known to be a key component in the production and release of IL-1β, we hypothesized that the NLRP3 inflammasome modulates the response to trauma.

Methods: To test this hypothesis, we exposed a preliminary cohort ($n = 8$-9 per group/sex) of both male and female wildtype (WT) and NLRP3 knockout (KO) mice to a predator (cat) stress in an enclosed space for 10 minutes. The PTSD-like phenotype was assessed 1-2 weeks after predator exposure across 3 behavioral paradigms: open field, light-dark box, and a trauma-reminder test. A composite avoidance score was then calculated to measure the overall PTSD-like phenotype.

Results: In all three tests, no effect of genotype was found. In the open field, stress exposure tends to increase PTSD-like phenotype ($p = 0.10$) in mice, independently of their genotype. In the light-dark box, a main effect of stress was observed ($p<0.05$), with posthoc comparisons revealing elevated PTSD-like phenotype in stressed KO mice compared to non-stressed KO mice ($p<0.05$). In the trauma-reminder test, stress tended to exert a main effect ($p = 0.10$) and to interact with the NLRP3 genotype ($p = 0.11$). Predator stress tended to increase PTSD-like phenotype in WT, but not KO, mice.

Conclusions: Altogether these preliminary results suggest that the NLRP3 inflammasome may not contribute to the development of anxiety-like symptoms, but may modulate the avoidance of trauma-related cues in response to a traumatic event. Additional experiments need to be conducted using model to inquire further implications regarding sex-dependent effects of the NLRP3 inflammasome in PTSD risk. As current FDA-approved treatments are shown to have a limited efficacy in individuals diagnosed with PTSD, developing an in-depth understanding of the role of these inflammatory signaling pathways in PTSD pathophysiology may be useful for developing novel pharmacological treatments.

Keywords: PTSD, Inflammasome, Avoidance

Disclosure: Nothing to disclose.

M3. Glutamatergic Mechanisms Mediate Enduring Vulnerability to Drug Use Following an Acute Stressor

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Background: Converging epidemiological studies indicate that a history of acute life-threatening events increases the incidence of post-traumatic stress disorder (PTSD), and a diagnosis for PTSD carries 30–50% comorbidity with substance use disorder (SUDs). Thus, patients with comorbid PTSD/SUDs have greater drug use severity and show poorer treatment outcomes than patients diagnosed with either alone. Using an animal model, we found that exposure to a single stressful event experienced 3 weeks earlier can enhance cocaine, alcohol and heroin intake and trigger a number of enduring adaptations within corticostriatal synapses of the nucleus accumbens core (NAcore), resembling drug induced adaptations. Recently, we paired acute restraint stress with a novel odor and found that stressed rats showed increased reactivity when exposed to a stress-conditioned stimulus (stress-CS) in a defensive burying task and in a self-administration model the stress-CS was sufficient to reinstate cocaine, alcohol and heroin seeking in extinguished animals.

Drug-associated cue presentation evokes transient increases in the tetrapartite synapse, including pre and postsynaptic neurons, astrocytes and the extracellular matrix (ECM), that coincide with 15 min of drug cue-associated exposure. These transient changes are referred to as transient synaptic plasticity (t-SP) because they are reversed by 120 min after initiation of the behavior. Given the overlap between the enduring adaptations produced by acute restraint stress and withdrawal from cocaine and heroin use, we hypothesize that exposure to a stress-CS elicits t-SP and coping responses in the defensive burying task. Moreover, medium spine neurons (MSNs) constitute 90-95% of the neurons in the NAcore and are chemically coded into two subtypes that selectively express D1 or D2 dopamine receptors. These two populations appear to subserve distinct behavioral functions, with D1 activation generally promoting and D2 activation inhibiting behaviors. Thus, we also hypothesize that stress cue-induced t-SP will occur in D1-, but not D2-MSNs.

Methods: Male and female Sprague Dawley rats, and D1- and D2-Cre transgenic mice were restrained for 2 hours in plexiglas cylinders and exposed to an odor that became a stress-CS, or sham animals were left in new home-cage boxes exposed to an odor (neutral stimulus - NS). Three weeks after the stressful experience animals were exposed to a cage that contain bedding on one corner and stress-CS or NS on the opposite corner (noxious object which to be buried). After completing the defensive burying task, animals were perfused and tissue processed for spine morphology studies with DiI lipophilic colorant, MMPs activity with zymography gel microinjections or astrocyte morphology studies with GFAP-hM3dq-mCherry virus that specifically labels astrocytes. We also recorded single cell Ca2+ dynamics in D1- and D2-MSNs of freely moving mice using a miniature microscope (nVista) and virally expressed Cre-dependent Ca2+ indicator (GCaMP6f) during restraint stress, defensive burying and cross-sensitization.

Results: We have observed key features of stress-CS induced changes in tetrapartite synaptic plasticity. Stress-CS exposure is associated with synaptic potentiation in NAcore, quantified by an increase in dendritic spine head diameter and spine density, and increased matrix metalloprotease-9 activity that catalyzes proteins from the ECM. Stress-CS exposure also induced down-regulation of astroglial glutamate transporters (GLT-1) and retraction of astrocyte synaptic coverage compared to stress animals exposed to a neutral stimulus. Furthermore, we observed differential changes in Ca2+ activity of D1 vs D2-MSN during restraint stress, defensive burying test and cross-sensitization.

Conclusions: These data propose that these changes which coincide with 15 min defensive burying task seems to be correlated with transient synaptic plasticity in NAcore that lead to stress coping responses. Because t-SP is not observed during cued sucrose seeking, and stress-CS exposure did not induce sucrose-seeking in sucrose-trained rats, we hypothesize that the t-SP described here is potentially a pathological feature of stress disorder. Further experiments are needed to establish whether stress cue-induced t-SP occur in D1 and whether it correlates with changes in Ca2+ activity of D1 or D2-MSN.

Keywords: Post Traumatic Stress Disorder, Tetra Partite Synapse, Synaptic Plasticity, Nucleus Accumbens, Substance Use Disorder

Disclosure: Nothing to disclose.

M4. Parsing Distinct and Common Neural Mechanisms of Response to Learned Threat in Childhood Anxiety and Irritability

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Background: Symptomic comorbidity is pervasive in psychiatry. This clinical heterogeneity impedes diagnosis and target identification for the development of precise mechanistic treatments. Anxiety and irritability are among the most highly co-occurring problems in youth (e.g., Shimshoni et al., 2020). Children with both elevated irritability and anxiety, compared to children with anxiety alone or healthy controls, are more severely impaired in multiple domains of functioning (Shimshoni et al., 2020). Both anxiety and irritability involve high-arousal, phasic negative affect states (e.g., negative affectivity; Kircanski et al., 2018; Rothbart, 2007). A large literature links anxiety to aberrant threat responses, mediated by perturbations in amygdala-prefrontal cortex circuitry (LeDoux & Pine, 2016; Shin & Liberzon, 2010). In comparison, a substantially smaller literature examines the pathophysiology of irritability. A recent translational model of irritability posits that irritability is characterized by aberrant threat responses mediated by amygdala-prefrontal-hypothalamic-periaqueductal gray dysfunction (Brotman et al., 2017; Leibenluft, 2017). Threat processing thus plays an important, mechanistic role in the pathophysiology of irritability and anxiety (Brotman et al., 2017; LeDoux & Pine, 2016). Delineating common and unique patterns of threat processing could inform efforts to distinguish anxious and irritable youth. Here, we used a well-validated fear extinction recall paradigm during functional Magnetic Resonance Imaging (fMRI; Britton et al., 2013; Gold et al., 2020) to examine distinct and common neural mechanisms of childhood anxiety and irritability using a data-driven latent phenotyping approach (bifactor modeling; Kaczkurkin et al., 2020; Kircanski et al., 2018).

Methods: In a large transdiagnostic sample of youth (N = 331; Mage = 13.57 yrs; 45.92% male) with disruptive mood dysregulation disorder (n = 70), anxiety disorder (n = 95), attention-deficit/hyperactivity disorder (n = 39), and healthy controls (n = 127), we conducted bifactor analysis to derive latent factors representing the common and unique variances associated with symptoms of anxiety and irritability (Kircanski et al., 2018). Parents and children

completed the Screen for Child Anxiety Related Emotional Disorders (SCARED; Birmaher et al., 1999) and the Affective Reactivity Index (ARI; Stringaris et al., 2012) to assess anxiety and irritability, respectively. Of the 331 youths included in the bifactor analysis, 59 (28 anxiety disorder, 31 healthy controls) completed a threat conditioning and extinction paradigm, followed by an extinction recall (ER) fMRI paradigm (Gold et al., 2020). During fMRI of ER, participants made threat-safety discriminations under two attention conditions: current threat appraisal and recall of explicit threat contingencies. Specifically, participants rated their current levels of fear evoked by, and memory for, facial morph stimuli falling along a continuum with varying degrees of threat ambiguity (morphed images differing in similarity to the extinguished threat (CS+) and safety (CS−) cues). We extracted participants' factor scores from the bifactor model and conducted whole-brain analyses to examine associations between the bifactor-derived phenotypes and both neural activity and amygdala functional connectivity. Whole-brain activation and functional connectivity analyses were conducted in AFNI and considered a $p < 0.001$ voxelwise threshold with k□56 and p□0.05 FWE-corrected cluster extent threshold.

Results: Bifactor model in 331 youth showed good fit with the data (factor loading p's < 0.001; Comparative Fit Index=.973; Non-Normed Fit Index=.966; Root Mean Square Error of Approximation = .072; 90% confidence interval, CI [.065, .080]) and replicated the four factors (i.e., common factor [termed "negative affectivity"], parent-rated irritability, child-rated irritability, anxiety factor) identified in a previous study (Kircanski et al., 2018).

During explicit memory recall of threat-safety contingencies, negative affectivity (i.e., common latent factor) was associated with increased activation in prefrontal (ventrolateral, ventromedial, and dorsolateral prefrontal cortex), limbic (hippocampus, amygdala), parietal, and motor regions (r's = .17 to .30). In contrast, anxiety and parent- and child-reported irritability were associated with decreased activation in these regions (r's = −.11 to −.37), and parent-reported irritability was uniquely associated with decreased activation in thalamus (r=-.18). Moreover, during threat appraisal, high levels of negative affectivity were associated with increased connectivity between amygdala and inferior parietal lobule in response to threat/safety ambiguity.

Conclusions: Using a fear extinction recall paradigm, we identified dissociable neural correlates of negative affectivity vs. anxiety or irritability in youth. The regions identified are largely consistent with the extinction recall literature implicating that dysregulated amygdala-PFC circuit is involved in anxiety during recall of threat-safety stimuli. Parsing the distinct and common neural mechanisms of childhood anxiety and irritability would improve the understanding of the heterogeneity of anxiety and mood disorders and move the field toward a precision medicine approach to these common, impairing conditions.

Keywords: Anxiety, Irritability, Youth, fMRI, Bifactor

Disclosure: Nothing to disclose.

M5. Central Amygdala Projections to Lateral Hypothalamus Mediates Avoidance Behavior in Rats

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Background: A debilitating hallmark of post-traumatic stress disorder (PTSD) is persistent avoidance of trauma-associated stimuli. In our lab, we have shown that subsets of rats exposed to predator odor ("traumatic") stress will show persistent avoidance of a predator odor-paired context, mirroring individual differences in stress reactivity in humans. These 'Avoider' rats show greater central amygdala (CeA) activation, as measured by c-Fos induction, than their Non-Avoider counterparts and stress-naïve Controls, suggesting that the CeA plays an important role in modulating avoidance behavior. Indeed, the CeA is known to modulate downstream effector regions to produce physiological and behavioral adaptations to stress. One downstream area of interest is the lateral hypothalamus (LH), which is known to modulate approach vs. avoidance behaviors. The main goal of this study is to test the overarching hypothesis that CeA cells that project to the LH modulate avoidance behavior in Avoider rats.

Methods: Male Wistar rats were used in all experiments. First, we tested if CeA projections to LH form functional synapses with LH cells using a combination of optogenetics and slice electrophysiology (N = 4). Then, to test the hypothesis that activation of CeA-to-LH cells mediates avoidance behavior, rats were given intra-LH retrograde tracer (Retrobeads) injections, stressed and indexed for avoidance, re-stressed, and sacrificed for c-Fos immunohistochemistry (N = 7-8/group). We also tested if in vivo activation of CeA-to-LH cells is necessary and/or sufficient for avoidance behavior using in vivo chemogenetic inhibition or stimulation. Specifically, inhibitory (Gi) or stimulatory (Gq) DREADD receptors were targeted to CeA-to-LH cells using an intersectional cre-dependent viral expression strategy. Subsequently, we tested if DREADD inhibition of CeA-to-LH cells using clozapine-n-oxide (CNO) disrupted avoidance behavior in Avoider rats (N = 6-9/group) and if DREADD activation of CeA-to-LH cells using CNO supported avoidance behavior in stress-naïve rats (N = 7-8/group). Finally, synaptic transmission and intrinsic properties of CeA-to-LH cells were characterized using slice electrophysiology. Data were analyzed using factorial ANOVAs followed by Tukey's post-hoc tests. Subsets of electrophysiology data were analyzed using two-sample t-tests and Spearman rank-order correlations. Significance level was set at $p < 0.05$.

Results: Optical stimulation of channelrhodopsin-expressing CeA terminals in LH evoked inhibitory currents in LH cells that were blocked by the GABA(A) receptor antagonist, picrotoxin, indicating that CeA projections to LH form functional GABAergic synapses with LH cells. Using a combination of retrograde tracing and c-Fos immunohistochemistry, we found that Avoider rats have more c-Fos+ CeA-to-LH cells than Non-Avoiders and Controls (p's < 0.01), suggesting that activation of this circuit is important for supporting an avoidance phenotype. In support of this idea, we found that DREADD inhibition of CeA-to-LH cells attenuated avoidance in Avoider rats ($p < 0.001$), and that DREADD activation of this circuit recapitulated avoidance behavior in otherwise stress-naïve rats ($p < 0.05$). In our slice electrophysiology studies, a main finding was that CeA-to-LH cells of Avoider rats have greater voltage SAG amplitudes (reflective of HCN-mediated currents) than CeA-to-LH cells of Non-Avoiders and Controls (p's < 0.05). In addition, we found that the voltage SAG in CeA-to-LH cells of Avoiders is largely mediated by HCN2-4 channels, whereas the voltage SAG in CeA-to-LH cells of Non-Avoiders and Controls is largely mediated by HCN1 channels.

Conclusions: These findings show that CeA-to-LH cells form a functional circuit that is important for modulating avoidance behavior in rats. In addition, our results suggest that the activity of this circuit may be modulated by HCN channels, and that targeting specific subtypes of HCN channels may have therapeutic potential for the treatment of PTSD-related conditions. This work was supported by NIH grants AA027145 (MMW), AA023305 (NWG), AA026531 (NWG), and AA007577.

Keywords: Acute Traumatic Stress, Avoidance, Central Amygdala, Lateral Hypothalamus

Disclosure: Nothing to disclose.

M6. Propranolol Decreases Fear Expression by Modulating Fear Memory Traces

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Background: Post-traumatic stress disorder (PTSD) can develop following a traumatic event and results in heightened, inappropriate fear and anxiety. Although approximately 8% of the United States population suffers from PTSD, only two drugs have been approved by the FDA to treat it, both with limited efficacy. Propranolol, a non-selective □-adrenergic antagonist, has shown efficacy in decreasing exaggerated fear, and there has been renewed interest in using it to treat fear disorders.

Methods: Here, we sought to determine the mechanisms by which propranolol attenuates fear by utilizing an activity-dependent tagging system, the ArcCreERT2 x enhanced yellow fluorescent protein (eYFP) mice. 129S6/SvEv mice were administered a 4-shock contextual fear conditioning (CFC) paradigm followed by immediate or delayed context re-exposures. Saline or propranolol was administered either prior to or following the first context re-exposure. To quantify hippocampal memory traces, ArcCreERT2 x eYFP mice were administered a delayed context re-exposure with either a saline or propranolol injection prior to context re-exposure.

Results: Propranolol decreased fear expression only when administered prior to a delayed context re-exposure. Fear memory traces were decreased in the dorsal dentate gyrus and basolateral amygdala following propranolol administration in the ArcCreERT2 x eYFP mice. Propranolol acutely altered functional connectivity between hippocampal, cortical, and amygdalar regions.

Conclusions: These data indicate that propranolol may decrease fear expression by altering network correlated activity and by weakening the reactivation of the initial traumatic memory trace. This work contributes to the understanding of noradrenergic drugs as therapeutic aids for PTSD patients.

Keywords: Propranolol, Engram, Fear Conditioning, c-Fos, Arc

Disclosure: Nothing to disclose.

M7. Increased Theta EEG Power During Emotional Appraisal in Veterans With PTSD and Mild TBI

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Background: Combat veterans returning from Iraq or Afghanistan exhibit higher rates of mild traumatic brain injury (mTBI) than seen in previous conflicts. Patients with existing mTBI appear to be more susceptible to developing posttraumatic stress disorder (PTSD), and the combination further worsens outcomes for these veterans. In the present study, we seek out an electroencephalographic (EEG) biomarker to predict PTSD risk or severity in mTBI patients. PTSD is associated with over-activation of limbic and related areas that monitor potential threats to the organism. An established EEG signature of limbic over-activity is increased posterior theta power during emotional image appraisal, thought to arise from neural activity between limbic and visual areas being coordinated to enable greater awareness of potentially-dangerous stimuli. And indeed this pattern has been observed in subjects with PTSD. We hypothesized that, in combat veterans with mTBI, posterior theta power may serve as a biomarker of PTSD risk or severity.

Methods: Fifty-four (17 control; 17 mTBI-only; 20 mTBI+PTSD) male combat veterans from the military conflict in Iraq or Afghanistan completed the study. Clinical assessment involved administration of the Brief Traumatic Brain Injury Screen (BTBIS), the Mini-International Neuropsychiatric Interview (MINI) for DSM-IV-TR. the Clinician-Administered PTSD Scale (CAPS), and the Beck Depression Inventory II (BDI-II). Subjects were included if they: (1) reported experiencing one or more concussive events (mTBI) during combat (i.e., a blast exposure or a blow or jolt to the head) that resulted in a loss or alteration of consciousness of 20 minutes or less, on the BTBIS; and met criteria for mTBI based on questions adapted from Vasterling et al. Subjects who developed PTSD prior to combat were excluded from the study.

Task: Subjects were presented with 100 positive and 100 negative images in random order chosen from the International Affective Picture System (IAPS) and were asked to rate their emotional valence on a continuous scale. After answer was submitted, a one-second gap played prior to the next image being shown.

EEG acquisition and processing: EEG data were collected synchronously from 132 scalp and four infra-ocular electrodes with an active reference (BioSemi Instrumentation, Amsterdam, NL) at a sampling rate of 512 Hz with 24-bit analog-to-digital resolution. Artifacts were removed with independent component analysis (ICA) as implemented in EEGLAB. For source localization, we first identified putative brain ICs identified as those with < 15% residual variance from an equivalent dipole model localized within the brain volume. ICs were then hierarchically clustered based on anatomical location, by calculating the Euclidean distance between all dipole locations.

Statistical analysis: Group differences were compared using one-way analysis of variance (ANOVA) with demographic, clinical and behavioral variables, and bilateral occipital cluster peak P300 magnitude and theta power as dependent variables and group as between subject variable. Clinical correlations were tested using Pearson's r.

Results: For all groups, maximal ERP amplitudes were observed around 300 ms latency, corresponding to the ERP we refer to here as P300. Because the P300 amplitude appeared greater for mTBI +PTSD veterans, we searched for the IC cluster with the largest contribution to the P300 peak across subjects, which was bilateral occipital. A significant effect was found for occipital P300, with mTBI+PTSD veterans exhibiting greater P300 amplitudes than control or mTBI-only veterans, $F(2,44) = 3.358$, $p < 0.05$. Follow-up post-hoc testing revealed a significant difference between controls and mTBI+PTSD ($p < 0.03$), and between mTBI-only and mTBI+PTSD ($p < 0.04$), but not between control and mTBI-only ($p > 0.9$) veterans. For all mTBI-only and mTBI+PTSD veterans, greater bilateral occipital P300 magnitude correlated with greater overall CAPS PTSD severity score ($r = 0.329$ with $p < 0.038$; Fig. 4A). Correlation strength was greater and more significant with the avoidance sub-score ($r = 0.472$, $p < 0.006$; Fig. 4B), but not significant for hyperarousal and re-experiencing sub-scores ($r = 0.312$, $p < 0.057$, and $r = 0.230$, $p < 0.124$, respectively), than for total CAPS score. Finally, the P300 magnitude also significantly correlated with BDI-II scores ($r = 0.321$ with $p < 0.045$; Fig. 4C).

Conclusions: Among mTBI veterans, posterior theta EEG activity during emotional appraisal is associated with greater PTSD symptoms, especially when avoidance and hyperarousal are prominent symptoms.

Consistent with our hypothesis, veterans with comorbid PTSD and mTBI exhibited a larger emotion processing ERP from the occipital cortex. Furthermore, this is accompanied by greater coordinated theta power from occipital areas. The magnitude of both observed abnormalities also correlated with PTSD severity, consistent with a model in which the negative effects of PTSD on outcomes following mTBI arise from abnormalities in oscillatory corticolimbic activity.

Keywords: Combat PTSD, Mild Traumatic Brain Injury, EEG Biomarkers

Disclosure: Nothing to disclose.

M8. Neuromelanin-Sensitive MRI Signal is Associated With Functional Striatal Response to Social but Not Monetary Reward Processing: Potential Mechanisms for Social Anxiety

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Background: Although peer acceptance is a highly salient form of reward, neural response to reward has largely been studied in the monetary domain. Testing reward processing in the social domain is important when considering the neural mechanisms implicated in social anxiety. Direct tests of the association between social anxiety symptoms and neural responses across reward domains are rare. Moreover, most research examining relations between brain function and reward processing confound the intrinsic reward of being correct with receiving positively-valenced outcomes. Yet, symptoms of social anxiety may be differentially associated with dysregulated processing of intrinsic (being correct) and extrinsic (receiving a positively-valenced outcome) rewards across social and non-social domains. Our prior work used well-matched EEG- and fMRI-based tasks to disentangle the brain's response to the intrinsic reward of being correct from its response to positively and negatively valenced outcomes in social (acceptance, rejection feedback) and monetary (monetary gain, loss) domains. More severe symptoms of anxiety were associated with greater cortico-striatal engagement when accurately predicting social rejection feedback. Given the role of striatal dopamine in reinforcement learning, this suggests a dopamine-based mechanism by which negative peer feedback may be intrinsically rewarding to anxious individuals. We have recently demonstrated that neuromelanin (NM) MRI signal in the substantia nigra provides a putative proxy measure of striatal dopamine function. Although NM signal has been linked to dopamine-related disorders, it has rarely been assessed in conjunction with brain function during psychosocial processing. Here, we test the relation between NM signal and functional engagement of striatum during social and monetary reward processing in a novel sample of young adults with a range of social anxiety symptoms.

Methods: Participants (N = 36; 21 females; 21.06 ± 3.43 years) completed a neuromelanin-sensitive MRI (NM-MRI) and well-matched fMRI-based monetary and social outcome tasks. In the monetary task, a pair of doors appeared on the screen. Half of the trials had a positive valence goal: correctly predict the door that will result in a monetary gain (win trials). On the other trials, there was a negative valence goal: correctly predict the door that will result in a monetary loss (loss trials). Incorrect predictions resulted in a null outcome. The social task had identical attributes except doors were replaced with photos of age-matched peers. Participants were told that peers had rated them after receiving a text of their picture. Positive and negative valence goals were to correctly predict which peer had liked (acceptance trials) or disliked them (rejection trials), respectively. Incorrect predictions resulted in a null outcome, reflecting that the purported peer never received a text. NM-MRI signal was assessed from a subset of substantia nigra voxels we previously showed are related to striatal dopamine release capacity (measured with dopamine PET). Signal was extracted from these voxels and quantified as a contrast to noise ratio relative to a nearby reference region with minimal NM concentration. This provided a putative proxy measure of dopamine function such that higher NM signal reflects greater dopamine function in the ventral striatal pathway. Analyses (3dMVM, AFNI) tested the relation between NM-MRI signal and functional engagement in striatum during fMRI-based monetary and social tasks ($p < .005$, ke > 25). Spearman's ρ tested associations between symptoms of social anxiety and functional engagement related to NM-MRI signal.

Results: A Domain (social, monetary) X Valence (positive, negative) X Outcome (accurate, inaccurate) X NM-MRI signal interaction emerged in right caudate (MNI=15, 21,10; ke=26; $F(1,34) = 11.76$, $p < .005$, $\eta p2 = .26$). This was driven by a Valence X Outcome X NM-MRI signal interaction in the social ($F(1,34) = 15.44$, $p < .001$, $\eta p2 = .31$), but not monetary domain. To ease interpretation, participants were split into groups based on median NM-MRI signal. In the social domain, Valence x Outcome interactions were found in those with high ($F(1,17) = 6.78$, $p < .05$, $\eta p2 = .30$) and low ($F(1,17) = 7.30$, $p < .05$, $\eta p2 = .29$) NM-MRI signal. In those with high NM-MRI signal, there was greater striatal activation for accurate-vs-inaccurate predictions for accepting feedback ($t(17) = 3.06$, $p < .01$), but no difference for rejecting feedback. In those with low NM-MRI signal, the opposite pattern emerged: there was greater striatal activation for accurate-vs-inaccurate predictions for rejecting feedback ($t(17) = 2.91$, $p = .01$), but no difference for accepting feedback. Greater striatal engagement to accurate-vs-inaccurate predictions for rejecting feedback was associated with more severe symptoms of social anxiety ($rS = .37$; $p < .05$).

Conclusions: We demonstrate that NM signal relates to brain function during psychosocial processing. We also show preliminary support for a dopamine-related mechanism by which correctly predicting negative peer feedback may be intrinsically rewarding, particularly among those with more severe social anxiety. Testing the association between NM signal, social anxiety, and functional engagement during social reinforcement learning is an important next step. Results could reveal a biologically based, psychosocial target for novel interventions for social anxiety.

Keywords: Social Rejection, Monetary Reward, Social Anxiety, Striatum, Neuromelanin

Disclosure: Nothing to disclose.

M9. Long-Term Trajectories and Predictors of Post-Traumatic Stress Symptom Development in Military Servicemen Deployed to Afghanistan

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Background: Symptoms of post-traumatic stress disorder (PTSD) can manifest several years after the actual trauma exposure. As military deployment can put soldiers at increased risk for developing PTSD symptoms, longitudinal evaluations of PTSD symptoms and associated risk factors in deployed military personnel are essential to map the psychological burden on our servicemen and to elucidate the factors which may contribute to the development and worsening of PTSD symptoms.

Methods: In the current study longitudinal development of PTSD symptoms was examined in a cohort of Dutch Afghanistan veterans (N = 963). Participants were assessed seven times from pre-deployment up to ten years after deployment. Latent growth modeling was used to identify

distinct trajectories of PTSD symptom development. A random forest model integrating biological, psychological and social data was used to predict PTSD trajectory membership

Results: Four distinct trajectories of PTSD symptom development were identified: resilient (85%), improved (6%), severely elevated-recovering (2%), and delayed onset (7%). Only the delayed onset group reported increasing symptom levels between five and ten years post-deployment, even though this group reported high use of psychological care (77%). Results of the random forest model were not available at the time of the abstract submission, but will be presented during the mini-panel.

Conclusions: This study demonstrates the long-term dynamics in the development in PTSD symptoms in Dutch Afghanistan veterans. It identifies a group of veterans with further increasing PTSD symptoms that does not seem to benefit after seeking psychological help. Our prediction model provides insight in the role several risk factors may play in the development of symptoms over time.

Keywords: Combat PTSD, Symptom Trajectory, Longitudinal Analysis, Risk and Resilience

Disclosure: Nothing to disclose.

M10. Changes in Functional Connectivity After Theta-Burst Transcranial Magnetic Stimulation for Posttraumatic Stress Disorder: A Machine Learning Study

Abstract not included.

M11. Prenatal Citalopram Promotes Resilience in Offspring Exposed to Maternal Stress

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Background: Untreated maternal mood and anxiety disorders can have adverse effects on offspring, including increased risk for depression and anxiety disorders in offspring. However, it remains unclear how the use of selective serotonin reuptake inhibitors (SSRIs) during pregnancy, while beneficial to mothers, affects their offspring throughout development and during adulthood. Using mice as a model organism, we implemented a chronic unpredictable stress (CUS) paradigm in dams during the mid- to late stages of pregnancy. Some dams were also treated orally with the SSRI citalopram to test the hypothesis that SSRI treatment in mothers has beneficial effects for at-risk offspring.

Methods: Experimental groups were (1) maternal CUS, (2) maternal CIT, (3) maternal CUS+CIT, and (4) control. We sacrificed subsets of offspring from each litter at postnatal days 7, 14, and 21, which are important time points during serotonin system development. Neurochemical analyses were performed on the brains of postnatal offspring using high-performance liquid chromatography and protein concentration analysis. The remaining offspring matured until 3 months of age. At this time, we assessed changes in behavior through the use of four tests: elevated plus maze, open field test, forced swim test, and novelty suppressed feeding. Sample sizes were selected to control for litter effects, i.e., 10-12 dams per group.

Results: We observed that in pups born to stressed dams, serotonin, norepinephrine, tryptophan, and tyrosine levels were elevated at postnatal day 7 in the forebrain. Furthermore, pups born to stressed dams had elevated protein concentrations in the forebrain at postnatal day 7. Concomitant treatment with CIT during CUS attenuated stress effects on neurochemistry in P7 offspring. We found that adult male offspring born to stressed dams exhibited increased avoidance of anxiogenic environments in all tests. Similar to the developmental neurochemical findings, maternal treatment with CIT during pregnancy attenuated stress-associated behavioral changes in adult male offspring.

Conclusions: Behavioral alterations were not observed in adult female offspring suggesting that male offspring are selectively vulnerable to maternal stress. Our findings suggest that CIT treatment during pregnancy in mothers diagnosed with or at high risk for affective and anxiety disorders may promote structural plasticity and behavioral resilience in male offspring.

Keywords: Selective Serotonin Reuptake Inhibitors (SSRIs), Perinatal Stress, Transgenerational Epigenetic Effects, Fetal Exposures of Alcohol and Other Drugs, Neurodevelopmental and Behavioral Deficits, Stress and Anxiety Disorders, Citalopram, Risk and Resilience

Disclosure: Nothing to disclose.

M12. Delayed Emotion Circuit Maturation Following Childhood Abuse Exposure

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Background: Childhood abuse represents one of the most potent risk factors for developing psychopathology, especially in females. Recent evidence suggests that exposure to early-life adversity may be related to advanced maturation of emotion processing neural circuits. However, it remains unknown whether abuse is related to early circuit maturation and whether maturation patterns depend on the presence of psychopathology.

Methods: The current study examines these questions in a multi-site sample of 246 females (ages 8–18) completing clinical assessment, maltreatment histories, and high-resolution T1 structural magnetic resonance imaging (MRI). Girls were stratified based on abuse history and internalizing disorder diagnosis: Typically-Developing (TD; no abuse/no diagnosis), Resilient (abuse/no diagnosis), and Susceptible (abuse/diagnosis) girls. Machine learning models of normative structural brain development were aggregated into a stacked generalization framework, trained to predict chronological age using gray matter volume (GMV) in TD girls from whole-brain, emotion, and language circuitry parcellations. The "super learner" predicted chronological age and brain age gap estimates (BrainAGE; predicted age minus true chronological age) were calculated as indices of relative circuit maturation.

Statistical Details:

Exp. 1: Stacked generalizer, ridge regression, multilayer perceptron, support vector, random forest, and gradient boosting submodels were optimized using 10-fold cross validation. Chronological age was predicted from gray matter volume in $N = 79$ TD girls. Submodel predictions were aggregated with ridge regression trained on submodel hold-out predictions. Mean absolute error was evaluated on a test set of $N = 20$ TD girls.

Exp. 2: Group differences in BrainAGE. Three multiple regression models were conducted on BrainAGEs for each of the three circuit feature parcellations (whole-brain, emotion, language). Each included binary abuse exposure (yes: $N = 50$), binary psychopathology diagnosis (yes: $N = 85$), scanner site, IQ, model bias (chronological age), and neglect.

Exp. 3: Feature importance analysis. One-thousand bootstrap samples with replacement of abuse-related gray matter volume were taken from Resilient and Susceptible girls. BrainAGEs were recalculated with abuse-related perturbation for that feature in the model. Feature importance score was calculated as the mean change in performance across bootstraps, relative to chance expectations.

Results: There were no abuse- or diagnosis-related differences in whole-brain or language circuit BrainAGEs. However, abused girls showed reduced BrainAGE relative to TD girls in emotion circuits ($F[1,150] = 15.680$, $t[150] = -2.366$, $p = 0.014$). Additionally, younger emotion circuit BrainAGEs were related to increased hyperarousal symptoms ($t[96] = -2.050$, $p = 0.043$). Feature importance analyses revealed differential GMV contributors to BrainAGE in Resilient versus Susceptible abused girls,

especially in lateral prefrontal and parietal cortices, insular cortex, and hippocampus.

Conclusions: These results suggest that abuse exposure in girls is associated with delayed structural maturation in emotion circuitry. The relationship between delayed maturity and hyperarousal symptoms may represent an adaptive response to enhance threat detection in dangerous environments. However, the differential influence of fronto-parietal cortices and hippocampus on BrainAGE in Resilient girls may represent protective neurodevelopmental markers of reduced psychiatric risk following abuse.

Keywords: Child Abuse and Neglect, Prefrontal Circuit Maturation, Structural MRI, Stress Resilience and Susceptibility, Adolescent PTSD

Disclosure: Nothing to disclose.

M13. Hyperarousal Symptoms of PTSD Correlate to Neuromelanin-Sensitive MRI Signal in the Locus Coeruleus, a Putative Measure of Noradrengeric System Function

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Background: The central noradrenergic system plays a key role in arousal and consolidation of emotional memory. The locus coeruleus (LC), the primary site of noradrenergic neurons in the brain, has a topographic pattern of projections, with the caudal extent of the LC sending descending projections modulating autonomic signalling. Dysregulation of the noradrenergic system has been implicated in theoretical accounts of PTSD, particularly in regard to symptoms of hyperarousal. Despite a strong theoretical foundation, understanding of noradrenergic dysfunction in PTSD is incomplete, impeding research into novel treatments targeting this system in PTSD. Recent work has pioneered the use of a specialized neuroimaging technique, Neuromelanin-Sensitive MRI (NM-MRI), a non-invasive method to probe the function of the human noradrenergic system in vivo by examining signal contrast in the LC. NM-MRI signal here has been positively related to emotional memory performance and autonomic function (indexed by salivary alpha amylase or heart rate variability) but it has yet to be investigated in individuals with PTSD. On the other hand, low LC NM-MRI signal has been observed in major depressive disorder. We hypothesized that hyperarousal symptoms of PTSD would be positively correlated to NM-MRI signal in the caudal LC.

Methods: Participants were combat-exposed Canadian Armed Forces veterans suffering from PTSD or major depressive disorder ($n = 24$, age $= 48.0\pm8.5$, 71% male). Most participants were recruited from the Operational Stress Injury Clinic at the Royal Ottawa Mental Health Centre. The majority of these ($n = 19$) met diagnostic criteria for PTSD on the Clinician-Administered PTSD Scale for DSM 5 (CAPS). All participants underwent an MRI scanning session to obtain a T1-weighted anatomical image and a NM-MRI image (NM-MRI; a 2D-gradient recalled echo sequence with magnetization transfer contrast). NM-MRI signal from the locus coeruleus (LC) was calculated by segmenting the LC on the unprocessed NM-MRI image and calculating the contrast-to-noise ratio (CNR) of voxels within the LC relative to a central pons reference region containing minimal NM. The LC segmentation was performed by warping an over-inclusive LC mask from MNI space to native space and using this as a search space wherein to find the brightest cluster of 6 adjacent voxels (2.6 mm2), defined as the LC. This operation was repeated for the right and left LC. The automated segmentation was visually inspected and was found to perform 6.5% of operations suboptimally, requiring manual correction. Division of the over-inclusive LC mask into 3 rostro-caudal segments allowed subregional segmentation of the LC. NM-MRI Signal within the caudal LC was calculated by averaging the brightest LC voxels bilaterally on all slices defined as caudal LC according to the caudal segment of the warped over-inclusive LC mask. Caudal LC signal was correlated to hyperarousal and depressive symptoms using partial correlations controlling for age, sex, and PTSD diagnosis.

Results: Most participants endorsed at least mild symptoms of hyperarousal (mean CAPS hyperarousal score $= 11.8\pm5.3$) and depression (mean Beck Depression Index total score $= 25.7\pm10.7$). PTSD and non-PTSD participants did not differ on severity of either type of symptom (all $p>0.19$, 2-sample t-tests). Consistent with our hypothesis, NM-MRI signal in caudal LC was positively correlated to severity of the CAPS hyperarousal symptom cluster ($r=0.48$, $p = 0.030$, partial correlation controlling for age, sex, PTSD diagnosis, and depression severity). Similar to previous reports, LC NM-MRI signal was negatively correlated to depression severity ($r=-0.47$, $p = 0.035$, partial correlation controlling for age, sex, PTSD diagnosis, and hyperarousal severity). PTSD diagnosis ($n = 19$ PTSD vs $n = 5$ non-PTSD) was not significantly related to caudal LC signal ($t20=-1.0$, $p = 0.32$).

Conclusions: Our finding that the LC NM-MRI signal is correlated to hyperarousal symptoms is consistent with previous findings examining the LC NM signal and autonomic measures and also with evidence of hyperactivity of the LC in PTSD. Furthermore, this finding supports the hypothesis that the LC NM-MRI signal can interrogate LC function in a similar way that we have previously shown that the substantia nigra NM signal can interrogate dopamine function. Although the current sample was not well designed to test the effect of PTSD diagnosis on the LC signal, we did not observe evidence of such a diagnostic effect and, given the opposing effects of depression and hyperarousal symptoms on the LC signal and the common comorbidity of both symptom types in individuals with PTSD, a dimensional approach to PTSD symptomatology may be more appropriate for studies of this nature.

These preliminary findings, by linking a key symptom cluster of PTSD to a proxy neurochemical measure that is relatively easy to acquire, are a promising step in the effort to move toward a future where the heterogeneity of PTSD can be characterized based on pathophysiological profiles rather than clinical symptoms alone. Identifying patients with elevated noradrenergic function could help guide treatment selection and help develop novel drugs targeting this system that has been linked to burdensome PTSD symptoms including hyperarousal and nightmares.

Keywords: PTSD, Noradrenaline, Hyperarousal, Neuromelanin-Sensitive MRI

Disclosure: Terran Biosciences: Patent (Self)

M14. Autonomic Features in Posttraumatic Stress Disorder: "Les Prophéties" for Theta-Burst Stimulation Response?

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Background: Abnormalities in sympathetic response and decreased parasympathetic inhibition are associated with posttraumatic stress disorder (PTSD) and may manifest as maladaptive cardiovascular autonomic activity, resulting in a perpetual

hyperarousal state. These autonomic abnormalities are generally expressed as elevated heart rate and reduced heart rate variability (HRV). Application of intermittent theta-burst stimulation (iTBS), a novel repetitive transcranial magnetic stimulation technique, has demonstrated superior clinical outcomes as compared to sham stimulation in PTSD, and is a promising tool to modulate the frontolimbic network involved in pathological autonomic response associated with PTSD. Nevertheless, it remains unclear if iTBS affects the autonomic nervous system and whether HRV features may be a potential biomarker of clinical response to iTBS for PTSD.

Methods: Fifty veterans with PTSD participated in a randomized, controlled trial, where they received 10 daily-sessions of sham-controlled iTBS over the right dorsolateral prefrontal cortex (DLPFC), 1,800 pulses/day at an intensity of 80% of the active motor threshold. Using a per-protocol sample ($n = 37$ with viable data and at least one session of iTBS), we evaluated the effects of iTBS on autonomic response using ultra-short HRV features, including the root mean square of the successive differences (RMSSD) and low frequency/high frequency power ratio (LF/HF) at baseline (time 0) and after the last iTBS (time 1). Additionally, we applied univariate analysis to assess HRV parameters as potential predictors of clinical response considering outcomes obtained at time 1, one month after stimulation (time 2), and 1-year later (time3).

Results: In regard to HRV parameters, a nonparametric two-sample Wilcoxon matched-pairs signed rank and Wilcoxon rank-sum (Mann-Whitney) tests showed no statistical significance within and between-groups (iTBS1800 vs. sham), respectively (all $p \geq 0.05$). The effect sizes of HRV outcomes difference between-groups after intervention were small ($r \leq 0.10$). Neither RMSSD nor LF/HF were significant predictors of short or longer-term clinical outcomes. Exploratory analysis of HRV features in 4 periods (30s, 45s, 60s, and 195s) also did not reveal effective prediction for clinical response predicated on iTBS in PTSD.

Conclusions: Our findings do not support the hypothesis that iTBS affects autonomic activity quantified by various measures of HRV. While the trial was likely underpowered to address this question, the absence of findings across all outcomes is notable. However, conclusive evidence on whether autonomic activity can be used as a biomarker of clinical response to iTBS in PTSD remains elusive. Furthermore, the naturalistic patient population may have introduced confounding factors related to cardiovascular and associated clinical factors. Moreover, limitations of the study include those inherent to secondary analysis of clinical trials, namely recruitment did not focus on autonomic or related activity. Additionally, this study did not evaluate direct measures of frontolimbic activity (i.e. functional neuroimaging) and we are unable to conclude whether iTBS modulated these networks or insufficiently so in order to detect downstream changes. That stated, future trials might consider acquisition of ECG during iTBS, perhaps with a greater number of iTBS sessions, and specifically focus recruitment on patients with elevated hyperarousal symptoms in order to specifically address the question whether iTBS affects autonomic activity. Clearly, further studies are also required to characterize the physiological effects of iTBS and develop low-cost biomarkers to identify patients most likely to respond.

Keywords: Posttraumatic Stress Disorder, Theta-Burst Stimulation, Autonomic Nervous System, Heart Rate Variability, Frontolimbic Network

Disclosure: Nothing to disclose.

M15. Bridging the Gap Between Innate and Learned Behaviors: A Parental Role in Promoting Survival

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Background: My research investigates the relationship between the innate and the learned. I examine how an organism unlocks an innate behavior at the appropriate time (maternal instinct), and how learned information is passed to subsequent generations via paternal transgenerational epigenetic inheritance. Together, I investigate how learned behaviors in a parent can become innate behaviors in the offspring.

When faced with the sound of crying pups, virgin female mice routinely ignore the pups, and rarely retrieve them to their nest. However, after giving birth, new mothers exhibit a modulated set of behaviors, many of which are driven by auditory cues. I found that: (i) the oxytocin receptor is expressed preferentially in the left auditory cortex, and is required in the left, but not right auditory cortex for retrieval behavior; (ii) mothers, but not naïve virgins, exhibit time-locked neural responses to pup calls in the left auditory cortex; and most importantly (iii) virgin mice exhibit these signature responses when pup calls are paired with topical application of oxytocin or optogenetic stimulation of oxytocinergic neurons [Marlin et al., Nature 2015]. My initial discoveries have provided novel insights into the fundamental mechanisms underlying the induction of innate behaviors.

Building upon these findings in my postdoctoral work, I have expanded my focus from the emergence and refinement of innate behaviors, to the transmission of learned behaviors across generations. This phenomenon, known as transgenerational epigenetic inheritance (TEI), provides an intriguing mechanism by which parents could prepare their offspring for dynamic selective pressures that they have themselves experienced in their lifetime. In humans, following the Dutch Hunger Winter famine of 1944-1945, the children of famine survivors exhibited high rates of early mortality and metabolic illnesses such as obesity, diabetes and cardiovascular disease. This period of stress in famished parents was also associated with adverse psychological outcomes in their children, such as increased anxiety and depression [Harris and Seckl, 2011, Bygren, 2013]. These studies have raised the possibility that TEI is an important mechanism for the transmission of learned behavioral responses to aversive experiences across generations.

There is mounting evidence that stress-induced epigenetic changes impact the morphology of the brain, most clearly demonstrated through olfactory fear conditioning. The pairing of a stressful stimulus such as a foot-shock with a behaviorally neutral odor increases the number of olfactory sensory neurons (OSNs) responsive to the conditioned odor. Surprisingly, studies suggest this increase persists in the offspring [Dias and Ressler, 2014]. The changes in neural structure are paralleled by increased avoidance of a previously neutral odor.

Methods: I directed my expertise in the neural mechanisms of behavior to the phenomenon of TEI. My broad aim is to investigate how learning and the subsequent changes in neural organization and function in parents are transmitted to offspring. I adopted a quantitative approach to examine neuronal number in both conditioned parents and their offspring as follows. After olfactory conditioning, I remove the main olfactory epithelium (MOE), which contains the OSNs, and the main olfactory bulb, which houses their terminals. Using the immunolabeling-enabled three-dimensional imaging of solvent-cleared organs (iDISCO) tissue clearing method, I clear and stain the MOE for neurons that express a receptor for the paired odor in conditioned and unconditioned animals. Using light sheet microscopy to image the entire epithelium and bulb.

Results: I observed a significant increase in MOE cell number in response to the conditioned odor (Tukey's Multiple Comparison

Test p = 0.0088). This increase is not merely a consequence of stimulus-related activity, given that both the paired (odor co-terminating with shock) and unpaired (60 second delay between odor and shock) experimental groups are presented with the odor (p = 0.0218). An increase seems to require that the odor gains valence by being paired in time with shock. This poses an interesting problem: how is the information to increase cell number transmitted to immature cells in the main olfactory epithelium? Remarkably, the increase is also observed in F1, animals which are experientially naïve to the odor. My preliminary data has positioned me to ask: how are changes transmitted to the germline, maintained during development, and recapitulated in the epithelium of offspring?

Conclusions: Taken together, my research will examine the process in which a learned behavior can be transmitted across generations, providing innovative insight into mechanisms of epigenetic inheritance. Implicit in these findings is that the categorical distinction between innate and learned behaviors may be fundamentally flexible. Thus, a learned behavior in the parent can essentially become an innate behavior in the offspring. My studies, although emergent, illuminate a novel biological system supporting behavioral change at the time scale of a single generation.

Keywords: Epigenetics, Synaptic Plasticity, Behavior, Maternal Behavior, Transgenerational

Disclosure: Nothing to disclose.

M16. Estradiol Regulation Dopamine Release Plays a Critical Role in Addiction Vulnerability in Females

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Background: While sex differences in the pervasiveness and prognosis of addiction have long been known to exist, we still lack a complete understanding of why these sex differences emerge and the neurobiological mechanisms driving them. The mesolimbic dopamine system has been shown to be involved in the expression of many sex-specific motivated behaviors and is thought to be a critical mediator of female addiction vulnerability. Many sex differences exist at baseline - independent of the estrous cycle - and reflect differences in dopamine system organization/neuroanatomy. Additionally, there is also robust dopamine release regulation by ovarian hormones, such as estradiol. Here we developed novel reinforcement tasks to define sex-specific behavioral strategies and combined these with subsecond dopamine monitoring in the nucleus accumbens (NAc) to describe how hormonal control of dopamine microcircuit function gives rise to sex differences in motivated behavior for drugs and natural rewards.

Methods: To disentangle the interaction between drug-associated cues and the consummatory and appetitive responding driven by cocaine, we have developed a new behavioral procedure that combines Pavlovian-instrumental transfer with behavioral economic analysis. This task can be completed within a single session, allowing for studies looking at estrous cycle stage-dependent effects in intact cycling females, something that has been difficult in the past. We then combine this, and other operant tasks, with fast-scan cyclic voltammetry and pharmacology to define how hormonal regulation of dopamine release – via local acetylcholine microcircuits – are specifically related to these sex-specific motivational strategies for drug and non-drug rewards.

Results: We found no differences in cocaine self-administration across the estrous cycle in the absence of cocaine-paired cues; however, when cues were introduced, the cues that acquired value during estrus-but not during diestrus or in males-increased motivation. These data suggest that the processing of cues plays a critical role in determining sex differences in motivation. In the NAc, dopamine release is known to play a critical role in associative learning for reward-predictive cues and is heavily modulated by the activity of cholinergic interneurons signaling through nicotinic acetylcholine receptors located directly on dopamine terminals. We find critical differences in cholinergic regulation of dopamine terminals and their regulation by estradiol that underlies sex-specific behavioral strategies and cue-reward associations between males and females.

Conclusions: Together, these data suggest that fundamental differences in the motivational properties of psychostimulant drugs between males and females are complex and are driven primarily by the interaction between drug-associated stimuli and drug effects. We find critical differences in cholinergic regulation of dopamine terminals that underlies these differences in behavior between males and females. These findings are especially important to consider when thinking about how these differences lead to disease pathology and how to develop efficacious interventions in both sexes.

Keywords: Reinforcement Learning, Decision Making, Sex Differences, Brain, Dopamine, Adaptive Behavior, Learning, Cocaine

Disclosure: Nothing to disclose.

M17. Mental Health of Residents and Fellows at SUNY Downstate Health Sciences University During the Pandemic of COVID-19

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Background: In December of 2019, the novel coronavirus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was reported in Wuhan, China, by the Municipal government, and the disease was later named as COVID-19. The World Health Organization (WHO) designated COVID-19 as a global pandemic on March 11th, 2020, after the disease had spread to more than 70 countries. The United States is one of the most affected countries worldwide. New York State was one of the earliest and most affected states by the disease. On March 28th, 2020, New York Governor Cuomo declared State University of New York (SUNY) Downstate Health Sciences University a COVID-19 only facility, which allowed the hospital to accept suspected or confirmed COVID-19 cases only, and other patients would be distributed to nearby facilities. As part of essential healthcare workers, residents and fellows at SUNY Downstate were exposed to multiple stresses, such as working with limited resources and fearfulness of acquiring the illness as a result of caring for patients. Our study aimed at screening for symptoms of depression, generalized anxiety, panic disorder, post-traumatic stress disorder, and burnout in residents and fellows during the COVID-19 pandemic.

Methods: We sent an anonymous cross-sectional Qualtrics-mediated online survey to all residents and fellows affiliated with SUNY Downstate during the period from May - July 2020. The survey included questions about demographic information, COVID-19 exposure, Generalized Anxiety Disorder 7-items questionnaire (GAD-7), Patient Health Questionnaire-2 (PHQ-2), and screening questions for panic disorder and post-traumatic stress disorder. Data were analyzed using SPSS 27.

Results: We received 121 valid responses, which included residents and fellows from thirty different specialties and subspecialties, and with different training levels. While 24% (N = 29) of the sample mentioned spending all their time caring for COVID-19 patients, 17.4% (N = 21) mentioned caring for COVID-19 patients more than 75% of their time, and 10.7% (N = 13) mentioned spending 50–75% of their time caring for COVID-19 patients. We also inquired about the personal experience of losses, and 33.1% (N = 40) of the sample reported losing 1-10 patients, and 28.9% (N = 35) of the sample has lost more than ten patients to the illness. Moreover, 35.5% (N = 43) of the sample lost 1-10 colleagues, and 31.4% (N = 38) reported the loss of 1-10 supervisors to COVID-19. On the other hand, 5% (N = 6) of the sample lost 1-10 family members, and 9.1% (N = 11) reported the loss of 1-10 friends to COVID-19. As regards symptoms, 19.8% (N = 24) of the sample reported mild symptoms of GAD, 15.7% (N = 19) reported moderate symptoms of GAD, and 17.4% (N = 21) reported severe symptoms. 9.9% of the sample (N = 12) reported PHQ of 3 or more, and 23.1% (N = 28) reported at least one symptom of panic disorder, and 28.9% (N = 35) responded positively to PTSD questions in relation to COVID-19. There were no outliers in the data, as assessed by inspection of a boxplot. A Mann-Whitney U test was run to determine if there were differences in GAD, depression, and burnout scores between males and females. Distributions of scores were similar, and median scores were not statistically significantly different between males and females for GAD ($p = 0.201$), and depression ($p = 0.738$). Burnout scores were higher in females (Mean rank = 61.92) than males (Mean rank= 50.25) with tendency to significance ($p = 0.56$). A chi-square test for association was conducted between gender and symptoms of panic as well as PTSD. All expected cell frequencies were greater than five, and there were no statistically significant gender differences in panic ($p = 0.27$) or PTSD ($p = 0.137$) symptoms. A chi-square test for association was conducted between the personal experience of loss and the development of panic and PTSD symptoms. There was a statistically significant association between the recent loss of a colleague and the experience of a traumatic event related to COVID-19, $\chi2(1, N = 121) = 6.275$, $p = 0.012$.

Conclusions: Our results suggest an increased frequency of symptoms of anxiety, depression, panic, and PTSD among residents and fellows at SUNY Downstate during the COVID-19 pandemic, with PTSD more related to loss of colleagues.

Keywords: COVID-19, Depression, GAD, PTSD, Stress and Trauma

Disclosure: Nothing to disclose.

M18. Innate Fear Response is Reflected in the Blood Methylome of Rhesus Macaques and Overlaps With the Epigenetic Signature of Fear in Human

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Background: Anxiety is a complex phenomenon often emerging comorbid with neuropsychiatric disorders; however, our understanding of the underlying neuronal circuits and biological correlates is limited. Pathological anxiety appears to be a function of both genetic and environmental factors and features of anxiety in humans can be recapitulated in suitable laboratory animals. Here we investigated individual variations in fear response to a snake stimulus in free-ranging rhesus monkeys and discovered an unusual bimodal distribution of fearful and fearless behavior, presumably as a result of an environmental insult by a Hurricane. In a translational approach we determined the DNA methylation profile of fear in these monkeys and show evidence that this signature is relevant for the acoustic startle response in human. Our data highlight the importance and translational utility of non-human primate models for neuropsychiatric research and provide insight into biological correlates of fear.

Methods: A free-ranging population of rhesus monkeys (Macaca mulatta) from the Caribbean Primate Research Center at Cayo Santiago, Puerto Rico was tested for their innate fear response to a rubber snake (n = 171; age range: 4 – 25 years; 111 males and 60 females). Monkeys were provisioned daily with commercial food and water and are able to browse natural vegetation. Data were collected over a two-year period (2009 - 2011), May through September, during the hours of 7:00 AM - 12:00 PM. A subset was captured for blood drawing (n = 147) and 5 ml EDTA blood tubes were collected. After DNA extraction was performed, a final set of n = 90 animals were available for DNA methylation analysis using the Illumina EPIC arrays. Raw data in the form of IDAT files were exported and subsequently processed in R. Due to the close genetic relationship between human and rhesus monkey we use the Illumina EPIC methylation array platform as a translational tool to compare epigenetic signatures in rhesus monkeys and humans.

Results: Of 171 monkeys tested, 81% were fearful to the snake stimulus, whereas 19% were fearless. We observed a bimodal distribution of fear responses, with fearless monkeys being significantly older than fearful monkeys ($t = 7.35$; $p < 0.01$). In fact, monkeys born prior to the occurrence of Hurricane Georges in 1998 separated into distinct fearless and fearful subgroups, compared to those born after the hurricane who were mostly fearful. This was true for both males and females.

Mapping efficiency of the EPIC methylation array probes to the rhesus macaque genome was 51.5%, and after exclusion of probes with a mismatch in the 5 positions closest to the target site, 377,181 high-confidence human-macaque probes remained. After additional filtering of outlier, a final set 343,441 probes were available for the EWAS. A model with 1 SV was fitted and EWAS results did not show major inflation (lambda values = 1.01). 49 differentially methylation positions associated with fear response were detected at pFDR<0.05, 18 (37%) showed increased methylation with decreased fear response, whereas 31 (63%) showed increased methylation with increased fear response. In addition, a regional analysis identified 15 differentially methylation regions (8 hypermethylated, 7 hypomethylated) associated with fear response. For the gene set enrichment analyses, 4 significantly enriched KEGG pathways were found (cAMP signaling, AMPK signaling, cholinergic signaling and glucagon signaling, all $q < 0.05$)

In order to investigate a potential overlap between the DNA methylation signature of fear in rhesus and fear-related phenotypes and DNA methylation in human we leveraged data from the Grady Trauma Project, specifically focusing on startle psychophysiology data. Selecting trauma-exposed individuals with startle data available we had n = 134 individuals available for analysis. After processing, 780,559 probes remained with 46 of the 49 fear-associated sites found in the hurricane-exposed monkeys present in the processed GTP data. However, EWAS testing did not yield genome-wide significant results for baseline startle response, which was expected given the overall sample size. Of note, effect sizes for the monkey EWAS (log2 fold change range −0.79 – 0.74) were much higher than those in the human analysis (log2 fold change range −0.0013 – 0.0012). Nevertheless, the 46 sites associated with fear in rhesus had significantly lower p-values in our human dataset compared to all other sites (Wilcoxon rank sum test p = 3.03E-04; permutation p = 3.00E-04) providing evidence for an overlap between the DNA methylation signature of fear in

rhesus monkey and the DNA methylation signature of baseline startle response in human.

Conclusions: Fear and fear-related disorders are highly prevalent and knowledge on underlying causal mechanisms and biomarkers that are relevant for diagnosis, therapy and prevention is critically needed. We show evidence for fear-associated DNA methylation profiles in a non-human primate model. Overlap of the detected signature with methylation profiles in human clinical samples highlight the translational relevance of this approach.

Keywords: Rhesus, DNA Methylation, Fear

Disclosure: Alkermes Inc: Consultant (Self)

M19. Genetic Endocannabinoid Variation Does Not Predict Differential Neural Fear Activation During Fear Extinction in Healthy Humans: A Preliminary Study

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Background: The endocannabinoid (eCB) system moderates responses to anxiety- and fear-inducing stimuli. Preclinical research suggests that individual variability in this system may confer risk for anxiety or trauma-related disorders. Differential expression of fatty acid amide hydrolase (FAAH), a catabolic enzyme and primary regulator of eCB signaling in the brain, has been linked to a common single-nucleotide polymorphism (FAAH C385A; rs324420) and successful fear extinction. The present study examines fear-related neural activation in healthy adults during extinction recall that may differ due to FAAH C385A.

Methods: 33 healthy adults (ages 18-54) completed a novel Pavlovian fear-extinction paradigm using virtual reality coupled with fMRI in regions of interest: hippocampus, ventral medial prefrontal cortex (vmPFC), dorsal anterior cingulate cortex (dACC), and amygdala. During acquisition, two conditioned stimuli (CSs) were presented: CS+ was paired with an aversive unconditioned stimulus (US), whereas CS- was never paired with the US (safety cue). During extinction, both CSs were presented in the absence of the US. Participant genotype was determined from buccal samples using Taqman Genotyping.

Results: All participants were able to acquire differential fear to the CS+ and CS- and extinguish fear to the CS+. During fear extinction recall, dACC activation was significantly greater than zero to the CS+E ($p < 0.05$, 95% CI [0.008, 0.113], $r = 1{,}000$ bootstrapped samples). However, genetic eCB did not predict differential neural activation during fear extinction recall (p's > 0.05, $r = 1{,}000$ bootstrapped samples).

Conclusions: In healthy individuals, genetic eCB variation does not differentiate neural fear activation during extinction recall. These results are in opposition to previous preclinical research suggesting that FAAH C385A may alter neural responses to anxiety- and fear-inducing stimuli. Among healthy adults, genetic eCB is unrelated to stressor response. Variation in eCB genetics may play a larger role in stressor response in those with significant prior trauma or those with pre-existing anxiety disorders.

Keywords: Endocannabinoid System, Pavlovian Conditioning, Brain Imaging, fMRI, Genetic Variability

Disclosure: Nothing to disclose.

M20. Brain-Derived Neurotrophic Factor From Basolateral Amygdala Inputs to Lateral Septum are Necessary for Social Recognition in Mice

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Background: The lateral septum (LS) integrates sensory and affective information to modulate behavioral responses to environmental stimuli. LS dysfunction in humans is implicated in changes in social behavior, such as social aggression, and rodent lesion studies have reported changes in a variety of social behaviors, including social aggression and social recognition. However, the biological mechanisms by which the LS regulates social recognition remain unknown. Interestingly, levels of tropomyosin receptor kinase B (TrkB), the cognate receptor for brain-derived neurotrophic factor (BDNF), are highly expressed in the LS, while levels of BDNF itself are nearly absent. BDNF is an activity-dependent neurotrophin that has been implicated in the regulation of social behaviors. Following synaptic release, BDNF signals through its cognate receptor (TrkB) to promote neuron growth and survival, facilitate synaptic strength, and regulate synaptic plasticity. Here, we provide the first evidence that TrkB signaling in the LS is required for normal social recognition, and identify inputs to the LS from the basolateral amygdala (BLA) as the efferent source of BDNF for mediating this behavior.

Methods: We used viral transgenesis and circuit ablation techniques to investigate the role of BDNF-TrkB signaling and efferent inputs to the LS in short-term social recognition. All experiments used male mice. First, we used fluorescence in situ hybridization using RNAScope to quantify levels of Ntrk2 mRNA expression in specific cell types in the LS ($n = 3$ mice), and to quantify levels of BDNF in efferent inputs to the LS from BLA and ventral hippocampus (vHPC) ($n = 3$ mice). To examine whether the LS is active during social interaction, we used immunohistochemistry to examine activation of c-Fos in mice presented to a social stimulus (a novel mouse, $n = 4$ mice) and a non-social stimulus (a conical tube, $n = 4$ mice). We then examined the necessity of TrkB expression in the LS on short-term social recognition using virally transduced cre-mediated knockdown of TrkB receptors in the LS of mice with floxed Ntrk2 alleles ($n = 11$ mice). To examine the responsivity of neurons lacking TrkB to social stimuli, we generated supplemental mice with a knockdown of TrkB receptors ($n = 6$ mice) and mice with functional TrkB expression ($n = 6$ mice), and examined c-Fos expression in response to interaction with a socially novel mouse. Then we utilized circuit specific viral transgenesis of diphtheria toxin A to ablate LS inputs from the BLA ($n = 10$ mice) and vHPC ($n = 8$ mice) and examine their effects on short-term social recognition. Finally, we used viral transgenesis to induce cre-mediated knockdown of BDNF in BLA neurons projecting to LS using mice with floxed BDNF alleles ($n = 9$ mice). For each behavioral experiment, a control group ($n = 8$ to 10 mice) was generated using experiment matched viruses that only express a fluorophore, such as eGFP and mCherry. We performed RNAScope, immunohistochemistry, and western blot to confirm each of our viral manipulations was carried out successfully. Additional behavioral assays, such as a non-social odor discrimination task, were used to confirm that our behavioral phenotypes were specific to social experience.

Results: Greater than 85% of GABAergic interneurons in the LS express TrkB across both the rostral-caudal span of the LS and across the dorsal-ventral axis of the LS. Mice exposed to a social stimulus versus a non-social stimulus revealed selective activation of the middle portion of the LS ($t(6) = 5.543$, $p = 0.0044$), but not in the rostral LS ($t(6) = 0.5534$, $p = 0.839$) and the caudal LS ($t(6) = 0.3285$, $p = 0.840$). Utilizing a discrimination index derived from relative time spent examining socially novel and socially familiar

mice in the 3-chamber social interaction task, we demonstrated mice with functional TrkB receptors (Mdn = 8.883) discriminate socially novel from familiar mice greater than mice lacking functional TrkB receptors in the LS (Mdn = 2.555)(U(11,7) = 12, p = 0.0154). Mice lacking functional TrkB receptors also displayed decreased expression of c-Fos in response to social interaction in the middle portion of the LS compared to those with normal TrkB expression (t(9) = 3.069, p = 0.0396), but displayed no differences in c-Fos expression in the rostral LS (t(9) = 1.669, p = 0.242) and the caudal LS (t(9) = 1.665, p = 0.242). Mice with functional BLA neurons that project to the LS (Mdn = 8.017) discriminate socially novel from familiar mice greater than mice lacking BLA neurons that project to the LS (Mdn = 1.317)(U(10,10) = 17, p = 0.0115). Mice with functional vHPC neurons that project to the LS did not discriminate socially novel from familiar mice more than mice lacking vHPC neurons that project to the LS(Mdn = 9.600) (U(8,9) = 30, p = 0.8527). Mice that express BDNF in BLA neurons that project to LS (Mdn = 6.150) discriminate social novel from social familiar mice greater than mice lacking BDNF in BLA neurons that project to the LS (Mdn = 3.483)(U(9,9) = 9, p = 0.004).

Conclusions: Short-term social recognition is mediated by BDNF-TrkB signaling in the LS, and specifically by BDNF supplied through BLA inputs to the LS. Future studies include identification of intracellular mechanisms by which BLA-derived BDNF controls the transmission of social information to the LS and understanding how TrkB manipulation alters in vivo encoding of social stimuli in the LS.

Keywords: Lateral Septum, Basolateral Amygdala, Social Recognition Memory, Brain-Derived Neurotrophic Factor, TrkB

Disclosure: Nothing to disclose.

M21. Transcranial Magnetic Stimulation Modulates Glutamate/Glutamine Levels in Young Adults With Autism

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Background: The neurobiology of autism spectrum disorder (ASD) is thought to be associated with imbalanced neuronal excitation/inhibition, yet evidence of altered γ-aminobutyric acid (GABA) and glutamate + glutamine (Glx) levels in comparison to non-autistic controls are mixed. Repetitive transcranial magnetic stimulation (rTMS) may modulate inhibitory processes; thus, we utilized 1H magnetic resonance spectroscopy (MRS) to assess GABA+ and Glx levels in ASD participants that received active or sham rTMS to the dorsolateral prefrontal cortex (DLPFC) as part of a randomized, double-blind, sham-controlled clinical trial.

Methods: 28 ASD [23.3 (4.69) years; 7-female] and 19 control [23.8 (4.47) years; 6-female] participants underwent MRS as part of an rTMS clinical trial at the Centre for Addiction and Mental Health (CAMH, Toronto, Canada). ASD participants received active or sham rTMS 5 days/week for 4 weeks, totalling 20 sessions; they underwent another MRS session upon completion of the clinical trial. MEGA-PRESS data was acquired on a 3-Tesla GE scanner from a 20 x 40 x 30 mm3 voxel, positioned in the left DLPFC. Mean GABA+ and Glx levels were compared between ASD and control participants at baseline using ANCOVAs with age as a covariate. Pre/post-treatment metabolite GABA+ and Glx levels were compared between the active and sham treatment groups using 2 x 2 mixed model ANCOVAs, with rTMS treatment group (active vs. sham) as a between-subjects factor, time (pre- vs. post-treatment) as a within-subjects factor, and age as a covariate. The absolute change in GABA+ and Glx levels from pre- to post-treatment were compared between the active and sham groups, using ANCOVAs, including age as a covariate. Multiple linear regressions were performed to investigate if treatment group moderated the relationship between baseline metabolite level and metabolite change.

Results: ASD and control participants did not differ in mean GABA + (F(1,44)=0.59, p = 0.45) or Glx (F(1,44)=0.03, p = 0.86) levels at baseline. Moreover, mean GABA+ and Glx levels did not differ from pre- to post-treatment in either ASD treatment group; there was no main effect of group for GABA+ (F(1,26)=0.33, p = 0.57), Glx (F(1,25)=0.23, p = 0.64), and no group-by-time interaction, for GABA+ (F(1,24)=0.46, p = 0.50) or Glx (F(1,26)=0.72, p = 0.40). The absolute change in GABA+ level did not differ between treatment groups (F(1,19)=0.89, p = 0.36); however, the absolute change in Glx level was greater in the active vs. sham rTMS group (F(1,19)=6.54, p = 0.02, Cohen's f=0.59). rTMS moderated the relationship between baseline Glx and pre/post-treatment Glx change in the active group only (F(1,17)=4.78, p = 0.04, Cohen's f=0.53); baseline Glx predicted pre/post-rTMS Glx change (b=1.52, SE=0.32, t(17)=4.74, p<0.001), indicating that Glx levels increased in participants with lower baseline Glx levels, whereas Glx levels decreased in participants with higher baseline Glx levels.

Conclusions: Our findings indicate that baseline GABA+ and Glx levels may not differ between young adults with ASD versus matched controls; however, our pilot clinical trial suggests that rTMS may modulate Glx levels in young adults with ASD, such that the direction of change is associated with baseline Glx level. These results also demonstrate that MRS may be sensitive to changes in cortical metabolism following rTMS intervention in ASD.

Keywords: Autism, Repetitive Transcranial Magnetic Stimulation (rTMS), Magnetic Resonance Spectroscopy

Disclosure: Nothing to disclose.

M22. Sulforaphane as a Treatment for Autism: A Randomized Double-Blind Study

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Background: Some underlying biochemical abnormalities in Autism Spectrum Disorder (ASD) may be associated with oxidative stress and lower antioxidant capacity, depressed glutathione synthesis, reduced mitochondrial function and oxidative phosphorylation, increased lipid peroxidation, and increased neuroinflammation. Sulforaphane has chemical properties which may counteract some of these deficits. A double-blind study in the US found that sulforaphane ameliorated several measures of ASD symptoms, a finding supported by some open trials. We present results from a larger double-blind study of sulforaphane effects on children with ASD in China.

Methods: 110 children, male and female, ages 5-15, with a diagnosis of ASD, were enrolled in a 12 week randomized double blind study of sulforaphane or matched placebo, using Avmacol® tablets (Nutramax Laboratories) (Clinical trials.gov NCT02879110) Dosage was based on weight ranging from 2, 4, 6 or 8 tablets /day. Outcomes measures, evaluated at bassline and weeks 4, 8 and 12, included two clinician rated scales, Ohio State Autism Rating Scale (OARS-4), and Clinical Global Improvement Scale and several caregivers rated scales- Social Responsiveness Scale (SRS), Repetitive Behavior Scale – Revised (RBS-R), and social relatedness sub-scale from the Autism Behavior Checklist (ABC). The clinician rated scales included direct observation and caregiver input. Side-effects were rated using in the SAFTEE scale and laboratory

measures collected at baseline and 12 weeks. Statistical analysis used intent to treat mixed model analysis of covariance, using both differences score from baseline and actual scores at each time point.

Results: 94 patients were available for analysis of treatment effects if they had at least one post drug treatment study evaluation. The intent to treat mixed model analysis showed that sulforaphane improved ratings on the clinician rated OARS with significant decreases on OARS total average scores (P=0.002) and sub-scores of impaired social interaction (P=.0006) and communication barriers (P=.003) but not stereotyped behaviors (P=0.300). There was also statistically significant better improvement on the CGI-I scale in the sulforaphane group vs placebo (P<.001). Overall effect size, including all post-drug time points, ranged from d's of 0.21-0.33, but effect sizes at the 12-week time point alone were higher ranging from 0.54 -1.02. However, there were no significant changes in scores on the caregiver rated scales (SRS, RSR, ABC [social relating behavior sub-scale]). For OARS total average difference scores, and impaired social interaction scores, patients over 10 yrs. of age showed a greater decrease than younger patients. For OARS and CGI-I scores patients with lower surrogate IQ scores (IQ<60 score) showed a greater improvement that patients with higher scores (IQ≥60 score). Side effects were low; there were few differences between placebo and sulforaphane groups on SAFTEE scale and no clinically significant difference between the groups on changes in routine laboratory values. Additional biomarker correlates are being analyzed.

Conclusions: Sulforaphane produced significant decreases in autism scores on two clinician rated scales with some significant reductions in symptoms occurring as early as 8 weeks of treatment. This provides additional evidence in a different country that sulforaphane may be a useful adjunctive treatment for patients with autism. We cannot fully assess reasons for the lack of changes in parent-caregiver rated scores. However, it may be due to several potential factors: (a) parents and caregivers were not adequately trained in the rating scales; (b) relatively short length of treatment, since the initial Zimmerman study reported maximum changes in some of these scales at 18 weeks treatment; (c) the interpretation of more activated behaviors as problematic in a Chinese family culture which saw them as disruptive of orderliness; and/or (d) recent reviews suggest that the SRS scale may not be one of the best reliable indicators of clinical change in autism studies.

Keywords: Sulforaphane, Autism, Social Responsiveness, Communication Barriers

Disclosure: Nothing to disclose.

M23. Lateralization of Sensorimotor Behavior and Cortical Function in Autism Spectrum Disorder

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Background: Individuals with ASD show multiple sensorimotor abnormalities including atypical lateralization of manual skills. Systematic studies of functional sensorimotor abilities across right and left hands in ASD are needed to determine the extent to which lateralization of different behaviors is disrupted. Studies assessing brain function during right and left hand sensorimotor behaviors also are needed to clarify neurodevelopmental processes associated with lateralization of function in ASD. The present study aimed to 1) characterize strength, variability and regularity of right and left hand sensorimotor behaviors in ASD, and 2) define functional brain network processes associated with right and left hand sensorimotor control in ASD.

Methods: Twenty right-handed individuals with ASD and 20 right-handed typically developing controls matched on age (range: 10-34 years), sex, and nonverbal IQ completed a visually guided precision manual force task during functional magnetic resonance imaging (fMRI). Participants pressed with their thumb and forefinger against a force transducer while viewing two horizontal bars. The lower FORCE bar moved upwards with increased force towards a static TARGET bar set to 45% of each individual's maximum voluntary contraction (MVC) for that hand. Participants completed three 26 sec blocks of sustained force each separated by 26 sec of rest. They completed one run of the task with each hand. Grip strength (MVC), mean force, force regularity (approximate entropy, or ApEn), and force variability (SD) were examined separately for each hand. Percent BOLD signal change during force vs. rest was examined.

Results: Relative to controls, individuals with ASD when using their left hand showed reduced grip strength, reduced mean force, and increased force variability, as well as reduced BOLD activation relative to controls in multiple sensorimotor regions, including bilateral primary motor cortex (M1), right primary sensory cortex (S1), anterior cingulate cortex, left primary visual cortex (V1), right cerebellar Crus I, and pons. When using their right hand, individuals with ASD showed reduced ApEn and increased force variability, and greater BOLD activation than controls in bilateral V1 and extrastriate cortex, right S1, middle cingulate, right angular gyrus and right ventral premotor cortex.

Conclusions: Individuals with ASD showed reduced grip strength and mean force relative to controls for their left hand only suggesting disruptions affecting gross motor strength may be specific to the non-dominant hemisphere. Our fMRI results from left hand testing suggest individuals with ASD show reduced sensorimotor brain network activation relative to controls involving decreased activation in sensory cortical areas associated with processing visual feedback (S1, V1) and bilateral dampening of M1 activity. In contrast, alterations of precision sensorimotor control, including the ability to dynamically adjust precision output as reflected by the regularity of the time-dependent structure of the force time series (ApEn), are specific to the dominant hand implicating reduced lateralized dominance of brain networks supporting precision sensorimotor behavior. Right-hand fMRI testing indicated that individuals with ASD show greater involvement of sensory cortical areas and non-dominant motor cortical areas suggesting atypical lateralization of function to support precision sensorimotor adjustments. Overall, these findings suggest different sensorimotor behaviors show distinct patterns of lateralization in ASD, and neurodevelopmental processes involved in specialization of hemispheric function are disrupted.

Keywords: Autism Spectrum Disorder, Sensorimotor, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

M24. Associations Between Childhood Trauma Exposure and the Neural Correlates of Safety Cue Learning in Development

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Background: Exposure to trauma during childhood is prevalent and confers heightened risk for the development of psychopathology including anxiety disorders. Understanding threat and safety learning and related neural mechanisms is essential for the development of novel interventions and identification of mechanisms linking trauma exposure and psychopathology.

Methods: The present fMRI study examined conditioned inhibition via safety cue learning (SCL) in a sample of healthy adults and youth (ages 12-30; n = 67). The paradigm included stimuli representing threat, safety, and a safety compound (i.e., CS+ and CS- were paired). The Childhood Trauma Questionnaire (CTQ) assessed childhood trauma exposure. A general linear model examined neural activation in the anterior hippocampus, a key region of interest, and anterior hippocampal functional connectivity with the dorsal anterior cingulate cortex (dACC), a target neural pathway supporting SCL.

Results: There was a significant interaction between childhood trauma exposure and task condition ($F(3,62)=3.16$, $p = 0.031$). Specifically, youth and young adults with higher levels of trauma exposure (i.e., total CTQ score equal to or greater than median score) showed lower hippocampal activation to the safety compound and higher hippocampal activation to the threat cue than those with lower levels of trauma exposure. In addition, anterior hippocampal-dACC functional connectivity during SCL was positively correlated with age ($r=0.293$, $p = 0.025$), such that older individuals displayed greater functional connectivity between the anterior hippocampus and dACC during SCL.

Conclusions: These findings suggest that the neural mechanisms supporting conditioned inhibition may be disrupted following childhood trauma exposure. Furthermore, youth may be particularly vulnerable to this impact due to lower hippocampal-dACC functional connectivity during SCL.

Keywords: Stress and Trauma, Anxiety Circuitry, Fear learning

Disclosure: Nothing to disclose.

M25. Augmented Mindfulness Training for Adolescents With Early Life Stress Exposure: fMRI Neurofeedback Feasibility Study

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Background: Experience of significant early life stress (ELS) due to abuse, neglect, or family dysfunction affects one in four children in the United States. ELS is the strongest predictor of childhood- and adult-onset internalizing psychopathology and in adulthood associated with worse outcomes to evidence-based interventions. Although advances were made in delineating the neural processes resulting in ELS-related internalizing disorders, there is an urgent need to improve interventions. We proposed the posterior cingulate cortex (PCC) as target for fMRI neurofeedback manipulation to improve mindfulness training. PCC supports self-referential thinking, and its dysregulation result in impairments in threat detection, reward anticipation, and emotion regulation observed in ELS internalizing disorders. Notably, PCC has been identified as key target during mindfulness in adults. In adults with PTSD, mindfulness can help to reestablish functional connectivity between large-scale brain networks and can reduce trauma-related reactions. Therefore, we designed and conducted a proof-of-concept feasibility study to test engagement of PCC with real-time fMRI neurofeedback (rtfMRI-nf) during mindfulness training in adolescents. We hypothesized that adolescents would be successful at learning to self-regulate and deactivate the PCC activity during mindfulness training.

Methods: Seventeen healthy adolescents [age: mean(sd) = 15 (1) years; 41% female] were enrolled in and completed the rtfMRI-nf targeting the PCC. The rtfMRI-nf experiments were conducted on the GE MR750 3T MRI scanner (EPI parameters: TR/TE=2000/30ms, SENSE acceleration R=2, matrix 96×96, 46 axial slices, $2.5 \times 2.5 \times 2.9$ mm3 voxels). The PCC region-of-interest (7mm sphere, MNI coordinates = -7,-52, 23) was selected based on a meta-analysis investigating functional neuroimaging studies of the default mode network (DMN) and mindfulness meditation studies, including neurofeedback, adjusted further during pilot testing. Adolescents first underwent mindful breathing training outside of the scanner. Next, they completed the augmented mindfulness training (AMT) task, consisting of three conditions: Focus, Describe, and Rest. In the "Focus" condition, participants received ongoing rtfMRI-nf signal from the PCC presented as variable-height bar, and were instructed to lower it by focusing on the physical sensations of their breath where-ever they most strongly felt it. In the "Describe" condition, participants engaged in self-referential processing by mentally deciding whether an adjective described them. In the "Rest" condition, participants were not presented with any task. Each of three rtfMRI-nf runs started with a 66s "Rest" block, followed by alternating blocks of "Rest" (30s) "Describe" (20s) or "Focus on Breath" (70s) blocks. Prior to and after rtfMRI-nf runs, there were "Observe" and "Transfer" runs during which no neurofeedback was provided. Adolescents provided task ratings after each run. Clinicaltrials.gov identifier: NCT04053582.

Results: During neurofeedback, participants evidenced a signal change (i.e., Focus – Describe) in the PCC significantly smaller than zero [$t(16) = -4.04$, $p < .0005$; 95% CI: -0.36 to -0.14]. A whole-brain analysis (Focus – Describe; FDR corrected, $p < 0.0001$) further showed reduced activity in regions of the DMN (e.g., ventromedial PFC), as well as the inferior frontal gyrus (IFG), dorsal anterior cingulate cortex (dACC), posterior insula, and medial temporal lobe. Self-reported ease of focusing on physical sensations of breath during PCC rtfMRI-nf was negatively associated with PCC activity [$rs(16) = -0.64$, $p < 0.01$].

Conclusions: The findings of this study provide initial evidence of safety and feasibility of rtfMRI-nf in adolescents. Further, the findings show adolescent's capacity to decrease PCC neural activity with rtfMRI-nf during mindfulness training. Future studies with larger samples are warranted to determine whether neurofeedback augmented mindfulness training (a) re-regulates PCC activity, (b) alters disrupted connectivity within DMN and with regions of the salience network (e.g., insula, amygdala, and dACC) and (c) improves clinical outcomes in adolescents with ELS-related internalizing disorders.

Keywords: Adolescents, Real-Time fMRI Neurofeedback, Mindfulness, Proof of Concept, Early Life Stress

Disclosure: Nothing to disclose.

M26. Longitudinal Associations Between Socioeconomic Status and Subcortical Brain Structure in Adolescents

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Background: Individuals with low socioeconomic status (SES) have a higher probability of multiple exposures (e.g. poor nutrition, neighborhood violence) that affect subcortical structural brain development. Previously, we used high dimensional deformation mapping of structural 3T MRI to examine the relationship between income to poverty ratio (IPR) and subcortical surface morphology in a sample of 256 males and females in the 8th grade (T1). We reported that the relationships between IPR and local shape variation were mostly negative in females and mostly positive in males at this first time point. The present study reports the results of the same analysis in this sample two years later (T2).

Methods: Participants were 123 females and 68 males in the 10th grade from the greater Chicago community (mean age= 16

years, 85 white, 57 hispanic). All participants had previously undergone a structural MRI at T1 (mean age= 14 years) and their parent had completed an interview about their family SES. Family IPR was calculated at T1 from all sources of household income during the previous calendar year, along with the federal government's poverty threshold. A second MRI was collected two years later at T2. Surfaces of the amygdala, caudate, hippocampus, nucleus accumbens, pallidum, putamen and thalamus were automatically generated from each MRI using a FS+LDDMM pipeline. This combines Freesurfer's (FS) probabilistic voxel-based classification and a deformable, high-dimensional template-based method of large diffeomorphic metric mapping. Subcortical surfaces for each participant were rigidly registered to atlas space to calculate a population average, and for each participant, local shape variation was calculated from the population average of all participants at a vertex-wise level. Males and females were examined separately due to different pubertal statuses ($p = .001$ T1 and $p < .001$ T2). Linear regression analyses using SurfStat regressed morphometric values onto T1 IPR scores to localize significant regions of shape variation. Random field theory was applied using SurfStat to achieve significant clusters of vertices at the family wise error rate (FWER) of $p < .01$ and FWER of $p < .05$ within each ROI (corrected). Age, pubertal status and intracranial volume (ICV) from the corresponding visit (T1 or T2) were covaried in all models. Linear regressions were also performed on the overall volumes of each subcortical structure.

Results: Multiple regression analyses for males found that IPR was not associated with volume of any subcortical structure after Bonferroni correction ($p < .007$) at T1 or T2. In females, IPR was negatively associated with T1 putamen (standardized $\beta = -.19$, $p = .004$) and thalamus (standardized $\beta = -.21$, $p < .001$) and T2 caudate (standardized $\beta = -.20$, $p = .006$), putamen (standardized $\beta = -.22$, $p = .003$) and thalamus (standardized $\beta = -.21$, $p < .001$). Surface analysis showed that in females, IPR was negatively associated with shape deformation (concave relative to population) in the basal ganglia and thalamus ROIs and that these associations were less extensive at T2 than T1. In males, IPR was positively associated with shape deformation (convex relative to population) in the right caudate head at T1 and left caudate head at T2, the left anterior hippocampus at T1 and the right anterior hippocampus at T2, and the right anterior and posterior amygdala at T1 and the left posterior amygdala at T2.

Conclusions: Males and females showed distinct patterns of associations between IPR and subcortical morphology. IPR was positively associated with subcortical shape variation in males and negatively associated with subcortical volume and shape variation in females. As we have previously argued, this difference in direction of association is likely related to the sex differences in pubertal development and brain maturation in adolescents. Females, at a more advanced stage of development were on a downward gray matter maturational trajectory whereas males were still on an upward trajectory. Together, these results suggest that lower SES is associated with delayed maturation of subcortical brain regions. Future research should work to identify potential exposures and protective factors that mediate this association, as well as the neurobiological mechanisms by which socioeconomic factors affect adolescent brain development.

Keywords: Subcortical Shape Analysis, Socio-Economic Status, Adolescent Development

Disclosure: Nothing to disclose.

M27. Impact of Daily Caffeine on Actigraphically-Measured Sleep Duration Among Adolescents With and Without Attention-Deficit/Hyperactivity Disorder

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Background: Caffeine use is increasingly ubiquitous among adolescents and may interfere with sleep health. Specifically, greater caffeine consumption has been associated with shorter sleep duration in this age group, and research from adult studies suggests that timing of caffeine intake (morning versus evening) may affect the extent to which sleep duration is impacted. Clarifying the relationship between caffeine and sleep may be particularly critical for adolescents with attention-deficit/hyperactivity disorder (ADHD), who have been shown to have greater sleep disturbances as well as elevated caffeine intake compared to typically developing youth. A recent study of adolescents with ADHD suggested associations between afternoon and evening caffeine use and self-reported, but not actigraphically-measured, sleep health. However, that study used a self-report measure of caffeine use that aggregated intake over the past 30 days, which may not be sensitive enough to capture the effect of daily caffeine intake on subsequent sleep duration. The current study had two primary aims: (1) to evaluate the impact of daily caffeine intake on subsequent actigraphically-measured sleep duration in adolescents, and moreover, to determine whether this relationship is dependent on the timing of caffeine use, and (2) to examine whether the relationship between caffeine intake and objectively assessed sleep duration differs among youth with ADHD versus those without.

Methods: Eighty-nine adolescents aged 11-17 (mean age = 14.08 (SD = 1.78), 45.6% female) were recruited from the community as well as the Duke ADHD Program. Twenty-one youth had a prior diagnosis of ADHD, any presentation type, and sixty-eight did not have prior psychiatric history. Exclusion criteria included occult sleep disorders (i.e., obstructive sleep apnea, periodic leg movement syndrome), current use of prescribed or over-the-counter sleep aids (e.g., sedatives, melatonin), and diagnosis of an acute or chronic medical illness or other medication use that may interfere with sleep as determined by the research team. Following study intake, participants were instructed to wear an actigraph watch for seven consecutive days and nights on their non-dominant wrist. Each morning of the study, participants completed a daily electronic sleep diary that queried about sleep the prior night. The daily diary also queried the number of caffeinated drinks (e.g., coffee, tea, soda) consumed during the prior day, separately by the time of day (i.e., morning (before noon), afternoon, evening (after 6 pm)). Linear mixed models controlling for the prior night's TST assessed associations between daily caffeine intake and subsequent sleep duration (total sleep time; TST) that night as well as group differences in these relationships.

Results: Total daily caffeine use was associated with reduced TST in the full sample ($\beta = -.18$, $t = -2.53$, $p = .01$). A coefficient of -0.18 suggests that with every additional caffeinated drink taken in a day, a particular participant could experience on average 0.18 hours (~11 mins) less TST that night. When examined separately by timing of caffeine use, evening caffeine intake was associated with reduced subsequent TST in the full sample ($\beta = .38$, $t = -3.01$, $p = .002$), while morning and afternoon caffeine use were not (p's > .05). ADHD youth did not report greater caffeine intake than non-ADHD adolescents ($p = .17$); however, there was an ADHD by caffeine interaction on TST ($\beta = -.77$, $t = -2.28$, $p = .005$), such that greater caffeine intake in the afternoon contributed to shorter TST among youth with ADHD, but not youth without ADHD.

Conclusions: Results suggest that daily caffeine intake reduces subsequent actigraphically-measured sleep duration in

adolescents. Moreover, this is the first study to use a daily measure of caffeine to demonstrate that (1) similar to the adult literature, the timing of caffeine intake is critical to its impact on objectively assessed sleep duration in adolescents, and (2) the relationship of caffeine intake to sleep differs between adolescents with and without ADHD. Although a prior study using an aggregated measure of caffeine use suggested associations between afternoon/evening caffeine intake and self-reported sleep health among adolescents with ADHD, that study failed to find associations with actigraphy. By utilizing a more sensitive (daily) measure of caffeine, the current study builds on prior work to show relationships between caffeine intake – especially afternoon use – with subsequent sleep duration in adolescents with ADHD. This finding highlights the importance of assessing caffeine use among adolescents with ADHD, as eliminating or reducing intake – particularly later in the day – may represent one avenue for improving sleep in this population. Future studies may utilize experimental methods and standardized doses/timing of caffeine intake to clarify further the relationships between caffeine intake and timing with sleep health among adolescents with ADHD. In addition, further clarification of types of caffeine used (e.g., coffee, sodas, energy drinks) and the motivation for afternoon/evening intake (e.g., to improve focus) among adolescents with ADHD may further inform interventions aimed at improving sleep in this population.

Keywords: Sleep Disturbance, ADHD, Adolescents, Caffeine

Disclosure: Nothing to disclose.

M28. Exposure of Picrotoxin, a GABAA Receptor Antagonist, in Pregnant Mice Causes ASD-Like Behaviors in Offspring

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Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by impairment of social interaction. The inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in an immature brain has a key role in the development of neuronal circuits (e.g., neurogenesis and synapse formation). GABA also evokes depolarization in the immature brain, in contrast to hyperpolarization in the adult brain. Recent studies reported that excitatory/inhibitory imbalance in the brain was one of the pathologies of ASD. Activation of excitatory cells with optogenetics without altering inhibitory cell activity in the mouse prefrontal cortex results in impaired social behavior. Autism-related genetically modified and valproic acid-exposed mice showed reduced parvalbumin-positive cells, a marker of GABAergic neurons, in the mature brain. However, it is unclear whether and how disturbances of GABA signaling in embryo induce ASD-like behaviors. We aimed to establish a new model animal for ASD using GABAA receptor inhibition in the embryo and to find the causes of ASD by investigating the behaviors and immunohistochemistry.

Methods: We injected intraperitoneally GABAA receptor inhibitor picrotoxin into pregnant mice at gestation 12.5 days and used the offspring in behavioral and immunohistochemical experiments. Body maturation was assessed by measuring body weight and eye-opening. Motor function was assessed by the righting reflex and hanging wire tests from postnatal day 7 to 25. Social interaction test was conducted in ages 5-6 weeks and 10-11 weeks. Open field and elevated plus-maze tests were conducted in ages 8 weeks to 9 weeks. After the end of the social interaction test (10-11 weeks), the mice were deeply anesthetized with pentobarbital and transcardially perfused with saline, followed by 4% paraformaldehyde in sodium phosphate buffer. We also analyzed c-fos expression in the cerebral cortex, hippocampus, amygdala, and the cerebellum. All of the animal experiments were performed in accordance with the Guidelines for the Care of Laboratory Animals of the Tokyo Metropolitan Institute of Medical Science.

Results: There were no significant differences between the mice that were prenatally exposed to GABAA receptor inhibitor (GABA mice) and control mice were that treated with vehicle (control mice) in body maturation, motor function, nociceptive response, and anxiety-like behaviors. GABA mice exhibited a reduction of active interaction time compared with control mice in the social interaction test in both ages 5-6 weeks and 10-11 weeks. We found that c-Fos expression in the mPFC of GABA mice did not change after the social interaction test, while c-Fos expression in the mPFC of control mice after the social interaction test was increased.

Conclusions: These results suggest that exposure to GABAA receptor inhibitor in embryo induced ASD-like behaviors in offspring. It is a possibility that disturbances of GABA signaling in an embryo are a new mechanism that leads to ASD. Detailed immunohistochemical analyses will reveal the mechanisms underlying the impairments of social interaction.

Keywords: ASD, Social Interaction, GABA-A Receptors

Disclosure: Nothing to disclose.

M29. MEK Inhibition Ameliorates Social Behavior Phenotypes in a Spred1 Knockout Mouse Model for Rasopathy Disorders

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Background: RASopathies are a group of disorders that result from mutations in genes coding for proteins involved in regulating the Ras-MAPK pathway and exhibit an increased incidence of autism spectrum disorder (ASD). Legius syndrome is a rare RASopathy caused by loss-of-function mutations in the SPRED1 gene. The patient phenotype is similar to, but milder than, Neurofibromatosis type 1 – another RASopathy caused by loss-of-function mutations in the NF1 gene. Both disorders exhibit increased activation of Ras-MAPK signaling, and commonly manifest with cognitive impairments and ASD. The phenotypic overlap is underpinned by a biochemical interaction, whereby SPRED1 interacts with neurofibromin to recruit it to the membrane, suggesting shared molecular mechanisms underlying these disorders. Here, we investigated if a Spred1−/− mouse model for Legius syndrome recapitulates ASD-like symptoms relevant to NF1 and Legius syndrome, and whether targeting the Ras-MAPK pathway has therapeutic potential in this RASopathy mouse model.

Methods: Social behaviors in Spred1−/− and wildtype (WT) littermate controls were assessed with the automated tube test in cohorts of both male and female mice. Social communication was measured by analyzing ultrasonic vocalizations in adult male Spred1−/− and WT mice in response to a novel female conspecific. Nesting behavior was assessed in female Spred1 −/− and WT mice with a nestlet shredding assay. Postnatal ultrasonic vocalization in response to removal from the nest was assessed in Spred1−/− and WT pups from postnatal day 4 to postnatal day 12. Therapeutic mechanisms underlying the observed behavioral phenotypes were probed by pharmacological targeting of the Ras-MAPK pathway with the MEK inhibitor PD325901 in adult Spred1−/− and WT mice. PD325901 or vehicle

control was acutely administered daily at 5mg/kg starting 3 days prior to behavior testing.

Results: Spred1−/− mice have robust deficits of social dominance in the automated tube test compared to wildtype controls, winning more matches, a phenotype observed in both male cohorts (two tailed binomial test, $p < 0.05$, $n = 8$) and female cohorts (two tailed binomial test, $p < 0.05$, $n = 8$). Male Spred1 −/− mice vocalized less to female conspecifics compared to WT mice (unpaired t-test, $p < 0.001$, $n = 14$-15). Nesting building in female Spred1−/− mice was significantly impaired (unpaired t-test, $p < 0.01$, $n = 17$-19). In postnatal ultrasonic vocalizations, Spred1−/− pups did not show differences in the rate of vocalization, but there were significant differences in spectral properties of calls including frequency (2-way ANOVA, effect of genotype $p < 0.0001$, $n = 22$-24) and amplitude (2-way ANOVA, effect of genotype $p < 0.01$, $n = 22$-24). MEK inhibitor treatment with PD325901 reversed deficits in social dominance in adult Spred1−/− mice, returning percentage of wins to chance levels (two tailed binominal test, no significant differences, $n = 8$ mice). Impairments in nesting behavior of Spred1−/− mice were also rescued with PD325901 treatment (2-way ANOVA, effect of treatment $p < 0.0001$, $n = 8$ mice per genotype).

Conclusions: Spred1−/− mice model social and communicative deficits relevant to ASD and RASopathy disorders. These results demonstrate for the first time that social behavior phenotypes in a mouse model for RASopathies can be acutely reversed with a pharmacological intervention. Furthermore, our work suggests a key role for Ras-MAPK signaling in the regulation for social behavior. As PD325901 is currently in clinical trial for treating non-cognitive manifestations of NF1, these results highlight the importance of monitoring the effects of this drug on cognitive and behavioral symptoms in current and future clinical trials

Keywords: ASD, Neurofibromatosis Type 1, RASopathies

Disclosure: Nothing to disclose.

M30. Reduction of MEF2C in Microglia Leads to Structural and Transcriptomic Changes in Mouse Models of MEF2C Haploinsufficiency Syndrome

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Background: MEF2C Haploinsufficiency Syndrome (MCHS) is neurodevelopmental disorder characterized by autism-related behaviors, like social interaction deficits, stereotypies, and communication difficulties. MCHS is caused by de novo mutations or microdeletions in MEF2C, which codes for a transcription factor that is important for experience-dependent synaptic plasticity and synapse development, and global Mef2c heterozygous mutant mice (Mef2c+/-) display numerous MCHS-relevant behavioral phenotypes. Mef2c is expressed in neurons and microglia in the developing brain, so we generated a microglial-selective Mef2c conditional heterozygous mutant mouse. These Mef2c cHetCx3cr1 mice displayed autism-like behaviors, including social deficits in both sexes and repetitive behavior, in male, but not female, mice. This finding revealed a critical sex-specific role for MEF2C function in microglia during neurotypical development. In the current study, we sought to determine the pathophysiological effects of microglial MEF2C hypofunction. We took a multi-pronged approach in both global and microglia-restricted Mef2c heterozygous mutant mice to examine cortical electrophysiology, brain morphology and microglial differential gene expression.

Methods: To investigate brain morphology and white matter tract myelination, mouse brain sections were incubated with Fluoromyelin stain. Corpus callosum myelination, thickness and axon morphology were examine using bright-field, epifluorescence, confocal imaging and electron microscopy. Patch-clamp recordings of layer 2/3 barrel cortex neurons were performed using standard ex vivo slice preps. To examine differential gene expression, isolated microglia or whole brain tissue was processed to isolate mRNA and RNA-seq analysis was performed. Both sexes are included in these studies.

Results: In both the global Mef2c+/- and Mef2c cHetCx3cr1 mice, there is no difference in cortical thickness compared to controls. However, both the global Mef2c-Het and microglia-restricted Mef2c cHetCx3cr1 showed a significant ($p<0.05$) increase in corpus callosum thickness. Ongoing studies are examining the nature of these changes in the corpus callosum. In addition, functional electrophysiological studies in Mef2c cHetCx3cr1 mice are ongoing, but initial findings revealed a reduction in evoked EPSCs in layer 2/3 somatosensory cortex ($p < 0.05$). RNA-seq data of isolated microglia from Mef2c+/- mice and of whole cortex from Mef2c cHetCx3cr1 mice have revealed multiple differentially expressed genes in heterozygous Mef2c microglia. Analysis of gene ontology and validation of DEGs from these studies is in progress.

Conclusions: Mef2c-Het and Mef2c cHetCx3cr1 male mice display significant changes in corpus callosum morphology, cortical synaptic transmission and microglial gene expression. The male-specific effects on ASD-like behaviors in the Mef2c cHetCx3cr1, suggest an important sex-specific role for MEF2C in microglial function and brain development. RNA-seq from microglia from Mef2c-Hets and cortex from Mef2c cHetCx3cr1 mice could help to generate new hypotheses as to the role of microglia in MCHS, and it might provide new insights into the role of microglia in ASD-like behavior and neuroimmune function.

Keywords: Microglia, Autism Spectrum Disorder and Related Syndromes, Sex Differences, Brain Development, Myelination

Disclosure: Nothing to disclose.

M31. High-Throughput Functional Analysis of Autism Risk Genes

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Background: Whole-exome sequencing has led to a growing list of "high confidence" risk genes associated with autism spectrum disorders (ASDs), which are beginning to converge on common pathways. However, our understanding of the mechanisms by which ASD risk gene disruption alters vertebrate brain development, resulting in behavioral dysfunction, remains limited. The goal of this research is to investigate the functions of multiple high confidence (hcASD) risk genes in fundamental processes of vertebrate brain development in parallel using in vivo, high-throughput approaches, which will provide a much-needed avenue for illuminating the biological mechanisms underlying ASD and a path forward for drug discovery.

Methods: Here we leverage the unique advantages of zebrafish as an in vivo, biologically relevant system for the functional analysis of multiple ASD genes in parallel. Using CRISPR/Cas9, we generated zebrafish mutants of 10 hcASD risk genes. To investigate how gene disruption alters basic sensory processing and arousal behaviors, we performed quantitative behavioral profiling to identify the behavioral "fingerprints" of these mutants. In addition, we use whole-brain activity mapping to assess how loss of ASD risk gene function affects baseline brain activity.

Results: Using quantitative behavioral profiling, we characterized the behavioral "fingerprints" of 10 hcASD risk gene mutants. We identified points of phenotypic convergence and divergence across mutant behavioral profiles of the following risk genes: CHD8, CNTNAP2, CUL3, DYRK1A, GRIN2B, KATNAL2, KDM5B, POGZ, SCN2A, and TBR1. In parallel, we screened >750 FDA-approved drugs to identify their effects on these behaviors. By comparing the effect of these drugs in wild-type fish to the mutant behavioral profiles, we aim to identify dysregulated pathways in mutants and potential suppressors of mutant behavioral phenotypes. Using pharmaco-behavioral profiling, we previously showed that estrogenic compounds could selectively rescue the cntnap2 mutant behavioral phenotype. Based on the identification of compounds that correlate and anti-correlate with the mutant behavioral profiles, we are currently conducting pharmaco-behavioral screens to identify compounds that selectively rescue these mutant behavioral phenotypes. Further, we identify differences in baseline brain activity resulting from ASD risk gene loss of function using whole-brain activity mapping.

Conclusions: These studies show how the high-throughput functional analysis of ASD risk genes in zebrafish has the potential to reveal novel pharmacological pathways and brain activity phenotypes for further investigation.

Keywords: Autism Spectrum Disorders, Zebrafish, Drug Discovery - New Approaches, Brain Development, Brain Circuitry

Disclosure: Nothing to disclose.

M32. Sex-Specific Effects in the Development of Social Recognition in Rats

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Background: The ability to recognize previously encountered conspecifics is crucial for normal social interaction. This social recognition ability is well described in adults of both sexes, but remains largely unexplored in juveniles. Therefore, we characterized social recognition in male and female juvenile rats, determined how social recognition ability changes with age in female rats, and investigated the potential role of circulating ovarian hormones in modulating female social recognition. In order to identify a potential neurobiological mechanism underlying sex and age specific regulation of social recognition, we targeted the bed nucleus of the stria terminalis (BNST) for investigation of neural activity following exposure to a novel social stimulus. The BNST expresses receptors for various neurochemicals implicated in the regulation of social recognition, including estradiol, and has previously been demonstrated to play a sex-specific role in social discrimination in adults.

Methods: We first determined whether juvenile male and female rats (n = 6 per sex) show similar temporal patterns of social recognition to adults, by testing their ability to recognize a previously encountered same-sex stimulus rat 30, 60, or 90 min following initial investigation, as defined as a significant preference to investigate a novel stimulus animal over the previously encountered (familiar) stimulus. We then determined at what age during development social recognition ability is established in female rats, by testing females (n = 24) as juveniles (31-32 days old), adolescents (42-43 days old), young adults (60-61 days old), and adults (80-81 days old). Next, we determined whether age differences in the release of circulating ovarian hormones contribute to developmental differences in female social recognition ability by examining the effects of estradiol benzoate (EB) administered 48 hours prior to testing for social recognition in juvenile female rats (n = 12 per treatment). Finally, we determined whether male and female juvenile rats (n = 8 per sex and condition) show different levels of social investigation induced neural activation in the anterior, intermediate, or posterior BNST, as indicated by expression of the immediate early gene cFos. All experiments were reviewed and approved by the Michigan State University Institutional Animal Care & Use Committee and conducted in accordance with National Institutes of Health guidelines.

Results: Similar to adults, juvenile males showed social recognition 30 and 60 min, but not 120 min, following the initial encounter. Juvenile females, however, did not show social recognition at any time point tested. We then determined at what age during development social recognition ability is established in female rats and found that only juvenile females were unable to show social recognition, while adolescent, young adult and adult females showed the expected preference for a novel over a previous encountered (familiar) stimulus rat. This developmental difference in social recognition ability was driven by a decrease in the amount of time females investigated the familiar stimulus. EB treatment in juvenile females induced a preference to investigate the familiar over a novel social stimulus, the opposite behavioral pattern observed in typical rodent social recognition. Also, EB treatment increased investigation of the social stimulus during the initial investigation period. Interestingly, exposure to this initial investigation period resulted in sex-specific effects on activation of the BNST. Females, but not males, showed an increase in activation of the anterior BNST and a trend toward increased activation of the posterior BNST. Although there was no effect of social investigation on BNST activation in males, when analyzing across both sexes, activation of the posterior BNST was negatively correlated with time spent investigating the social stimulus.

Conclusions: These findings provide the first evidence of a development-specific sex difference in social recognition ability in rats. While circulating ovarian hormones are able to modulate social recognition behavior, they do not explain the lack of social recognition seen in juvenile females. The BNST, which is known to regulate social discrimination in adult male and female rats, may contribute to sex differences in this behavior in juveniles. Ongoing work seeks to tease apart the underlying hormonal and neurobiological mechanisms of sex differences in the development of social recognition.

Keywords: Social Recognition Memory, Sex Differences, Development, Social Behaviors

Disclosure: Nothing to disclose.

M33. Sex-Biased Microglial Mitochondrial Dysfunction and Social Behavior Alterations Following Perinatal Immune Challenge

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Background: Autism Spectrum Disorders (ASDs) are a collection of complex neurodevelopmental disorders characterized by repetitive behavior and alterations in social interaction and communication. Postmortem analyses of brains from children with ASD revealed significant decreases in expression of mitochondrial electron transport chain (ETC) subunits, while other analyses have found evidence for immune activation in these brains. However, reports often fail to distinguish between the specific cell type affected or sex of the patient. Emerging evidence suggests that mitochondrial respiratory function is inhibited during proinflammatory activation of microglia. ASD occurs in ~1 in 59 children in the US, with a strong sex bias in prevalence (~3-4 males diagnosed to every female). This

male bias suggests that some aspect of ASD susceptibility depends upon sex-specific brain organization. There is a surge in levels of gonadal hormones that occurs only in males during a critical period surrounding birth that is responsible for masculinizing the male brain. Injection of female pups at birth with these sex hormones has been shown to divert the female brain phenotype towards a masculinized male-like state. Here, we hypothesized that male sex hormones during a perinatal critical period of brain development induce vulnerability to immune alterations in male microglial mitochondria, and that these microglial alterations lead to impaired brain development and aberrant social behavior.

Methods: Female mice were masculinized by subcutaneous injection on postnatal days (PN) 0 and PN1 with 100 ug estradiol benzoate dissolved in sterile sesame seed oil. PN9 male, female, and masculinized female mice were subcutaneously injected with 10 mg/kg lipopolysaccharide (LPS) dissolved in sterile saline or vehicle (Sal). Mice underwent social exploration behavioral testing on PN15, and then 3-chambered sociability and social novelty preference testing starting at PN30. A separate cohort of mice were sacrificed on PN30 for molecular and immunohistochemical assays. Microglia were isolated from prefrontal cortex using CD11b method, and mitochondrial electron transport chain gene expression was assessed by PCR array and microglial mitochondrial oxygen consumption was determined using MitoXpress Oxygen Consumption Assay. Immunohistochemistry for mitochondria (Tomm20) within microglia (Iba1) was performed, and mitochondrial morphology and network connectivity was determined using ImageJ and Imaris softwares.

Results: Using both whole transcriptome profiling by RNA sequencing ($n = 4$-7 per group) and PCR Array ($n = 3$-4 per group) of isolated microglia (CD11b bead isolation) from prefrontal cortex of male, female, and masculinized female mice injected perinatally with the bacterial endotoxin lipopolysaccharide (LPS), we found that 92% of ETC genes were significantly diminished acutely by LPS in male microglia with 54% of these genes remaining significantly downregulated 3 weeks following immune challenge, whereas 0% of these same ETC genes were significantly decreased in microglia isolated from female brains. Microglia isolated from prefrontal cortex of masculinized female mice challenged at PN9 with LPS showed a similar pattern to males, with 45% of ETC genes remaining significantly downregulated by PN30. Male and masculinized female microglial mitochondrial dysfunction was also observed in PN30 prefrontal cortex by deficits in oxygen consumption and alterations in mitochondrial morphology (significant decrease in mitochondrial length, volume, and network connectivity ($n = 3$-5 per group; significance defined by Two-way ANOVA (sex x treatment) $p < 0.05$), suggesting that reduced mitochondrial function may be implicated in microglial activation, particularly in males. Importantly, two-way ANOVA (sex x treatment) indicated that this same perinatal LPS model resulted in male and masculinized female-specific deficits in sociability (Interaction: $F1,43=16.62$, $p < 0.0001$) and social novelty preference (Interaction: $F1,42=9.814$, $p = 0.0003$) at PN30-40.

Conclusions: These findings suggest that perinatal sex hormones induce male-specific vulnerabilities in microglial mitochondrial function as well as male-specific behavioral susceptibility to perinatal immune challenge. We are currently exploring the necessity of microglial inflammatory signaling for these sex-biased behavioral alterations.

Keywords: Microglia, Mitochondria, Brain Development

Disclosure: Nothing to disclose.

M34. Juvenile Brain-Derived Neurotrophic Factor From Microglia Regulates Social Behavior in Adult Mice

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Background: Childhood experiences are highly associated with severity and treatment responsiveness of psychiatric disorders such as schizophrenia and depression. We have used juvenile social isolation (j-SI) mice as an animal model to investigate adverse childhood experiences. While j-SI impairs social behavior in adult mice, the underlying mechanism remains unclear. Since brain-derived neurotrophic factor (BDNF) has been known as a pivotal molecule to be implicated in psychiatric disorders and social behavior, we hypothesized that BDNF might be implicated in the impairment of social behavior.

Methods: j-SI mice were made by individually housing from P21 to P35. Mice with BDNF overexpression in microglia was made by mating Iba1-tTA mice and tetO-BDNF mice. Microglia in the cerebral cortex were sorted using magnetic-activated cell sorting (MACS) system. Excitatory postsynaptic current (EPSC) and inhibitory postsynaptic current (IPSC) into the pyramidal cells in the layer V of the prefrontal cortex (mPFC) were recorded by the whole-cell patch-clamp technique.

Results: There were no differences in mPFC-BNDF expression between j-SI mice and regular-housing mice, but when microglia were purified from the cerebral cortex, BDNF expression in microglia was markedly increased in j-SI mice. Following these findings, we examined mice with microglia-specific BDNF overexpression (Iba1-tTA::tetO-BDNF mice) and found that these mice showed impaired social behavior. Furthermore, the frequency of EPSC was decreased and that of IPSC was increased in layer V of the mPFC of microglia-specific BDNF overexpression mice compared to control mice as shown in j-SI mice, indicating that microglia-specific BDNF-induced E-I imbalance in the mPFC potentially related to the impaired social behavior. Finally, we normalized the BDNF expression in adulthood using Tef-off system, the social behavior and E-I balance in the mPFC stay abnormal.

Conclusions: These results suggest that juvenile BDNF expression from microglia regulate social behavior through E-I imbalance in the mPFC.

Keywords: Social Isolation, Prefrontal Cortex, BDNF, Microglia, Social Behavior

Disclosure: Nothing to disclose.

M35. Stability of Motor Threshold in Adolescents Undergoing High-Frequency Deep Transcranial Magnetic Stimulation for Depression

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Background: Transcranial magnetic stimulation (TMS) is an established treatment for depression and obsessive-compulsive disorder, and its neuromodulatory effects are posited to occur in part through changes induced in cortical excitability and plasticity. Deep TMS (dTMS) permits stimulation of deeper cortical structures, such as the medial prefrontal and anterior cingulate cortices, that are involved in various neuropsychiatric disorders. Stimulation intensity in both standard repetitive TMS and dTMS typically is calibrated using the individual's motor threshold (MT), a measurement of the magnetic pulse strength necessary to evoke action

p-

otentials in the primary motor cortex (M1). Prior work has identified developmental changes in the MT, thought to reflect maturation of cortical networks, which may impact the effects of repetitive stimulation paradigms. This study sought to evaluate cortical excitability changes, indexed by the MT, in an open-label, pilot trial of dTMS for adolescents with major depressive disorder (MDD).

Methods: Adolescents (12–18 years) with treatment-resistant MDD underwent dTMS, delivered to the left dorsolateral prefrontal cortex (DLPFC) in 30 treatments over six weeks. Each session consisted of 1980 stimuli delivered at 10 Hz in 55 36-pulse trains, with an intertrain interval of 20 seconds. Treatment stimulus intensity was variable (80% MT, $n = 3$; 100% MT, $n = 3$; 120% MT, $n = 5$). MT was measured via single-pulse TMS to M1 at baseline and weekly during the treatment course. Within individual participants, variability of MT was assessed with the coefficient of variation (CV). In the entire sample, the effect of the dTMS treatment course on MT was evaluated via a linear mixed model, with MT as the dependent variable and treatment week as independent variable. Additional models tested the effects of age, sex, baseline depression severity (as measured by the Children's Depression Rating Scale, Revised; CDRS-R), treatment stimulation intensity (80% MT vs. 100% MT vs. 120% MT), and responder status (≥50% reduction in CDRS-R score) on the MT across the dTMS treatment course.

Results: Eleven adolescents (7 female, 4 male; mean age 16.33 ± 1.56 years, range 12–18 years) underwent MT measurement and dTMS. Depression severity at baseline was in the moderate to severe range (mean CDRS-R score 64.27 ± 12.98). All eleven participants completed the dTMS treatment protocol, and four achieved clinical response. Individual participants demonstrated little variability in MT across the treatment course (CV range 0.0%–6.0%, mean 2.3%). In the entire sample, there was no significant main effect of treatment week on MT ($F_{5,48.608} = 0.414$, $p = .837$). Age, sex, baseline depression severity, and responder status did not have significant effects on MT when included in the models. However, there was a significant effect of treatment week × stimulation intensity ($F_{5,43.765} = 3.559$, $p = .009$) on the MT.

Conclusions: In this sample of depressed adolescents receiving high-frequency dTMS to the left DLPFC, the MT remained largely stable across the course of treatment. Our preliminary findings suggest that motor cortical excitability is not grossly affected in youth treated with dTMS to the DLPFC. However, the intensity of the treatment stimulus may influence changes in MT. Larger samples undergoing stimulation at various intensities and pulse frequencies will be necessary to ascertain whether the MT and other indices of excitability can be modulated with different dTMS stimulation parameters. Additionally, larger studies comparing adolescents who experience clinical response to dTMS with nonresponders will help to elucidate whether excitability changes are mechanistically related to antidepressant effects in this population. This could inform decisions on routinely repeating MT measurements for individual calibration purposes during a course of dTMS in adolescents; however, age- and maturation-related effects, stimulus intensity, and individual seizure risk would need to be considered on an individual basis. An understanding of how dTMS impacts excitability in the frontal cortex and other regions will require further studies utilizing additional measures of cortical excitability and inhibition that can be collected directly from the DLPFC via electroencephalography.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Cortical Excitability, Adolescent Depression

Disclosure: Nothing to disclose.

M36. The Role of Family Variables in the Outcomes of Court-Involved Youth: Potential Targets for Clinical Interventions

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Background: According to the National Center for Juvenile Justice, in 2017, approximately 800,000 youths under the age of 18 received dispositions through the juvenile court system in the U.S. In this population, there is an over-representation of racial and ethnic minorities and children from families with limited economic resources. There has been a trend towards utilizing interventions that divert youths toward mental health treatment and prevent recidivism. Research in other areas has demonstrated a significant effect of family functioning, parental mental health, and parenting practices, such as parental monitoring, on youth behavior. However, few studies have examined the impact of parental practices on recidivism. We explored the impact of family functioning and parental monitoring on the frequency and severity of delinquent acts in court-involved youth over 6 months. We hypothesized that family functioning and parental monitoring would significantly predict youth delinquent behaviors.

Methods: We provided surveys to 157 adolescent-parent dyads recruited from 2 eastern U.S. cities during their court-mandated mental health treatment and followed them over 6 months measuring frequency and type of delinquent acts. Youth completed the Symptom Checklist-90-Revised including the Global Severity Index as well as the Adolescent version of the Parental Monitoring Questionnaire and Family Assessment Device. Youth were between the ages of 11 and 17 (X=15.19) and had an open petition with the partnering Family Court at time of referral. Data was analyzed using bivariate comparisons and then with stepwise linear regression and multiple logistic regression.

Results: Sixty percent of the sample self-identified as ethnic or racial minorities. Analyses using t tests demonstrated that adolescents who reported more types of delinquent acts had higher rates of drug and alcohol use (both $p < .001$), poorer family functioning ($p = .002$), lower parental monitoring ($p < .001$), and higher frequency of committing delinquent acts ($p < .001$). Multiple logistic regression indicated that after controlling for youth psychiatric symptoms and general family functioning, parental monitoring significantly predicted greater versus fewer types of delinquent acts ($p = .012$). General family functioning was significantly correlated with frequency of delinquent acts ($p = .0113$), whereas youth psychopathology and parental monitoring were not ($p = .878$, $p = .292$ respectively). Stepwise linear regressions demonstrated that after controlling for age and youth psychopathology, family functioning significantly predicted frequency of delinquent acts ($p = .013$).

Conclusions: This study provides evidence that mental health treatment alone is not enough to promote optimal behavioral outcomes for court-involved youth. Therefore, in order reduce recidivism, clinical interventions are needed that specifically target family functioning and parental behavior. Future directions for this research should include improving our understanding of the biopsychosocial barriers and disparities faced by these at-risk youth and their families in order to develop relevant and scalable interventions that effectively target these familial factors.

Keywords: Court-Involved Youth, Clinical Interventions, Recidivism

Disclosure: Nothing to disclose.

M37. Elucidating Genetic Contributions to Accelerated Biological Degeneration Related to Antipsychotic Induced Weight Gain

Abstract not included.

M38. A Multidisciplinary Approach to Mental Illness: Do Inflammation and Telomere Length Form a Loop?

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Background: Psychiatric disorders are associated with excess morbidity and mortality resulting in a substantially reduced life expectancy compared to the general population mostly attributed to the increased incidence for chronic comorbid conditions, to a great extent age-related, such as cardiovascular and metabolic disorders. Several independent studies reported shorter telomere length (TL) and increased levels of circulating inflammatory markers in psychiatric disorders. Telomere shortening (TS) is a hallmark of aging, and accelerated shortening can be caused by stress and inflammation. However, there is still uncertainty on whether TS and inflammation exert an independent or rather an interacting effect on the risk of developing psychiatric disorders. In this study we investigated inflammation and TS in bipolar disorder (BD), schizophrenia (SZ) and major depression (MDD), and explored their interaction and correlation with age-related disorders.

Methods: The cohort comprised 40 patients with BD, 37 with MDD, and 41 with SZ recruited from patients followed-up and treated at the community mental health center of the Unit of Psychiatry of the Department of Medical Science and Public Health, University of Cagliari and University Hospital Agency of Cagliari, and the Unit of Clinical Pharmacology, University Hospital Agency, Cagliari, Italy. Patients were stratified according to the presence or absence of comorbid cardio-metabolic disorders. Diagnosis was made according to DSM-IV criteria and SADS-L (BD patients), and Structured Clinical Interview for DSM IV-TR Axis I Disorders (SCID) (MD and SZ patients). A total of 36 NPC with no personal or familial history of psychiatric disorders in first degree were recruited based on the same exclusion criteria described for patients. TL and plasma inflammatory markers [hsCRP, TNF-α] were evaluated in fresh blood using quantitative fluorescence in situ hybridization (qFISH) and ELISA kits, respectively. The association of categorical variables with molecular measures was tested with t-test or Mann-Whitney test. Linear regression models with each molecular measure as dependent variable were run with a series of covariates including the presence of cardiometabolic disorders.

Results: TL was significantly different among the four groups (model F6=20.128, p = 8.73x10-17, partial eta squared 0.469; effect of diagnosis, F3=31.870; p = 1.08x10-15; partial eta squared = 0.411; Figure 2A). There was also a significant contribution of age to the model (F1=14.811, p = 0.0001, partial eta squared = 0.098), but diagnosis was the most significant variable explaining the largest proportion of variation. Post-hoc analysis with Bonferroni correction showed that patients with SZ and MDD had significantly shorter TL compared to NPC (SZ versus NPC, p = 0.002; MDD versus NPC, p = 0.039) and to BD (SZ versus BD, p = 1.91x10-13; MDD versus BD, p = 4.22x10-12). Patients with BD had the longest TL compared to all the other groups. The rank analysis of covariance with age, sex and BMI as controlling variables showed that hsCRP levels were higher in patients with severe psychiatric disorders (model F4 = 4.18; p = 0.004, partial eta squared=0.107), with diagnosis being the most significant independent variable (F3=4.681; p = 0.004; partial eta squared =0.095; contribution of sex, F1=5.423, p = 0.021, partial eta squared=0.039, Figure 2B). The highest hsCRP levels were observed in patients with SZ (post-hoc analysis: SZ versus NPC, adjusted p = 0.027), with a mean value of 3.62 mg/L (Standard Deviation ± 2.28; mean value in NPC = 2.09, Standard Deviation ± 1.99). Moreover, hsCRP levels were inversely correlated with TL when controlling for age and BMI (partial correlation coefficient = -0.180; p = 0.042). As for TNFα levels, there was a statistically significant differences among the four diagnostic groups (F9=2.067, p = 0.037, partial eta squared = 0.131), with the sole significant contribution of diagnosis (F3=6.159, p = 0.004, partial eta squared = 0.103. Table S3). The latter finding was driven by the higher levels of TNFα in MDD compared to NPC (Bonferroni corrected p = 0.010).

Conclusions: We showed that patients with SZ and MDD had shorter TL compared to NPC, while BD patients presented the longest telomeres of all groups. While we suggest that telomere shortening and increased inflammation might represent signatures of psychiatric disorders (in particular SZ and MDD), our study design did not allow us to explore the causative role of the biological measures investigated. That considered, future studies should try to implement a longitudinal prospective design to clearly elucidate the role of telomere length, inflammation and aging in severe psychiatric disorders.

Keywords: Lithium, Leukocyte Telomere Length, Inflammation, qFISH

Disclosure: Nothing to disclose.

M39. Cannabidiol Reduces Withdrawal Symptoms in Nicotine Dependent Rats

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Background: Cannabidiol (CBD) reduces craving in animal models of alcohol and cocaine seeking and is known to modulate nicotinic receptor function, suggesting that it may alleviate symptoms of nicotine withdrawal; however, preclinical evaluation of its efficacy is still lacking.

Methods: Male and female Wistar rats were made dependent on nicotine using osmotic minipumps (3.15 mg/kg/day) for two weeks, after which minipumps were removed to induce spontaneous withdrawal. Three groups received CBD injections at doses of 7.5, 15, and 30 mg/kg/day for two weeks, starting one week into chronic nicotine infusion. The control group received vehicle injections of sesame oil instead of CBD. Finally, another control group received a saline minipump and sesame oil injections (double vehicle). Throughout the experiment, serum was collected for determination of CBD and nicotine concentrations, mechanical sensitivity threshold and withdrawal scores were measured, and body weight was recorded.

Results: CBD prevented rats from exhibiting somatic signs of withdrawal and hyperalgesia during acute and protracted abstinence. There was no dose-response observed for CBD, suggesting a ceiling effect at the doses used and the potential for lower effective doses of CBD.

Conclusions: This preclinical study suggests that using CBD as a strategy to alleviate the withdrawal symptoms upon nicotine-cessation may be beneficial.

Keywords: Nicotine, Cannabidiol, Withdrawal

Disclosure: Nothing to disclose.

M40. PFC PV Interneurons Facilitate Visual Attention and are Disrupted in a Genetic Model of Absence Epilepsy

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Background: Absence epilepsy involves brief periods of unconsciousness accompanied by a lapse in motor function, and occurs in 10–12% of all epilepsy cases across multiple types of epilepsy syndromes. In childhood absence epilepsy, about 35% of patients present with prominent comorbid impairments in attention. General inattentiveness cannot be explained by the momentary interruptions in consciousness induced by absence seizures, as it occurs during normal cortical electroencephalography (EEG) rhythms. Further, these deficits are unresponsive to current absence seizure treatments, and persist into adulthood when absence seizures no longer occur. The prefrontal cortex (PFC) has been linked proper attention function as well as attention deficits in patients with absence epilepsy. Additionally, the activity of parvalbumin (PV)- expressing GABAergic interneurons in the PFC are believed to support successful information processing and performance during attention tasks. We hypothesized that in a mouse model of absence epilepsy, mice would have attention impairments that would be underlain by altered activity in the PFC, and potentially a loss of PV interneuron activity. We explored this using a combination of electrophysiology, Calcium (Ca2+) imaging of PV interneurons, optogenetics, and behavior.

Methods: To examine the mechanisms of attention impairments in absence epilepsy, we used mice with a heterozygous loss-of-function mutation in Scn8a (Scn8amed, Kohrman, Harris, and Meisler 1996) henceforward referred to as Scn8a+/-. Male and female Scn8a+/-. mice expressing Cre: in PV interneurons were injected with a FLEX-gCaMP6s-GFP virus unilaterally in the prelimbic medial PFC (mPFC). Following virus injection, the mPFC was implanted with an optical fiber for light emission and collection of bulk Ca2+ activity from PV interneurons. Fiber photometry signals were acquired using a branched patchcord (Doric Lenses) and signals were projected onto a scientific complementary metal-oxide semiconductor camera (sCMOS, ORCA-Flash4.0 V3). The gCaMP signal (488) was normalized to the control signal (405), and the change in fluorescence over time was calculated. In experiments involving viral injection or implantation of optical fibers, the viral and implant location was confirmed prior to inclusion in final analyses. For optogenetic experiments, the surgical procedure was the same, except the injections and optical fiber implants were bilateral, and the virus was a FLOX-chR2, to direct channelrhodopsin to PV interneurons. Animals were maintained on a reverse-light cycle and food restricted to ~85% of their free-feeding weight, to increase the wakefulness and motivation during the behavioral tasks. The primary behavioral task examined visual attention, by training animals that a light cue that could flash below a reward port on the left or right predicted the location of a food reward. During testing, the cue length was varied psuedorandomly to increase attentional load. Most data were compared using a two-Way ANOVA, with cue length and group as the variables, and statistical testing was done in PRISM.

Results: We observed that during the VAT Scn8a+/− mice perform significantly worse than their WT littermates, in addition to having lower levels of PV interneuron activity during cue-presentation (two-way ANOVA, $F(1,70) = 16.17$, $p = 0.0001$, $n = 8$ for both WT and Scn8a+/-). Further, across animals, the peak amplitude, and time to peak of the PV fiber photometry trace was predictive of overall performance ($r2 = 0.5981$, $p = 0.0145$ and $r2 = 0.5960$, $p = 0.0089$ respectively), suggesting increases in PV activity during cue-presentation were necessary for proper attention performance. To confirm that increased PV activity is also sufficient for proper cue-perception during an attention task, we used optogenetics to increase PV activity, specifically during the cue. We found that increasing PV activity at a gamma frequency, significantly increased performance in both WTs and Scn8a+/- mice (repeated measures two-Way ANOVA, light effect: $F(1.682, 10.09) = 4.921$, $p = 0.0306$), suggesting this may represent a therapeutic target in patients with absence epilepsy and treatment-resistant attention deficits.

Conclusions: We found that recruitment of PV interneurons in the PFC was significantly reduced in Scn8a+/- mice during cue perception, and this was strongly associated with impairments in selecting the correct reward port. To our knowledge, these results provide the first evidence of PV interneurons modulation during cue perception during normal attention, and complement other studies highlighting their importance in maintaining vigilance prior to cue presentation. Moving forward, it will be useful to determine whether pharmacological augmentation of GABAergic interneurons could improve attention performance in rodents and potentially in clinical populations.

Keywords: Attention, Epilepsy, Cognition, Fiber Photometry, Optogenetics

Disclosure: Nothing to disclose.

M41. The Effects of Psilocybin on Binge-Like Feeding Behaviour in Rats

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Background: Psilocybin is a psychoactive serotonergic compound that belongs to a class of drugs called "psychedelics". Psilocybin is metabolised to psilocin, which is a partial agonist at multiple serotonin receptors. Recently, studies of psilocybin therapy have demonstrated evidence for its potential efficacy in treating depressive and anxiety symptoms when administered in a clinical setting. These promising results have inspired the undertaking of larger controlled trials in related disorders. Several receptors targeted by psilocin, including 5-HT1A, 2A, and 2C receptors, are expressed in brain regions such as the nucleus accumbens and hippocampus, suggesting that it may be capable of modulating behavioural domains beyond mood and emotion. To explore the possible effects of psilocybin on dysfunctional feeding behaviour, we investigated the impact of psilocybin treatment in a model in which irregular access to a highly palatable food induces a binge-like feeding behaviour in rats.

Methods: Studies were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986. The study commenced with a 28-day training phase during which 50 female Wistar rats were trained to binge eat on chocolate. Training consisted of placing a pot of chocolate into animal cages for 2 hour periods on an irregular schedule (once per day, on days 1, 2, 4, 6, 7, 9, 12, 14, 15, 18, 23, and 28). Throughout the training period, a control group of nine female Wistar rats were presented with an empty pot on the same days. On Day 30, the treatment phase of the study began. As the study drug (psilocybin) and positive control (lisdexamfetamine) were administered according to different routes and schedules, all animals were administered with an intraperitoneal injection on Day 30 and Day 37 and dosed orally daily from Day 30-38. Specifically, the "no-binge control" group ($n = 9$) that had not been trained to binge eat were dosed with vehicle only, while animals that had been trained to binge eat on chocolate were allocated into five different treatment groups. Three study drug groups ($n = 10$ per group) received a single intraperitoneal injection of psilocybin (1, 3, or 10 mg/kg, respectively) on Day 30 and Day 37 and vehicle via oral gavage on Days 30-38. A positive control group ($n = 10$) received a single vehicle injection on Day 30 and Day 37 and daily oral dosing of lisdexamfetamine (0.8 mg/kg) on Days 30-38. The final group that had been trained to binge eat ("binge control", $n = 10$) were administered with vehicle on Days 30 and 37 (via injection) and

Days 30-38 (via oral gavage). During the treatment phase, binge trained animals were exposed to a pot of chocolate on Day 30 (1 hour following injection), Day 35 (5 days following injection), and Day 38 (24 hours following the second injection) in order to test the effects of vehicle or psilocybin on chocolate intake at multiple timepoints post-administration. In order to assess a 24-hour timepoint without the confound of a binge session the day before (i.e. the 1-hour timepoint, we administered a second injection on Day 37. Animals were allowed ad libitum access to water and normal chow throughout the entire study. Data were analysed using an ANCOVA, incorporating the appropriate pre-treatment baseline measure as a covariate. Comparisons to the binge control group were made using the Williams' test for the psilocybin groups and by multiple t-test for the lisdexamfetamine group.

Results: The positive control lisdexamfetamine significantly reduced chocolate and normal chow intake during all binge sessions conducted at post-treatment timepoints, compared to the binge control group (Chocolate: Day 30 $p<0.001$, Day 35 $p<0.001$, Day 38 $p<0.001$; Normal chow: Day 30 $p<0.001$, Day 35 $p<0.001$, Day 38 $p<0.001$). Single injections of 1, 3, and 10 mg/kg psilocybin significantly reduced chocolate intake, but not normal chow intake, during the binge session conducted 1 hour post-injection (Day 30), compared to the binge control group (1 mg/kg, $p = 0.002$; 3 mg/kg, $p = 0.002$; 10 mg/kg, $p<0.001$). In addition, 10 mg/kg psilocybin also reduced chocolate but not normal chow intake 24 hours post-injection (Day 38), compared to the binge control group ($p = 0.016$). No changes in chocolate or normal chow intake were observed at 5 days (Day 35) following psilocybin treatment.

Conclusions: This study demonstrated that psilocybin specifically reduced bingeing on chocolate at 1 hour post-treatment, with no significant effect on consumption of normal chow, with a similar effect seen for only the high dose of psilocybin (10 mg/kg) at 24 hours post-treatment. These results suggest that psilocybin treatment is able to modulate hyperphagia associated with access to a highly palatable food in rats. Future work should consider the involvement of different serotonin receptor subtypes in the expression of this response.

Keywords: Psychedelics, Psilocybin, Feeding Behavior

Disclosure: COMPASS Pathways: Employee, Stock/Equity (Self)

M42. Food-Seeking Behavior is Mediated by Fos-Expressing Neuronal Ensembles Formed at First Learning

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Background: We previously demonstrated that neuronal ensembles in the infralimbic cortex (IL) develop after prolonged food self-administration training and mediate food seeking behavior. However, rats demonstrated evidence of learning the food self-administration response as early as day 1, with responding quickly increasing to asymptotic levels. Since the contribution of individual brain regions to task performance shifts over the course of training, it remains unclear whether IL ensembles are gradually formed and refined over the course of extensive operant training, or, if functionally-relevant ensembles might be recruited and formed as early as the initial acquisition of food self-administration behavior. In the present study we aimed to determine the role, if any, of IL ensembles at the earliest possible point after demonstrable learning of a response-outcome association.

Methods: We first allowed rats to lever press for palatable food pellets and stopped training rats when their behavior first evidenced the learning of the response-outcome association (learners), and compared their food-seeking behavior and neuronal activation (Fos protein expression) to rats trained for a comparable period of time, but that did not form this association (nonlearners). To determine the functional relevance of Fos-expressing ensembles to subsequent food seeking, we tested region-wide inactivation using muscimol+baclofen and neuronal ensemble-specific ablation using the Daun02 inactivation procedure.

Results: We found that learners had greater food-seeking behavior and neuronal activation within the prelimbic cortex (PL) and IL, suggesting that mPFC subregions might encode initial food self-administration memories. We found that inactivating the IL with muscimol+baclofen and inactivating Fos-expressing IL ensembles both reduced food seeking in learners.

Conclusions: Together, these data suggest that IL neuronal ensembles are formed in tandem with the learning of food self-administration behavior, and furthermore, that these ensembles play a functional role in food-seeking.

Keywords: Engram, Medial Prefrontal Cortex, Learning and Memory, Feeding Behavior

Disclosure: Nothing to disclose.

M43. A Limbic Circuit Selectively Linking Active Escape to Food Suppression

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Background: Stress and anxiety are precipitating factors for eating disorders, but the neural basis linking stress to alterations in feeding is not well understood. Responses to stress can differ and can be characterized as either active coping (e.g. fight or flight) or passive coping (e.g. freezing, immobility). These responses are also associated with other adaptive behavioral effects including reduced feeding until the period of danger has passed, and these stereotyped feeding responses are conserved across organisms ranging from flies to mice and humans. While an association between stress and reduced food intake is well established, the specific neural circuits that are activated by active vs. passive coping strategies and reduce food intake have not been delineated.

Methods: Here we used a combination of chemogenetics, optogenetics, molecular profiling, viral tracing and fiber photometry to identify and population of neurotensin neurons in the lateral septum that link stress to anorexia.

Results: LSNTS neurons specifically increase their activity during active escape, responding only to stressful experiences when flight is a viable option, but not to stressful experiences associated with freezing or complete immobility. Chemogenetic activation of LSNTS neurons leads to a decrease of food intake and body weight in mice, without altering other behaviors associated with stress, such as locomotion and anxiety. Molecular profiling of LSNTS neurons showed that these neurons co-express several molecules, including Glp1r (glucagon-like peptide 1 receptor). and pharmacologic and genetic manipulations of Glp1r signaling in the LS recapitulates the behavioral effects of LSNTS activation. Activation of LSNTS nerve terminals in the lateral hypothalamus (LH), a well-established feeding center, also decrease food intake.

Conclusions: Taken together, these results show that LSNTS neurons are selectively tuned to link active escape stress to reduced food consumption via effects on hypothalamic pathways regulating food intake.

Keywords: Lateral Septum, Neurotensin, Anorexia Nervosa, Acute Stress

Disclosure: Nothing to disclose.

M44. Sex Differences in Conditioned Taste Aversion

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Background: The appropriate evaluation of reward and punishment is critical to mammalian survival, and its disruption contributes to numerous psychiatric illnesses including anxiety and post-traumatic stress disorder. Anxiety disorders are the most prevalent psychiatric disorders, and disproportionately affect women. Few animal models capture the assessment of reward and punishment. To address this deficit, the present study employed conditioned taste aversion (CTA), in which a palatable substance is paired with an aversive stimulus, resulting in aversion at subsequent exposure. CTA utilizes a critical decision-making mechanism for assessing the relative danger or safety of a stimulus, which has broad implications in human psychology and psychiatric disorders. Despite the known female predominance of anxiety disorders, the neural circuitry of CTA is fundamentally understudied in females.

Methods: The purpose of the present study was to delineate the sex differences in the neural mechanisms underlying reinforcement versus aversion in male and female rats. Male and female Sprague-Dawley rats (PN 60-62) were placed in the feeding cage, allowed to drink Boost® for one hour and then treated with either saline or lithium (LiCl, 38 mg/kg). Nausea-related behaviors were observed for one hour, after which they were returned to home cage. Behaviors observed following LiCl injection were pica (eating of corncob bedding), lying on belly, and ptosis. Twenty-four hours later, subjects were returned to the test cage. Ultrasonic vocalizations (USVs), used as an indication of the animal's affective state, were recorded for the first 10 minutes after being returned to the test cage, prior to being offered Boost®. They were again offered Boost® or water and allowed to drink for one hour, after which they were perfused and brains collected for analysis of c-fos as a marker for neural activation. CTA expression was analyzed as a ratio of Boost® consumption on day 2 compared to day 1. Immunohistochemistry was performed on relevant brain regions and c-fos+ cells were manually counted. USVs were analyzed by quantifying the number of 1-minute blocks with >10 55 kHz calls (indicative of a positive affective state), or the presence of 22 kHz calls (indicative of a negative affective state).

Results: We found that CTA expression (day 2/day 1 ratio of Boost® consumption) between male and female rats was similar. Males and females in estrus engaged in 55 kHz calls when Boost® was paired with saline, while females in diestrus showed a more variable call pattern. All animals suppressed 55 kHz vocalizations when Boost® was paired with LiCl. Few animals engaged in 22 kHz calls, even when returned to the context in which they previously received LiCl. Both sexes engaged in nausea behaviors in response to LiCl. Of these behaviors, only lying on belly correlated with CTA expression. Expression of c-fos was higher in both sexes when Boost® was paired with saline, and suppressed when it was paired with LiCl. Females showed a higher level of c-fos expression than males in the insula and basolateral amygdala in the saline-paired Boost® condition. Females also expressed more c-fos globally than males. Both sexes showed robust c-fos expression in the central nucleus of the amygdala in response to both the aversive stimulus (LiCl) and the reinforcing stimulus (re-exposure to Boost® previously paired with saline).

Conclusions: While both sexes showed similar behavior, the neural circuitry showed some striking differences between males and females. Brain regions such as the insula, important for interoceptive signaling, and basolateral amygdala, which assesses affective valence, showed a more robust response in females than males in both saline and LiCl conditions.

One surprising outcome was how similar the aversive and rewarding c-fos response appeared in the central nucleus of the amygdala (CeA) in both sexes. While the CeA is well known to respond to aversive interoceptive signals, its function in reward processing is still largely unknown. Further studies to better delineate the specific cell types responding to these stimuli, in order to better understand the variable function of this brain region to these opposing stimuli, are underway.

Another novel finding of our study was the expressing on USVs in response to the reinforcing stimulus, and their suppression when that same stimulus was rendered aversive due to previous pairing with LiCl. These behaviors provide a deeper understanding of the animal's affective state.

These studies offer one of the first views of the neural circuit of CTA in female rats. Further understanding the neural mechanisms and circuits that underlie these behaviors will offer greater insight into how males and females differentially process rewarding and aversive stimuli, and potentially reveal new therapeutic targets for neuropsychiatric disorders.

Keywords: Neural Circuit and Animal Behavior, c-Fos, Sex Differences

Disclosure: Nothing to disclose.

M45. Mitochondria-Related Nuclear Gene Expression After High Fat Diet in Male and Female C57BL/6 Mice

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Background: Rates of obesity have been steadily on the rise nationally, garnering public health concern. Obesity is comorbid with altered reward processing and often with depression. Both diet composition and overall weight impact many aspects of reward related behavior. Diet-induced obesity is associated with several cellular and molecular changes in reward-related brain regions impacting neuronal and circuit function. One such cellular function impacted by diet is metabolism, which is driven and regulated by mitochondrial function. Mitochondrial function in the context of diet-induced obesity has been extensively studied in muscle tissue, pancreas, and other body systems, but less work has been done characterizing the impact of high fat diet (HFD) on mitochondria in the brain, specifically in reward-related brain regions. Mitochondrial function has been shown to play an important role in both cellular activity and structural plasticity. Further, altered mitochondrial function can be sufficient to alter behavior. Here we examine how multiple lengths of HFD exposure impacts mitochondria-related nuclear gene expression in both male and female C57BL/6 mice.

Methods: Male and female mice were group housed and exposed to HFD (45% calories from fat) or standard lab chow for 24hr, 1wk, 1mo or 3mo. Body weight and food intake were measured twice a week for the duration of exposure (or at the start and end of exposure for the 24hr time point). Brain tissue was collected on the final day of exposure and RNA was extracted. Brain regions analyzed included the nucleus accumbens, dorsal striatum, lateral hypothalamus, and ventral tegmental area. We used quantitative real time PCR (qPCR) to investigate the expression of several nuclear genes involved in mitochondrial function including, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, dynamin related protein 1, Early

growth response protein 3, mitochondrial fission protein 1, and mitofusin 1 and 2.

Results: HFD increased body weight in male and female mice at the 24hr and 3mo time points. Weight was also significantly increased in male mice at 1mo. Neither male nor female mice showed significantly different weight gain at the 1wk time point. Altered expression was observed for all the genes examined, although the patterns of increased or decreased gene expression at different time points in different brain regions were unique between sexes. Interestingly, in both sexes, more genes were significantly altered at the earlier time points (24hr and 1wk) than the later time points (1mo, 3mo), indicating rapid changes that normalize over time.

Conclusions: Together our results indicate that HFD does alter mitochondria-related nuclear gene expression in male and female mice, and the changes associated with initial exposure differ from those observed at longer time points. These findings represent an initial probe into mitochondrial molecular mediators in reward-related brain regions and further studies are needed to probe how these changes in gene expression correspond to behavioral and cellular changes associated with HFD.

Keywords: Mitochondria, High Fat Diet, Gene Expression

Disclosure: Nothing to disclose.

M46. The Role of White Matter Microstructure in Impulsive Behavior

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Background: Impulsivity is a complex behavioral construct integrating affect and cognitive controls and the associated cortical and subcortical neural circuits. Increased impulsivity is a hallmark trait in some psychiatric illnesses including addiction and externalizing disorders. We hypothesize that altered cerebral white matter microstructure may also underwrite normal individual variability in impulsive behaviors and tested this in healthy individuals.

Methods: We collected impulsivity and diffusion tensor imaging (DTI) data in N = 74 healthy adults (32 females, 42 males; age 36.6 ± 13.6 years-old). Impulsivity was evaluated using the Barratt Impulsiveness Scale-11 (BIS-11), providing a total score and three subdomain scores: attentional, motor, and non-planning impulsiveness. DTI was processed using ENIGMA-DTI analysis pipeline to measure whole-brain and regional white matter fractional anisotropy (FA) values in 24 tracts.

Results: Whole brain total average FA was significantly and inversely correlated to motor impulsiveness (r=-0.32, p = 0.007) and positively correlated to non-planning impulsiveness (r=0.30, p = 0.012). Additional nominally significant correlations were observed for the motor impulsiveness and regional FA values for cingulate (r = -0.28, p = 0.016), cingulum (r = -0.28, p = 0.017), and corona radiata (r = -0.26, p = 0.03) tracts; and for non-planning impulsiveness and regional FA values for the corona radiata (r = 032, p = 0.006) and the anterior corona radiata (r=0.27, p = 0.025).

Conclusions: These results provide initial evidence that the motor and non-planning subdomains of impulsive behavior are linked to specific white matter structural connectivity. The data further provides support to the notion that impulsivity is in part a network-based construct involving white matter microstructural integrity in otherwise healthy populations.

Keywords: Impulsivity, White Matter Fractional Anistropy, Healthy Controls, DTI

Disclosure: Nothing to disclose.

M47. Alterations of Peripheral Monoamine and DNA Methylation in Behavioral Addiction

Abstract not included.

M48. Sex-Specific Effects of Early Life Adversity on Hormones and Reproductive Function

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Background: Early life adversity can alter development and has been linked to changes in hormonally-regulated endpoints including pubertal timing and reproductive behaviors. These effects seem to depend on the timing, type, and severity of adversity experienced, yet the mechanisms by which these changes occur are not well understood. One form of early life adversity that is common in humans is growing up in poverty or having limited access to resources. Using a rodent model of limited resources called the limited bedding and nesting model (LBN), our laboratory has previously shown that male rats have elevated adult plasma estradiol levels following exposure to early life adversity. Female rats, on the other hand, show no effect of early life adversity on plasma hormone levels, pubertal timing, or estrous cycle duration (Eck et al. 2019). These data suggest that males may be more susceptible than females to the impact of early life adversity on hormonally-regulated endpoints, leading us to investigate the effects of LBN on male reproductive behaviors.

Methods: All experiments were conducted under the approval of Temple University's Institutional Animal Care and Use Committee. Long Evans rats were reared in either LBN or control housing conditions from postnatal day 2 through 9. In the LBN condition, dams and pups were placed in a limited resource environment where a metal grate prevented access to cob bedding and dams were given a single paper towel to use as nesting material. Control animals were reared in standard laboratory housing conditions, with ample cob bedding, plastic enrichment, and cotton nestlets. On PND10, LBN animals were moved back to standard laboratory housing conditions. Adult control and LBN males (n = 9-12 per group) were tested for male reproductive behaviors three times over the course of three consecutive weeks. All males were virgin prior to the first test. Each week, males were paired with a novel, ovariectomized, hormonally primed female rat for 30 minutes and their interactions were video recorded for later behavior analysis. For each week, the number of mounts, intromissions, and ejaculations exhibited in those 30 minutes was recorded as well as the latency to the first instance of each behavior. Repeated-measures ANOVAs were used to investigate how these behaviors changed over the course of the three weeks. Planned comparison were used to compare the number of each behavior as well as the latencies to mount and intromit during each week of testing. Due to some animals taking longer than the maximum of 30 minutes to ejaculate, Mann-Whitney U tests were used to compare latencies to ejaculate at each time point.

Results: A repeated-measures ANOVA revealed that rats in both control and LBN conditions increased the number of mounts [$F(2, 36)=9.25$, $p.=001$, $\eta p2 = .34$], intromissions [$F(1.46, 29.50) = 29.50$, $p = .024$, $\eta p2=.19$], and ejaculations [$F(2, 40)=4.88$, $p = .013$, $\eta p2=.20$] exhibited over the course of the three weeks. Both control and LBN rats also showed decreased latencies to exhibit mounts [$F(1.45, 23.25)=6.16$, $p = .012$, $\eta p2=.28$] and intromissions [$F(1, 17)=2.57$, $p = .128$, $\eta p2=.33$] over the course of the three weeks. There were no significant differences between LBN and control males for the number of mounts, intromissions, or

ejaculations exhibited in any of the three sessions (p>.05 in all cases). However, LBN males showed a shorter latency to mount than controls during week 2 [t(19)=2.15, p = .044, d=.92] as well as a shorter latency to ejaculate during week 2 (U=29, p = .023, r=-.48) and a trend toward a shorter latency to intromit during week 1 [t(11.36)=2.18, p = .051, d=.93].

Conclusions: Both LBN and control males show improvements in reproductive behaviors over time in terms of both number of behaviors and latency to behave. However, LBN males have shorter latencies to engage in certain behaviors during weeks 1 and 2 before group differences even out by week 3. These data suggest that LBN males acquire reproductive behaviors more quickly than their control counterparts. This idea could represent an evolutionarily adaptive response to the experience of early life adversity, allowing animals with limited access to resources, and therefore a potentially shorter lifespan, the opportunity to reproduce and pass on their genes earlier. These results are also in line with our previous finding that LBN males have elevated estradiol levels in adulthood, as estradiol is known to be critical for the expression of male sexual behavior. Together our findings point to enhanced male reproductive behavior in LBN males compared to controls and may indicate increased masculinization of these animals following early life adversity.

Keywords: Early Life Stress, Gonadal Hormones, Reproduction, Motivation

Disclosure: Nothing to disclose.

M49. Developing Pharmacological Probes for Interrogation of the Circuitry of the Nucleus Accumbens

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Background: With the support of a diverse ensemble of auxiliary proteins tightly regulating their function, voltage-gated Na+ (Nav) channels serve as the fundamental molecular determinants of neuronal excitability. Crucially, recent studies have shown that disruption of protein:protein interactions (PPIs) that regulate the function of Nav channels leads to neural circuitry aberrations that are implicated in the etiology of psychiatric disorders. From a pharmacological perspective, these PPI interfaces that become perturbed are highly specific and flexible, and could, therefore, serve as ideal surfaces for the development of targeted chemical probes and neurotherapeutic lead compounds. Here, we present recent advancements in our high-throughput screening (HTS) campaign, which entails experimental modalities ranging from in silico to ex vivo, to identify modulators of the PPI between Nav1.6 and its auxiliary protein fibroblast growth factor 14 (FGF14). With the PPI between these two proteins being known to modulate intrinsic firing properties of medium spiny neurons (MSNs) in the nucleus accumbens (NAc), lead compounds emerging from this campaign could lead to PPI-based neuropsychopharmacological agents.

Methods: We employed medicinal chemistry to synthesize and optimize, as well as chemoinformatics, the split-luciferase assay (LCA), surface plasmon resonance (SPR), and whole-cell patch-clamp electrophysiology to validate, pharmacological probes to interrogate neural circuitry.

Results: We screened 44,480 compounds in singlicate from the ChemBridge and Maybridge libraries against the FGF14:Nav1.6 PPI interface to identify small molecules capable of inhibiting assembly of the two proteins using the LCA. This preliminary screening revealed 1,184 small molecules that seemingly inhibited FGF14:Nav1.6 complex assembly (Z ≤ -5 relative to vehicle only) without conferring cytotoxicity. Re-screening of these 1,184 compounds in triplicate and subsequent counter-screening against the full-length luciferase enzyme validated the modulatory effects of 236 small molecules on FGF14:Nav1.6 complex assembly. This pool of candidate compounds was further narrowed to 93 compounds based upon a Lipinski's analysis coupled with structural analyses to identify likely pan-assay interference compounds (PAINS). Subsequent dose-response analyses of these 93 compounds identified 17 PPI inhibitors displaying low micromolar potency. After selectivity studies revealed that 10 of these compounds displayed omnivorous modulation of FGF:Nav PPI interfaces, the remaining seven compounds were profiled for their protein:ligand binding affinities toward both the C-terminal domain (CTD) of Nav1.6 and FGF14 using a protein thermal shift (PTS) assay and surface plasmon resonance (SPR). Three of these compounds, 5674, 7605, and 7647, showed appreciable binding affinities toward both proteins, a desirable feature as synergistic protein:ligand interactions with both cognates potentiates a compound's likelihood for functionally relevant modulation of a PPI. Given this, these three compounds were selected for functional evaluation using whole-cell patch-clamp electrophysiology in heterologous cell systems and acute brain slice preparations. Although all 3 exhibited modulatory activity against Nav1.6-mediated currents and MSN firing, 7605 stood out for its effects on fast and long-term inactivation, as well as for its ability to suppress sodium persistent currents and firing in MSNs in an FGF14-dependent manner. Additionally, molecular dynamics simulation predicted favorable blood-brain barrier (BBB) permeability of 7605, which was subsequently confirmed by in vivo studies. Evaluation of 7605 as a probe for interrogating the circuitry of the NAc and for its modulatory effects on motivated behavior is currently ongoing.

Conclusions: These results not only demonstrate the feasibility of centering a drug discovery campaign on a PPI in the brain, but provide an innovative strategy that could lead to novel PPI-based neuropsychopharmacological agents.

Keywords: Ion Channels, Medium Spiny Neuron, Nucleus Accumbens

Disclosure: IonTx Inc.: Stock / Equity (Self)

M50. Small Molecule Inhibition of the TRIP8b-HCN Interaction

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Background: Major Depressive Disorder (MDD) is a critical public health problem with a lifetime prevalence of nearly 17% in the United States. Many patients remain symptomatic despite appropriate treatment for MDD, which indicates a need to develop mechanistically distinct antidepressant therapies. One target that has recently gained attention is the interaction between hyperpolarization-activated cyclic-nucleotide gated (HCN) channels and an auxiliary subunit of the channel, tetratricopeptide repeat-containing Rab8b-interacting protein (TRIP8b). HCN channels are key regulators of neuronal excitability in the mammalian hippocampus, and recent work from our lab and others has established that antagonizing either HCN or TRIP8b promotes antidepressant-like effects on behavior. We have previously described the development of a high-throughput screening assay to identify novel small-molecules that are capable of disrupting the TRIP8b-HCN interaction.

Methods: The crystal structure of TRIP8b in complex with the C terminus of HCN has been solved (Bankston et al 2012). Utilizing

this crystal structure, we have virtually screened millions of compounds. These hits were subsequently filtered using in vitro biophysical assays (Han et al 2015) including AlphaScreen and NanoBIT assays. One of the promising hits, NUCC-0200590 was validated by flow cytometry (to detect HCN surface expression) and electrophysiology in HEK cells.

Results: In this report, we have identified a new small molecule capable of disrupting the TRIP8b-HCN interaction. We demonstrated a strategy for the synthesis of this compound and established the efficacy of the NUCC-0200590 in disrupting the protein-protein interaction in biochemical and cell-based assays. The binding was further established using STD-NMR which also provides epitope mapping to show areas of the molecule that closely bind to the protein. Finally, we found that NUCC-0200590 is capable of disrupting the TRIP8b-HCN interaction and regulating HCN function in cells. Several groups have shown that the TRIP8b-HCN interaction may be a therapeutic target for the treatment of MDD, hence the development of compounds such as NUCC-0200590 may lead to mechanistically distinct antidepressants and provide new chemical tools useful in studying its function.

Conclusions: These results provide evidence that NUCC-200590 may be used as a research tool to study the TRIP8b-HCN interaction as well as HCN functions in the brain and might someday be used as a novel class of antidepressant.

Keywords: HCN, TRIP8b, Antidepressants

Disclosure: Nothing to disclose.

M51. Estrogen Modulation of Midbrain Neural Output Drives Shifts in Social Motivation

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Background: Estrogen exerts physiological effects upon cortical, limbic and midbrain structures, influencing different behaviors such as memory, mood and reward processing. Disruptions in estrogen levels can be caused by chronic stress, hormonal replacement, specific cancers and disrupting chemicals in the environment. Importantly, these estrogen imbalances have been indicated to increase susceptibility to disruptions in mental health. Several studies have reported an important role of estrogen signaling in the ventral tegmental area (VTA) in response to stress, alcohol and drugs. Specially, estrogen has a role in mediating dopamine neuron function, possibly contributing to sex differences in susceptibility, prevalence, and symptoms of multiple neuropsychiatric disorders. This occurs through the complex action of estrogen receptors α and β (ERα and ERβ) and the 7-transmembrane G protein-coupled estrogen receptor (GPER/GPR30) expressed in the VTA. In particular, mood disorders are more prevalent in females who undergo life-long estrogen fluctuations, while susceptibility increases in males as they age and plasma testosterone, a precursor to estrogen, drops. We demonstrate that disruptions in motivational drive for social interaction, a common feature of multiple mood disorders, is tightly regulated by estrogen signaling in the VTA. Here, we found that rapid estrogen signaling, via GPER, modulates dopamine neuron potassium channel activity to alter non-reproductive social drive as measured by an effort-based social interaction test.

Methods: In in vivo pharmacological experiments, male and freely cycling female C57BL/6J mice were bilaterally cannulated and locally infused with specific ER agonist and antagonist to the VTA prior to behavioral testing for social drive. To quantify non-reproductive social drive mice were tested on an effort-based social interaction test, which consists of a two weighted swinging door choice to access an age and sex-match conspecific. We used the following antagonist: ERβ+ antagonist PHTPP (0.21 or 2.12ng/hemisphere), ERα antagonist MPP dihydrochloride hydrate (0.28 or 2.78ng/hemisphere) or GPER antagonist G15 (0.3 or 2.6ng/hemisphere). (6 mice per group x 4 groups x 2 sexes =48 mice). We used the following agonist: GPER agonist G1 (10pM), ERβ agonist DPN (10 pM) and ERα agonist PPT (10 pM). (6 mice per group x 3 groups x 2 sexes = 36 mice). We utilized in vitro slice electrophysiological cell recording of VTA dopamine neurons in combination with pharmacological agents, to isolate potassium channel function and measure directly the action of ER agonist and antagonist on DA neuron activity.

Results: We observed diminished social drive in both male and female mice following VTA infusion with GPER antagonist, with reduced entries to the chamber with the social target ($p < 0.05$). This effect disappears on day 4 of testing, and is similar to that found in estrous cycle differences in conditioned place preference for cocaine, possibly showing a similar dopaminergic mechanism. These results support the hypothesis that estrogen receptors can actively and rapidly alter social motivation. In vitro studies revealed that GPER activation rapidly reduces the firing rate and excitability in VTA dopamine neurons when they are recorded from males and females that are in estrus at the time of recording. Further, in vitro studies reveal unique potassium channel activation dependent upon specific ER activation.

Conclusions: We have revealed a cellular mechanism by which estrogen drives changes in ionic activity in the VTA resulting in changes in motivational drive. Understanding estrogen's effects on channel function in the dopaminergic pathways, and its influence on social motivation, is informative for the development of potential sex-specific therapeutics for mood disorders.

Keywords: Dopamine, Estrogen Receptor, Social Motivation

Disclosure: Nothing to disclose.

M52. H3K4me1 in VTA Mediates Early Life Stress-Induced Stress Sensitivity

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Background: Early life stress (ELS) is one of the strongest predictors of mood, anxiety, and substance use disorder risk, particularly after facing additional stressful events in adulthood. At the root of this risk may be alterations in motivation and reward processing. To study the cellular and molecular correlates of lifelong stress vulnerability, we use a "two-hit" stress paradigm in mice in which a first hit of stress in early life increases susceptibility for depression-like behavior in response to a second stress in adulthood. This latent behavioral vulnerability is accompanied by latent transcriptional alterations in key brain reward regions that are implicated in motivation, including the ventral tegmental area (VTA). We hypothesized that such latent transcriptional alterations would be primed by post-translational histone modifications. In particular, histone 3 lysine 4 mono-methylation (H3K4me1) is associated with primed chromatin. Several earlier findings indicated increased H3K4me1 in adult male VTA after ELS.

Methods: Levels of H3K4me1 in VTA were determined by Western blot (P21; n = 7 males and females each/group) or mod-spec (adult; n = 3 pooled male samples/group) in control and ELS mice. In order to test whether increased levels of H3K4me1 mediate ELS-enhanced sensitivity to adult stress, we generated an AAV to overexpress Setd7, an enzyme that monomethylates H3K4. We overexpressed Setd7 or control Gfp in VTA of standard-reared mice at postnatal day 10-15 during the stress sensitive period, allowed mice to mature to adulthood, and then tested them on a battery of behavioral tests including sucrose preference, social interaction, and splash

test (N = 7 males and 7 females per group). Finally, mice were subjected to sub-threshold social defeat stress and tested again to determine sensitivity to adult stress.

Results: ELS moderately increased H3K4me1 levels in VTA at both acute [P21; t(1,12)=1.89, p = 0.041] and adult time points [t(1,4)=2.09, p = 0.052], although this effect was specific to males. We validated that our AAV-Setd7 increases both SETD7 protein levels and H3K4me1 in VTA in vivo relative to AAV-Gfp control. Juvenile Setd7 expression in VTA did not alter behavior at baseline. However, there was a significant interaction between juvenile Setd7 overexpression and adult social defeat stress exposure on social interaction ratio [F(1,23)=4.41, p = 0.047], such that social interaction was significantly reduced after subthreshold defeat only among mice with VTA Setd7 overexpression (t(1,12)=3.92, p = 0.002).

Conclusions: Early life stress promotes a more open/permissive chromatin state in the VTA associated with transcriptional priming. Mimicking early life stress by increasing H3K4me1 in VTA primed an enduring sensitivity to adult stress, primarily manifest in reduced motivation to explore a social target. This provides causal evidence linking early life stress to epigenetic modifications in reward circuitry that directly mediate lifelong stress sensitivity.

Keywords: Epigenetics, Ventral Tegmental Area (VTA), Early Life Stress, Social Defeat Stress

Disclosure: Nothing to disclose.

M53. Elucidating the Role of Eukaryotic Elongation Factor 2 Kinase in Rapid Antidepressant Action

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Background: Major depressive disorder is a prevalent and serious form of mental illness. Traditional antidepressants, which target the monoamine system, are commonly used for the treatment of depression but typically take several weeks to exert a clinical effect, with a sizable fraction of the patient population failing to respond to treatment. There has been a major unmet need for the development of pharmacological therapies that can quickly and effectively alleviate symptoms associated with depression. Clinical data shows that a single sub-psychomimetic dose of ketamine, a noncompetitive glutamatergic N-methyl-D-aspartate receptor (NMDAR) antagonist, has rapid antidepressant responses in patients with treatment-resistant major depressive disorder. We previously showed that ketamine blocks NMDARs activated by spontaneous glutamate release (also referred to as "at rest") that couple to eukaryotic elongation factor 2 kinase (eEF2K) signaling, which is essential for the rapid antidepressant action of ketamine in mouse models. Subsequently, eEF2K is inhibited leading to desupression of protein translation. Our hypothesis is that ketamine blocks resting NMDAR activity in the hippocampus, inhibiting eEF2K activity, and subsequently increasing protein synthesis including brain-derived neurotrophic factor (BDNF) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propinic acid receptors (AMPARs) to trigger a homeostatic form of synaptic plasticity that is necessary for rapid antidepressant effects. While our work proposes eEF2K is a key molecule for rapid antidepressant action, there is little known regarding the precise role of eEF2K in neurons and synaptic plasticity.

Methods: We combined immunochemical, genetic and electrophysiological strategy to delineate the role of eEF2K in neurons. Cultured hippocampal neurons were prepared from whole hippocampi of mice. To identify localization of phosphorylated eEF2, cultured hippocampal neurons were fixed by paraformaldehyde (PFA) and stained using a specific antibody against phosphorylated eEF2. To perform loss of function experiments, we infected hippocampus neurons with lentivirus encoding eEF2K shRNA or non-targeting shRNA as a control. To measure AMPAR-miniature excitatory postsynaptic currents (mEPSCs), we performed whole-cell voltage patch-clamp recording. AMPAR-mEPSCs were isolated by adding tetrodotoxin (TTX), D(-)-2-Amino-5-phosphonopentanoic acid (AP-5) and picrotoxin (PTX) in the external solution.

Results: We examined the localization of eEF2 phosphorylation in sub-compartments of mature hippocampal neurons to elucidate specific localization of eEF2K. We observed high levels of phosphorylated eEF2 in dendrites and spines suggesting eEF2K can acts as a regulator of dendritic protein translation at postsynaptic sites. As previous studies demonstrated that spontaneous NMDAR activity maintains synaptic homeostasis, we examined whether blockade of NMDARs at resting conditions mediates homeostatic synaptic scaling. Cultured hippocampal neurons were treated with the NMDA receptor antagonist AP-5 or ketamine in the presence of the sodium channel blocker TTX, and AMPAR-mEPSCs were recorded. We found that the TTX/AP-5 or ketamine treatment induced synaptic scaling in cultured control neurons. To assess the requirement of eEF2K in synaptic scaling, we knocked down eEF2K in neurons and performed similar experiments. We observed that eEF2K knockdown neurons occluded synaptic scaling suggesting that eEF2K is required for the induction of synaptic scaling.

Conclusions: These finding demonstrate that NMDAR blockade at rest mediates homeostatic plasticity. These data also show eEF2K is localized in postsynaptic neurons and is essential for the induction of synaptic scaling. Collectively, these data provide novel insight into the intrinsic role of eEF2K in rapid antidepressant action.

Keywords: Rapid Antidepressant, Homeostatic Plasticity, NMDA Receptor

Disclosure: Nothing to disclose.

M54. A Randomized, Double-Blind, Placebo-Controlled Study of SEP-4199 for the Treatment of Patients With Bipolar Depression

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Background: SEP-4199 is a fixed ratio of 85% aramisulpride and 15% esamisulpride with stereoselective affinity for 5-HT7 and dopamine D2 receptors, respectively. The pharmacology of SEP-4199 has been optimized to amplify 5-HT7-mediated antidepressant effects while reducing D2 antagonist activity to a level of receptor occupancy that minimizes D2-related side effects while providing the potential for both antidepressant and anti-manic effects. The aim of the current study was to evaluate the efficacy and safety of SEP-4199 for acute major depressive episodes associated with bipolar I disorder.

Methods: Adult patients meeting DSM-5 criteria for bipolar I disorder who currently were experiencing a major depressive episode were randomized to 6 weeks of double-blind, placebo-controlled treatment with SEP-4199 at a fixed daily dose of 200 mg or 400 mg. The primary efficacy endpoint was week-6 change in the MADRS total score. The primary analysis sample consisted of patients in the US and Europe (N = 295 randomized); the pre-specified full intent to treat (ITT) analysis population consisted of all randomized patients (N = 344; including 49 patients in Japan). Efficacy measures included the Quick Inventory of Depressive Symptomatology, Self-Report (QIDS-SR-16), the

Hamilton Anxiety Rating Scale (HAM-A), and the Clinical Global Impression, Bipolar, Severity of Depression scale (CGI-BP-S, depression). Primary and secondary efficacy outcomes were assessed using an MMRM analysis.

Results: Baseline characteristics were comparable for each study treatment group. In the primary US/Europe analysis sample, the LS mean difference in week 6 MADRS change scores showed a strong trend to improvement versus placebo for the SEP-4199 200 mg dose (-3.29 [95%CI: -6.49, -0.09]; adjusted P=0.054; effect size [ES], 0.31), and the 400 mg dose (-3.13 [95%-CI: -6.27, 0.02]; adjusted P=0.054; ES, 0.29). In the full ITT analysis, LS mean difference in week 6 MADRS change scores (vs. placebo) showed greater improvement versus placebo for both the SEP-4199 200 mg dose (-3.68 [95%-CI: -6.65, -0.70]; P=0.016; ES, 0.34), and the 400 mg dose (-3.38 [95%-CI: -6.31, -0.45]; P=0.024; ES, 0.31). Greater improvement was also observed (versus placebo) on the QIDS-SR-16 for the 200 mg dose (P=0.049; ES, 0.28) and the 400 mg dose (P=0.038; ES, 0.29); and in anxiety severity as measured by the HAM-A (P=0.013; ES=0.35 and P=0.005; ES=0.39, for the 200 mg and 400 mg doses, respectively); no difference was observed for either dose compared with placebo on the CGI-BP-S depression scale. For the full ITT population, a similar proportion of patients completed the study in the SEP-4199 200 mg group (80.7%) the 400 mg group (87.8%) and the placebo group (86.1%). Discontinuation due to an adverse event occurred in 8.8% of patients in the SEP-4199 200 mg group, 7.0% in the 400 mg group, and 1.7% in the placebo group. The 3 most frequent adverse events for SEP-4199 200 mg, 400 mg, and placebo, respectively, were as follows: nausea (2.7%, 3.5%, 2.6%), galactorrhea (2.7%, 3.5%, 0%), and somnolence (1.8%, 4.4%, 0.9%). There were no clinically significant differences for SEP-4199 versus placebo on laboratory measures, except for elevations in prolactin levels. Mean endpoint change in QTcF interval was +6.3 msec in the SEP-4199 200 mg group, +9.3 msec in the SEP-4199 400 mg group, and +0.1 msec in the placebo group. No patient showed a QTc interval of >500 msec.

Conclusions: In this 6-week, double-blind trial of bipolar I patients with an acute major depressive episode, treatment with SEP-4199, in fixed doses of 200 and 400 mg/d, showed a strong trend to improvement for both doses on the MADRS compared with placebo in the primary analysis subgroup (P=0.054 and 0.054, respectively). In the full ITT analysis population, significant improvement was observed on the MADRS and the QIDS-SR-16 for both doses of SEP-4199 versus placebo. SEP-4199 doses of 200-400 mg/d were safe and well-tolerated during short-term treatment. Based on these proof of concept results, we believe that SEP-4199 (a novel compound with prominent 5-HT7 antagonist effects) is a promising compound that warrants further investigation as a treatment for bipolar depression.

Keywords: Bipolar Disorder, Major Depression, Atypical Antipsychotics

Disclosure: Sunovion Pharmaceuticals, Inc.: Employee (Self)

M55. Childhood Physical Abuse Differentiates Ketamine Treatment Response Among Adults With Treatment Resistant Depression

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Background: Not all patients with treatment resistant major depression who receive the N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine improve. The identification of predictors of ketamine treatment response would benefit treatment planning. In a data-driven, agnostic approach, we estimated distinct treatment response trajectories to repeated intravenous ketamine infusions in a naturalistic sample of depressed adults seeking treatment at a private ketamine clinic. We additionally examined demographic and clinical variables that possibly differentiate between response trajectories.

Methods: Growth mixture modeling (GMM) was performed to identify latent treatment response classes across 385 depressed patients (mean age 41.75; 49% female) who visited a ketamine clinic for IV ketamine. Patients received approximately 05 mg/kg ketamine. The period between infusions differed across patients and was included in the analysis as covariate. Treatment response was determined with the Quick Inventory of Depression Symptoms – Self-Report (QIDS-SR) filled out at baseline before the first infusion, and before the 2nd, 3rd, and 4th infusion. All subjects received at least one infusion, and missing data was inserted with Full Information Maximum Likelihood.

Results: GMM identified three latent classes of treatment response. One class of patients had moderate QIDS-SR scores at baseline and minimal improvement over the course of ketamine administrations. Two classes of patients had severe level QIDS-SR scores at baseline. One class had a rapid and robust antidepressant response; the other class showed minimal improvement over time. Comparisons between the two high depression trajectories for demographic and clinical variables revealed a history of high Childhood Trauma Questionnaire (CTQ) physical abuse in the class with compared to the classes without a significant ketamine treatment response. Other demographic and clinical variables did not differ between the two high depression classes. Childhood maltreatment did not differentiate between the low depression and minimal improvement and the two high depression classes.

Conclusions: Our outcomes show that IV ketamine should be considered as a primary treatment option for adults presenting with severe, treatment resistant depression and a self-reported history of childhood physical abuse. IV ketamine may not be as effective for moderately depressed individuals irrespective of childhood maltreatment.

Keywords: Depression, Childhood Trauma, Treatment-Response, Ketamine

Disclosure: Nothing to disclose.

M56. The Effect of Selective D3 Agonism on Anhedonia Symptoms and Reward Neurocircuitry in Subjects With MDD and Prominent Anhedonia

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Background: Anhedonia, which encompasses lack of pleasure, motivation, and/or interest, occurs at a clinically significant level in 37% of patients with major depressive disorder (MDD). It is among the most important risk factors for non-remission on conventional antidepressants, increased risk of suicide, and disability. Anhedonia has been associated with hypoactivation in the ventral striatum (VS), a key node in reward neurocircuitry. Pramipexole is a preferential D3 receptor subtype agonist that is FDA-approved for Parkinson's Disease and Restless Legs Syndrome, and target engagement studies using functional neuroimaging in healthy subjects have shown that a single dose of pramipexole augments activation in a key reward region in the VS called the nucleus accumbens. Hence, using pramipexole as a novel treatment to selectively target D3 receptors is a reasonable strategy for modulating the VS in MDD. Pramipexole has been shown to improve overall depressive symptoms with modest efficacy in

treatment resistant subjects with MDD when used as an adjunctive combined with standard antidepressant therapy and in a pilot trial comparing monotherapy with adjunctive therapy. Furthermore, an open-label study found that pramipexole specifically improved measures of anhedonia with large effect sizes, and lower baseline VS dopamine release was associated with greater improvement in overall illness severity. However, the specific effects of pramipexole on anhedonia, reward-related behavior, and neurocircuitry in MDD subjects with prominent anhedonia remain understudied.

Methods: In this open-label pilot study, 5 subjects with prominent anhedonia and MDD completed treatment with pramipexole for 8 weeks. Prominent anhedonia was defined as > 35 on the Mood and Anxiety Symptoms Questionnaire (MASQ) Anhedonic subscale. Consistent with recommendations from the literature, patients were started at 0.25mg daily at bedtime and increased every 3 days with a goal dose of 2.0 daily. Clinical measures, cognitive behavioral testing, and functional neuroimaging scans were conducted at baseline and 8-weeks post-treatment.

Results: The 5 subjects that completed treatment ranged in age from 25–45 years old, 80% were male, and all completed college or graduate education. Two additional subjects dropped out before completion of the study due intolerable side effects (nausea, poor sleep, and headache). No subjects experienced serious adverse events, such as extrapyramidal symptoms or impulsivity. The subjects who completed the study had significant improvement in the MASQ Anhedonic subscale ($t(4) = 4.51$, $p = 0.02$, Cohen's $d = 2.0$), overall depressive symptoms as measured by the Quick Inventory of Depressive Symptoms (QIDS, $t(7.5) = 2.39$, $p = 0.05$, Cohen's $d = 1.5$), and quality of life as measured by the Satisfaction with Life Scale (SWLS, $t(4) = -3.5$, $p = 0.02$, Cohen's $d = 1.6$). 80% (4/5) responded to the treatment at 8 weeks defined by a 50% improvement in QIDS. Reward task-evoked VS also significantly increased from baseline to post-treatment ($t(4) = -2.83$, $p = 0.05$, Cohen's $d = 1.3$), consistent with the results of a prior target engagement study. Furthermore, baseline VS activation and change in VS activation were negatively correlated with a strong effect at a trend level of significance ($r2 = 0.69$, $p = 0.08$). Although not significant, baseline VS activation was also negatively correlated with changes in MASQ Anhedonic score ($r2 = 0.24$, $p = 0.40$) and QIDS ($r2 = 0.48$, $p = 0.20$) with large effect sizes, suggesting that lower baseline VS activation is associated with greater improvement in symptoms in response to pramipexole. While the small sample size of this pilot study limits the capacity to draw inferential conclusions based on p values, the direction of effects and effect sizes are encouraging.

Conclusions: This study provides preliminary insights into the predictors and neural mechanisms of change in pramipexole response in subjects with prominent anhedonia and MDD. Furthermore, it informs an appropriately powered, randomized, placebo-controlled trial of pramipexole in the same subject group. This planned trial is a critical stepping stone for future neuromarker-guided studies of pramipexole and other D3 receptor agonists as tailored treatments for individuals with prominent anhedonia and MDD. Indeed, both symptom (prominent anhedonia) and brain measures (reward circuit dysfunction), in addition to other biomarkers, will likely be needed to specifically select subjects with the greatest chance of responding to a selective D3 receptor agonist.

Keywords: Anhedonia, Pramipexole, Major Depressive Disorder, Functional Neuroimaging, Precision Psychiatry

isclosure: Nothing to disclose.

M57. Examination of Changes in Hippocampal MRI Volume Measurements After Ketamine Administration in Patients With Major Depression and Healthy Controls

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Background: Changes in the hippocampus have been implicated in the pathophysiology (MacQueen and Frodl, 2011; Sheline et al., 1996) and treatment (Duman et al., 1999) of major depression (MDD). In particular, findings of reduced hippocampal volumes, as measured by magnetic resonance imaging, in MDD are thought to be potentiated by early life stress which is worsened by repeated bouts of depression (Sheline, 2011). Some pre-clinical models suggest that some of the stress related reduction in changes can be reversed by anti-depressants such as ketamine (Duman et al., 1999). No direct in-vivo measurement of hippocampal plasticity or dendritic spine density is yet possible but volumetric differences from MRI have been used to estimate these changes. In this study we have investigated the longitudinal difference in hippocampal and amygdalar subfields volumes between MDD patients and healthy controls (HC) over the course of a double-blind placebo-controlled study of ketamine at fields strengths of 3T and 7T.

Methods: A total of twenty-six unmedicated MDD (median (range) age=33.0 (20-55), 16 female) patients and twenty HCs (median (range) age=30(20-56), 13 female) participants are included in this analysis. Scans were nominally acquired at 5 timepoints: baseline, post (day 2-3) and interim (day 10-11) intervals after randomized ketamine and placebo infusions. 3T T1 weighted scans were performed on a GE HDx (Milwaukee, WI) scanner with the system 8-channel head coil at 1mm isotropic resolution. 7T T1 weighted scans were performed on a Siemens Magnetom (Erlangen, Germany) scanner using a 32-channel head coil (Nova Medical, Wilimington, MA) at 0.7mm isotropic resolution. Hippocampal and amygdalar segmentation was performed using FreeSurfer v. 6.0 (Fischl, 2012) (Martinos Center for Biomedical Imaging, Charlestown, MA, USA) using the longitudinal pipeline for all scans. Statistical modeling was done in R where mixed effect models were calculated separately for the whole hippocampus and amygdala, each hemisphere and subfield separately. Estimated total intracranial volume (eTIV), sex, and age were included as covariates. Percent change difference in volume, ICC and dice overlap coefficients were also calculated between each scan timepoint.

Results: At 3T, the mixed model for whole hippocampal volume at baseline revealed a main effect of sex (95% CI [25,344], $F1,33=5.5$ $p = 0.03$) for the right hippocampus, and left (95% CI [95,302], $F1,33 = 15.2$, $p < 0.001$) and right amygdala (95% CI [114,325], $F1,33=18.0$, $p < 0.001$). No differences were found between groups for any of the subfield regions at baseline.

No substantial changes in whole hippocampal were found at either post or interim scans after ketamine or placebo administration for either hemisphere or group. The left amygdala showed an increase of 36.72 mm3 ($SE = 14.79$, $t1,91 = 2.48$, $p = 0.01$) between post and interim scans after ketamine infusion. No other changes in amygdalar value were found for any other group, hemisphere or scan. Similar results were obtained at 7T, however, the 3T volumes were estimated to be about 11-14% larger.

The whole hippocampus (mean difference= -0.048%, SE=0.15) and subiculum (mean difference=-0.122%, SE=0.20) show the most consistency between scans whereas the fimbria (mean difference = 2.6%, SE= 0.64) and hippocampal fissure (mean difference = 2.9%, SE= 0.60) show the least consistency. The amygdala and subfields are in general more variable than those of the hippocampus with the anterior amygdaloid area (mean difference = 2.9%, SE= 0.60) and lateral nucleus (mean difference = 1.1%, SE= 0.52) being the most consistent and the central (mean difference = 3.8%, SE= 0.72) and medial (mean

difference = 5.7%, SE= 1.2) nuclei being the least consistent. The amount of variability in percent difference between scans was consistent for a given subfield.

The subfields with the highest Dice or overlap coefficient calculated between the baseline and each subsequent scan are the basal and lateral nuclei (0.92) of the amygdala. The medial and paralaminar nuclei had the lowest dice values. ICC values across scans were uniformly very high for all subfields with most being above 0.99 showing excellent agreement of volume measurement between scans within a field strength.

Conclusions: No differences in total hippocampal volume were found between MDD and HCs at baseline or at any point the study. There was a measurable increase in whole left amygdalar volume in MDD patients between ketamine and placebo scans 10 days post infusion. No other differences in whole amygdalar volume were found between MDD and HCs. The few differences in hippocampal and amygdalar subfield volumes after ketamine infusion did not survive multiple comparisons correction. Subfield and whole amygdalar volume measurements were fairly consistent across scans but differed between field strengths. In sum, despite having excellent agreement in hippocampal and amygdalar volumes estimated from repeated longitudinal anatomical MR images, we did not find convincing evidence for changes induced by an acute infusion of ketamine in MDD or HCs.

Keywords: Amygdala-Hippocampal Differences, Ketamine, Major Depression Disorder

Disclosure: Nothing to disclose.

M58. Pramipexole to Improve Cognition in Bipolar Disorder: A Negative Study Using an Experimental Therapeutics Approach

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Background: Adults with bipolar disorder (BD) often experience neurocognitive impairment, even during euthymic mood (Burdick et al. 2011). This impairment negatively impacts functioning and quality of life (Bowie et al. 2009; Burdick et al. 2010), but has received relatively little attention as a potential treatment target.

Pramipexole (PRAM) is a dopamine agonist; across neuroimaging and molecular genetic studies, dopamine has been implicated in cognitive function. Additionally, previous trials have found that other dopamine agonist agents improve cognition in healthy volunteers (Kimberg et al. 1997; Kimberg et al. 2003; Luciana et al. 1998).

In an 8-week, double-blind, placebo-controlled, adjunctive trial of PRAM to improve neurocognitive function, a subset of participants with BD exhibited significant improvements in cognition (Burdick et al. 2012). Results suggested that individuals who were affectively stable, but still experiencing cognitive impairment were most likely to benefit from PRAM augmentation.

The goal of the present study was to evaluate the effect of PRAM treatment on cognitive function in participants with BD experiencing clinically significant cognitive impairment despite affective stability. In this study, we utilized an experimental therapeutics strategy in which we sought to confirm target engagement through use of the dopaminergically-mediated Iowa Gambling Task (IGT). We (Burdick et al. 2014) and others (Riba et al. 2008) have shown that PRAM reliably engages reward circuitry as reflected by impaired performance on the IGT; therefore, we expected that once target engagement was confirmed (via changes to IGT performance), cognitive benefits of PRAM treatment would be optimized.

Methods: Participants were recruited from two psychiatry departments in the New York metro area. Eligibility criteria included being between the ages of 18-65; diagnosis of BD I or II; mood stability over a 4-week period (between screening and baseline); clinically-significant neurocognitive impairment (defined as > 1 SD below average on a global composite score derived from a set of standard cognitive assessments). Participants were randomized to receive PRAM or a placebo, dose was initiated at 0.125 mg BID and increased every 3 days to a target of 4.5 mg/day. Participants were seen 10 times over the course of the study, assessments were completed at baseline and weeks 4, 8, 16.

In order to test the proposed mechanism by which PRAM improves cognition (i.e., dopamine agonist), participants who showed a pattern of increasing monetary losses on the IGT over time were categorized as demonstrating target engagement. The primary study outcome of cognitive functioning was assessed using the MATRICS Consensus Cognitive Battery (MCCB).

Multilevel models, accounting for the nesting of assessments within participant, and controlling for subthreshold manic (Young Mania Rating Scale scores) and depressive (Hamilton Depression Rating Scale scores) symptoms, tested whether target engagement (IGT total money change score) predicted MCCB change. Intention to treat analyses were conducted, including all enrolled participants.

Results: Fifty-two participants were enrolled in the study; 26 in each group. Average age was 41.8 years, 62% were female. Baseline depression symptoms in the two groups were minimal (PRAM=5.73, placebo=6.40) and equivalent (t=0.46, p = 0.645). Baseline manic symptoms were also minimal, but the PRAM group scores were slightly higher (3.24 vs. 1.63, t=-1.09, p = 0.041). IGT (total money) change scores did not differ between the two groups (t=-0.25, p = 0.802), indicating that target engagement did not differ by group. MCCB overall change scores were also statistically equivalent between the groups (t=0.586 p = 0.561). Using our a prior definition of target engagement, twenty-three participants showed target engagement (PRAM n = 12, placebo n = 11); in the multilevel models, this was not a significant predictor of MCCB overall T-score (B=-0.53, p = 0.880). Only session was a significant predictor (B=0.50, p<.0001), suggesting improvement in cognition across both groups.

Conclusions: In a two-step clinical trial, we aimed to evaluate both the cognitive impact of PRAM in cognitively impaired adults with BD, and the proposed mechanism of action. In this two-site trial, PRAM was not associated with significant improvement on cognitive outcome measures relative to placebo. Our selected measure of target engagement did not track with cognitive change. This may have occurred for a variety of reasons. First, the IGT outcome measure may not be psychometrically-sensitive to engagement of the DA reward circuitry, and therefore, was not a valid measure of target engagement. Second, PRAM treatment in this trial did not adequately engage the brain target. The latter is unlikely, given ample prior evidence that PRAM reliably binds to the D3 receptor in brain regions associated with reward processing, even in single dose, lower-dose studies (Riba et al. 2008). Secondary post-hoc analyses will allow us to further investigate other IGT outcomes and results from another reward task in an effort to confirm target engagement. Regardless, the failure to see improvement on cognitive outcomes in the PRAM group indicates a negative trial. Neurocognitive impairment has significant consequences for individuals with BD, further pursuit of treatments to ameliorate these deficits is important to improve patient outcomes and quality of life.

Keywords: Cognitive Impairment, Bipolar Disorder, Dopamine, Clinical Trial

Disclosure: Nothing to disclose.

M59. Maternal Distress Predicts Altered Dentate Gyrus Microstructure, Dentate Gyrus-Orbitofrontal Functional Connectivity, Decreased Behavioral Flexibility, Increased Rumination and Depressive Symptoms in Adult Offspring

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Background: Early life stress leads to changes in the dentate gyrus (DG) of the hippocampus, such as decreased adult neurogenesis. DG, and its connectivity with orbitofrontal cortex (OFC) are important for behavioral flexibility. In rodents we found that exposure to maternal distress (a form of early life stress) reduces behavioral flexibility in offspring. In humans, impaired flexibility is thought to increase risk for rumination and depression, but the effects of maternal distress and DG structure are less understood. Children of depressed mothers might be especially susceptible to mother's distress. Using a longitudinal study of offspring of individuals with and without major depression (MDD), we evaluated whether maternal distress during offspring childhood is associated with DG microstructure, DG-OFC functional connectivity (FC), behavioral flexibility, rumination, and depressive symptoms when offspring are adults.

Methods: Sample: The sample includes 74 children, G2, and grandchildren, G3, of probands (G1) with and without major depressive disorder (MDD). G2 and G3 offspring of G1 probands with MDD were classified as high-risk for MDD; those of probands without MDD as low risk. (Gen 2 mean ± SE age at MRI scan: 46.8± 0.89yrs, 53% female; Gen 3, 22.8 ± 0.72 yrs, 39% female).

Imaging: MRI scanning was performed with a GE Signa 3 Tesla whole-body scanner with an 8-channel, phased array head coil. To evaluate structural differences, Freesurfer 6.0 was used on T1-weighted structural scans for (para)hippocampal segmentation "FS60" into 12 subfields, of which we evaluated the DG regions CA4 and granule cell/molecular layer. To investigate hippocampal microstructural difference, mean diffusivity, a measure thought to reflect neural integrity, was assessed. DG mean diffusivity was assessed using diffusion MRI with MRtrix analytic pipeline. Resting-state fMRI data were preprocessed and denoised with the CONN toolbox. Functional connectivity was calculated as Pearson correlation between timeseries from seeds (CA4 and granule cell/molecular layer) and time series from OFC regions (orbital gyrus and frontomarginal gyrus).

Measures: Diagnoses were assessed at each wave using the Schedule for Affective Disorders and Schizophrenia (SADS). Maternal distress, the degree to which mothers feel competent, conflicted, restricted and supported in their role as mother, was measured with Parental Stress Inventory approximately 15 years before the offspring MRI data were collected. Behavioral flexibility was measured with the STROOP task interference score and Continuous Performance Test II (Commission errors, T-score) at time of MRI data collection. Rumination was measured using the Nolen-Hoeksema et al. scale and depressive symptoms were measured using the PHQ-9 and were collected 5 years post MRI scan.

Statistical Analyses: Statistical analyses were conducted in R using regressions in a generalized estimating equation (GEE) framework to account for family structure in the data.

Results: All analyses were adjusted for gender and age at MRI. Data were collapsed across risk groups for depression; however being at family risk for depression ($\beta = 0.81$, $p = 0.01$) as well as mother's lifetime history of depression ($\beta = 0.96$, $p = 0.0001$) were associated with higher levels of maternal distress. Higher levels of maternal distress during offspring childhood predicted increased right CA4 ($\beta = 0.24$, $p = 0.03$) and left granule cell/molecular layer (GCML; $\beta = 0.25$, $p = 0.05$) mean diffusivity in adult offspring, signaling decreased neural integrity. Left GCML mean diffusivity remained significantly associated when also controlling for offspring MDD, maternal depression and family risk status.

CA4 functional connectivity with left orbital gyrus ($\beta = 0.26$, $p = 0.0007$) and left frontomarginal gyrus ($\beta = 0.37$, $p = 0.0008$) was higher in offspring of mothers with higher distress and this remained significant when controlling for offspring depression, risk status and mother's depression. Similarly, left GCML functional connectivity with orbital gyrus ($\beta = 0.30$, $p = 0.01$) and frontomarginal gyrus ($\beta = 0.24$, $p = 0.008$) was also increased although this did not remain significant when controlling for mother's depression.

Mother's distress significantly mediated the association between mother's depression and increased left CA4-frontomarginal gyrus functional connectivity (indirect effect: 0.25, $p = 0.05$; total effect: 0.82, $p = 0.003$).

Mother's distress significantly predicted behavioral flexibility at time of offspring MRI scan (STROOP: $\beta = 0.42$, $p = 0.0004$; CPT:$\beta = 0.23$, $p = 0.03$) as well as rumination ($\beta = 0.25$, $p = 0.02$) and depressive symptoms ($\beta = 0.21$, $p = 0.04$) 5 years after MRI scan. Mother's distress mediated an association between mother's depression and offspring rumination (indirect effect: 0.17, $p = 0.04$; total effect: 0.40, $p = 0.04$). CPT commissions predicted rumination ($\beta = 0.26$, $p = 0.04$), but did not significantly mediate the association between mother's distress and follow-up rumination and symptoms.

Conclusions: Findings indicate that maternal distress during offspring childhood predicts compromised adult DG microstructure, DG-OFC connectivity, behavioral flexibility and increased rumination and depressive symptoms even when controlling for family risk for depression and maternal depression. Reduction of maternal distress may improve developmental and long-term cognitive and depressive outcomes in offspring at risk.

Keywords: Parenting Distress, Intergenerational Depression, Dentate Gyrus, Orbitofrontal Cortex, Behavioral Flexibility

Disclosure: Nothing to disclose.

M60. Changes in Brain Circuitry Underlying Emotion Regulation, Hopelessness, and Suicidal Thoughts and Behaviors Associated With a Psychobehavioral Intervention in Adolescents and Young Adults With Bipolar Disorder

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Background: Hopelessness is one of the strongest clinical predictors of suicidal thoughts and behaviors (STBs). Previous research suggests that hopelessness may result from disturbances in emotion regulation (ER), and we recently reported prefrontal cortex (PFC) dysfunction common to emotion dysregulation, hopelessness and STBs. We have designed a novel psychobehavioral intervention, named BE-SMART-ER (Brain Emotion Circuitry-Targeted Self-Monitoring And Regulation Therapy for Emotion Regulation), to target this area of PFC and its circuitry by providing adaptive self-strategies for ER processes that are disrupted in hopelessness. We are studying adolescents and young adults with bipolar disorder (BD), as BD is marked by difficulties in ER and is associated with the highest suicide risk. Moreover, adolescence and young adulthood is a period when BD and STBs often begin to emerge, and of dynamic neurodevelopment and maturation in the PFC circuitry. Using functional magnetic resonance imaging (fMRI) and clinical research assessments, we therefore aimed to examine changes from before to after BE-SMART-ER in brain circuitry, ER, hopelessness and STBs.

Methods: Clinical research assessments and fMRI were conducted before and after 12 weeks of BE-SMART-ER for 9

adolescents and young adults with BD (ages 16-24 years). Assessments included measures of emotion dysregulation (Difficulties in Emotion Regulation Scale; DERS, total and subscale scores), emotion reactivity (Emotional Reactivity Scale; ERS), manic symptoms (Young Mania Rating Scale; YMRS), depressive symptoms (Hamilton Depression Rating Scale; HDRS), hopelessness (Beck Hopelessness Index; BHI, total and subscale scores), suicidal thoughts ("thoughts of suicide" item on Quick Inventory of Depressive Symptomatology; QIDS), and propensity for suicidal behavior (Concise Health Risk Tracking; CHRT). Functional activation was examined from scans during which individuals performed an explicit emotion regulation paradigm while viewing faces depicting positive and negative emotions. Analyses were performed to identify changes in assessment scores, brain circuitry, and their associations.

Results: From pre- to post- BE-SMART-ER, there were significant reductions in scores on the DERS subscale of difficulties in engaging in goal-directed behavior ($p = 0.02$), ERS ($p = 0.04$), YMRS ($p = 0.003$), BHI loss of motivation subscale ($p = 0.04$), and suicidal thoughts on the QIDS ($p = 0.039$). There was a trend toward decreases on the HDRS ($p = 0.06$). FMRI data showed significant increases in the engagement of the ventral PFC including in the medial orbital and dorsal PFC and insula ($p<0.05$, cluster 20 voxels). There were significant correlations between the increases in the PFC and reductions in scores on the DERS difficulties in engaging in goal-directed behavior and BHI loss of motivation subscales, and the CHRT ($p<0.05$).

Conclusions: The findings provide preliminary evidence suggesting beneficial effects of BE-SMART-ER on PFC circuitry during ER associated with reductions in emotion dysregulation and hopelessness, particularly for their subconstructs of goal-directed behavior and motivation, and suicidal thoughts and behaviors, in adolescents and young adults with BD.

Keywords: Hopelessness, Suicidality, Emotion Regulation, Bipolar Disorder, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

M61. Aberrant Reward Functioning in Adolescents at High Familial Risk for Major Depressive Disorder

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Background: A parental history of major depressive disorder (MDD) is an established MDD risk factor. Reward processing deficits is a potential mechanism underlying familial risk transmission. Early adolescence, specifically the ages spanning 12-14, is marked by enhanced sensitivity to rewarding stimuli and increased MDD rates, suggesting that it is a crucial developmental period for studying reward functioning.

Although impaired reward responsiveness characterizes adolescents with MDD, behavioral evidence has been mixed amongst those at risk for MDD, with much of the current work falling outside the early adolescent period, which may preclude from identifying premorbid reward processing deficits. Additionally, no studies have applied computational modeling analyses to determine the specific reward processes that are impaired in high-risk adolescents, which may clarify these mixed findings.

Neuroimaging studies examining ventral striatum (VS) and medial prefrontal cortex (mPFC) correlates of reward functioning show evidence of blunted VS activation in response to rewards amongst high-risk youth versus low-risk youth. The direction of mPFC abnormalities has been mixed, which may be due to reward task design differences. Examining VS and mPFC activity at rest removes task-design confounds. Resting neural activation can be examined by computing the fractional amplitude of low frequency fluctuations (fALFF), derived from dividing the resting amplitude of a given power spectra by the total power spectrum. To date, one study has been conducted in a high-risk sample, with the high-risk group showing greater mPFC fALFF compared to the low-risk group. Importantly, emerging evidence has shown that VS and mPFC resting activation is linked to reward learning, suggesting that VS and mPFC fALFF may serve as promising neural markers of reward deficits in high-risk youth.

Methods: The sample consisted of 12-14-year-old female and male healthy adolescents, both with (high-risk, $N = 27$) and without (low-risk, $N = 74$) a mother with MDD. Mothers and adolescents completed clinical interviews. Adolescents filled out self-report questionnaires assessing depression, anhedonia, and anxiety symptoms and were administered the probabilistic reward task (PRT), which utilizes signal detection theory to assess a person's propensity to modify behavior based on reinforcement history. Adolescents completed a resting state fMRI scan at a separate session.

With respect to the PRT analyses, response bias (a measure of propensity to choose the more frequently rewarded stimulus) and discriminability (a measure of the ability to discriminate the more frequently rewarded stimulus from the less frequently rewarded stimulus) were computed. Computational modeling analyses were conducted on the PRT data to determine whether reward sensitivity, an index of consummatory pleasure, versus learning rate, an index of reward learning, is the core reward process implicated in MDD-risk.

Regarding the resting-state fMRI data, after standard preprocessing steps, fALFF analyses were conducted. For each voxel, the filtered time series was transformed to the frequency domain using a Discrete Cosine Transform. Given that VS resting signals are maximal in a "slow-4" frequency band (027-0.073 Hz) and mPFC resting signals are maximal in a "slow-5" frequency band (0.01-0.027 Hz), Z-score normalized mean fALFF values were extracted from the VS and mPFC within their respective maximal frequency bands.

Results: Given significant differences in anxiety symptoms ($p = .004$), and established relationships between internalizing symptoms with reward processing deficits, all statistical models included all clinical symptoms as covariates. A Sex x Group x Block ANCOVA on response bias revealed a main effect of Group, with high-risk adolescents exhibiting a blunted response bias compared to low-risk adolescents, $F(1,94) = 4.897$, $p = .029$, $np2 = .050$. An analogous ANCOVA on discriminability revealed no significant effects, all $ps > .150$. With respect to computational modeling analyses, two Sex x Group ANCOVAs showed a main effect of Group on reward sensitivity, but not learning rate. The high-risk group showed a reduced reward sensitivity, $F(1,94) = 4.024$, $p = .048$, $np2 = .041$, but an intact learning rate, $F(1,94) = .685$, $p = .410$, $np2 = .007$, relative to the low-risk group.

Regarding fALFF analyses, we conducted two multiple linear regressions, with VS fALFF and mPFC fALFF serving as dependent variables, and Group, Response Bias, and Group x Response Bias as predictors covarying for sex and clinical symptoms. We did not find significant group differences in VS and mPFC fALFF ($ps > .600$); however, we found a Group x Response Bias interaction for mPFC fALFF, ($\beta = -.288$, $B = -1.388$, $t = -2.282$, $p = .025$, $f2 = .063$), with higher mPFC fALFF associated with a lower response bias only amongst high-risk adolescents (partial $r = -.563$, $p = .008$).

Conclusions: Results suggest that reward processing impairments may be a premorbid vulnerability marker for MDD that is especially prominent during early adolescence.

Computational modeling results revealed that reduced reward sensitivity may be a more salient risk-marker. Although we did not find fALFF group differences, mPFC fALFF-response bias

associations among high-risk adolescents suggests that those at familial risk for MDD who are also exhibiting a reduced reward response bias and hyperactive mPFC fALFF may be especially vulnerable to MDD-onset.

Keywords: Adolescent Depression, Reward Functioning, Familial Risk, Fronto-Striatal Networks, Fractional Amplitude of Low Frequency Fluctuations

Disclosure: Nothing to disclose.

M62. Chronic Stress Impairs Reward- and Motivation-Behaviors in Both Males and Females

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Background: We recently developed a novel chronic stress paradigm called chronic nondiscriminatory social defeat stress (CNSDS) that induces an altered affective state in both male and female mice (Yohn et al, 2019 Neuropsychopharmacology). Here we show that CNSDS exposure alters reward- and motivation-related behaviors in a sex-dependent nuanced fashion.

Methods: We examined the effect of CNSDS on instrumental reward and effort-related choice behaviors in male and female mice. Mice were exposed to Control or CNSDS ($n = 20$ males and 20 females per group), and then completed a social interaction test, instrumental acquisition, progressive ratio, and outcome devaluation. A separate cohort was assessed in effort-related choice following control or CNSDS exposure. Parameteric statistics (eg ANOVAs) were used where appropriate to analyze results.

Results: Preliminary data indicate that CNSDS induces maladaptive behaviors in both males and females in instrumental behaviors. When exposed to CNSDS, both sexes show decreased sensitivity to devalued reinforcers ($p = 0.0121$), decreased breakpoint in progressive ratio ($p < 0.0001$), and decreased motivation in an effort-related choice test. However, there are nuanced sex differences in the effects of CNSDS on instrumental acquisition.

Conclusions: Reward- and motivation-related rodents behaviors may offer more translational relevance for mood disorders than tests historically associated with anxiety or depression. We previously developed CNSDS as a chronic stress paradigm that is effective in both male and female rodents and found that CNSDS induces increased avoidance behaviors (Yohn et al 2019, Neuropsychopharmacology). Here we show that CNSDS induces maladaptive reward and motivation behaviors in both males and females.

Keywords: Motivation, Reward-Based Decision-Making, Depression, Rodent Models, Behavioral Tasks

Disclosure: Nothing to disclose.

M63. Neurobiological Underpinnings of Placebo Response Assessed by PET/MR Neuroimaging in Major Depressive Disorder

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Background: Progressively increasing placebo response rate in Major Depressive Disorder (MDD) clinical trials is a major obstacle in developing new interventions. We designed a double-blind, placebo-controlled, randomized clinical trial (RCT) to generate a large cohort of placebo responders. We aimed to investigate the role of mesolimbic dopaminergic mechanisms implicated in the MDD placebo response using PET/ MR and fMRI neuroimaging techniques. The study is currently ongoing, and assignment of subjects is under blind to the investigators.

Methods: We recruited adult subjects with MDD ($n = 64$). In accordance with the sequential parallel comparison design (SPCD), the 8-week, double-blind RCT was divided into 2 phases. During Phase 1, participants were randomized to active drug (bupropion 300mg) or placebo, with imbalanced placebo: drug ratio (7:1). During Phase 2, placebo non-responders were re-randomized. The nucleus accumbens, caudate and putamen were defined as a priori regions of interest (ROIs). Imaging sessions took place at baseline and after Phase 1. Subjects performed the Monetary Incentive Delay (MID) task in the PET/MR scanner. The 11C-raclopride radiotracer was utilized for assessing availability of dopamine D2 receptors. PET data were modeled using a modified parametric ntPET model (lp-ntPET; Normandin et al., 2012) that estimated the kinetics of neurotransmitter release from dynamic PET data.

Results: fMRI results demonstrated MID-task induced increased striatal activation in the caudate, putamen & nucleus accumbens (NAcc; $p<0.05$) in response to reward relative to neutral feedback during the baseline and follow-up scans. PET results demonstrated significant tracer displacement (interpreted as indexing dopamine release) in the NAcc (follow-up, $p < 0.05$), caudate (baseline & follow-up, $p < 0.01$) and putamen (baseline, $p < 0.01$ & follow-up, $p < 0.05$).

Conclusions: The preliminary results of blinded data implicate a potential role for mesolimbic DA mechanisms in mediating the placebo response in MDD. Analyses of unblinded data will help to further understand the role of dopaminergic system in the neurobiological basis of placebo response in MDD patients.

Keywords: Depression, Placebo Response, Raclopride, Reward, Monetary Incentive Delay Task

Disclosures: Akili Interactive Labs, BlackThorn Therapeutics, Boehringer Ingelheim, Compass Pathway, Otsuka Pharmaceuticals, Takeda Pharmaceuticals: Consultant (Self); Millennium Pharmaceuticals, NARSAD, NIMH: Grant (Self); BlackThorn Therapeutics, Stock / Equity (Self)

M64. The Kynurenine Pathway in Major Depressive Disorder, Bipolar Disorder, and Schizophrenia: A Meta-Analysis of 101 Studies

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Background: The importance of tryptophan as a precursor for neuroactive compounds has long been acknowledged. The metabolism of tryptophan along the kynurenine pathway and its involvement in mental disorders is an emerging area in psychiatry. We performed a meta-analysis to examine the differences in kynurenine metabolites in major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia (SZ).

Methods: Electronic databases were searched for studies that assessed metabolites involved in the kynurenine pathway (tryptophan, kynurenine, kynurenic acid, quinolinic acid, 3-hydroxykynurenine, and their associate ratios) in people with MDD, SZ, or BD, compared to controls. We computed the difference in metabolite concentrations between people with MDD, BD or SZ, and controls, presented as Hedges' g with 95% confidence intervals.

Results: A total of 101 studies with 10,912 participants were included. Tryptophan and kynurenine are decreased across MDD, BD, and SZ; kynurenic acid and the kynurenic acid to quinolinic acid ratio are decreased in mood disorders (i.e., MDD and BD), whereas kynurenic acid is not altered in SZ; kynurenic acid to 3-hydroxykynurenine ratio is decreased in MDD but not SZ. Kynurenic acid to kynurenine ratio is decreased in MDD and SZ, and the kynurenine to tryptophan ratio is increased in MDD and SZ.

Conclusions: Our results suggest that there is a shift in the tryptophan metabolism from serotonin to the kynurenine pathway, across these psychiatric disorders. In addition, a differential pattern exists between mood disorders and SZ, with a preferential metabolism of kynurenine to the potentially neurotoxic quinolinic acid instead of the neuroprotective kynurenic acid in mood disorders but not in SZ.

Keywords: Mood Disorders, Bipolar Disorder, Schizophrenia (SCZ), Meta-Analysis, Kynurenine Pathway

Disclosure: Nothing to disclose.

M65. Default Mode and Salience Network Alterations in Suicidal and Non-Suicidal Self-Injurious Thoughts and Behaviors in Adolescents With Depression

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Background: Suicidal ideation (SI) and non-suicidal self-injury (NSSI) are two distinct yet often co-occurring risk factors for suicide in adolescents. Elucidating the neurobiological patterns that specifically characterize SI and NSSI in adolescents is needed to inform the use of these markers in intervention studies and to develop brain-based treatment targets.

Methods: 49 adolescents with depression (15 males) and 21 healthy controls (9 males) underwent clinical interviews to determine SI and NSSI history; 28 of the depressed adolescents had a history of SI and 29 had a history of NSSI (20 overlapping). All participants underwent a resting-state fMRI scan. We compared groups in network coherence of subdivisions of the central executive network (CEN), default mode network (DMN), and salience network (SN). We also examined group differences in between-network connectivity and explored brain-behavior correlations.

Results: Depressed adolescents with SI and with NSSI had lower coherence in the ventral DMN compared to those without SI or NSSI, respectively, and healthy controls (all ps<0.033). Depressed adolescents with NSSI had lower coherence in the anterior DMN and in insula-SN (all ps<0.029), and higher CEN–DMN connectivity compared to those without NSSI and healthy controls (all ps<0.038). Lower network coherence in all DMN subnetworks and insula-SN were associated with higher SI (all ps < 0.00007).

Conclusions: SI and NSSI are both related to brain networks associated with difficulties in self-referential processing and future planning, while NSSI specifically is related to brain networks associated with disruptions in interoceptive awareness. Our findings highlight novel and specific targets for the treatment of these co-occurring behaviors.

Keywords: Adolescent Depression, Non-Suicidal Self-Injurious Behavior, Suicidal Behavior, Resting-State fMRI, Suicidal Ideation

Disclosure: Nothing to disclose.

M66. Spectral Embedding of the Structural Connectome Reveals Diffusion-Based Brain Subnetwork Correlates of Clinical Measures in a Transdiagnostic Psychiatric Cohort

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Background: Using brain network data to identify transdiagnostic correlates of psychiatric disorders is an ongoing challenge in the field of neuroimaging research. Such networks are represented as a set of vertices (brain regions) and edges (connections between brain regions), which are defined based on imaging modality. Commonly, white matter tractography based structural connectomes are used directly for either edge-based or graph theoretical analysis. However, edge-centric studies are limited to pairwise comparison, and predefined graph features limit access to potentially informative latent network structure. Alternatively, the mathematical properties of connectome graph laplacian can be utilized to model the "heat" or "information" diffusion characteristics, which take into account the entire network topology, of brain networks. In this study, we propose a novel method for representing the structural connectome by defining edge weights between nodes as a similarity metric based on the spectral embedding of each subject's brain graph. We then apply the network-based statistic (NBS) framework to identify subnetworks that correlate with clinical traits of interest.

Methods: Data used are diffusion tensor imaging based structural connectomes from an Research Domain Criteria (RDoC) study, with N = 66 patients (PT, mean age=27.5, male/female=20/46) with any form of internalizing psychopathology (e.g., major depressive disorder, generalized anxiety disorder, social anxiety disorder, post-traumatic stress disorder) and N = 23 age and sex matched healthy controls (HC, mean age=24.7, male/female=8/15). The Depression Anxiety and Stress (DASS) questionnaire was administered to each subject. The symmetric normalized laplacian is computed and eigen-decomposed to obtain the eigenmodes (eigenvectors of the laplacian matrix) for each subject's structural adjacency matrix. Each element of an eigenmode corresponds to the spectral embedding of a node such that diffusion occurs more quickly between nodes with similar eigenmode values. A subset of eigenmodes (the second, third and fourth in this study) is then used to determine the similarity (via Euclidean distance) of all nodes to one another in the embedding space. Next, an NBS-based framework is applied to the newly defined structural connectomes to identify subnetworks that either positively or negatively correlate with clinical traits of interest. As the connectomes used are similarity matrices based on the spectral embedding of nodes, a significant subnetwork using only positive correlations, for example, would indicate that faster diffusion in the subnetwork is positively correlated with the trait of interest.

Results: In a preliminary analysis, one subnetwork was found to correlate positively with the DASS depression subscale, indicating that faster diffusion within the subnetwork is positively associated with this scale. This subnetwork is composed of the bilateral precuneus, posterior cingulate cortices, amygdalae and left frontal cortical regions (rho threshold =0.35, p = 0.004). Edges in the subnetwork were predominantly adjacent to the bilateral precuneus. Interestingly, the precunei and posterior cingulate are hubs of the default mode network, a major functionally defined subnetwork that has previously been implicated in depression and anxiety.

Conclusions: This preliminary study both proposes a novel method for the identification of brain subnetwork based correlates of psychiatric disease, and employs this method to successfully identify a subnetwork that includes brain regions that have been previously implicated in depression and anxiety. These results provide evidence that structural network features of the brain regions in a canonically functionally defined subnetwork may be transdiagnostic markers of disease across the swath of internalizing psychopathologies.

Keywords: Brain Structural Connectivity, Graph Theory, MRI
Disclosure: Nothing to disclose.

M67. Heritability of Components of Actigraphy in Humans and Primates: Relevance to Bipolar Disorder

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Background: The growing use of actigraphy, which quantifies motor activity using a wrist-worn device that contains an accelerometer measuring movement/acceleration, as an objective measure of sleep and motor activity has provided new insights into rhythms and regulatory patterns of these domains in people with mood disorders. Whereas most actigraphy research on bipolar disorder (BD) has demonstrated sleep disturbances and greater evening orientation, the recent focus on motor activity as a core feature of this condition has facilitated a more accurate portrait of the full 24-hour cycle. Findings of more than two dozen studies converge in demonstrating a lower average and greater variability of motor activity.

The availability and feasibility of accelerometry as an unobtrusive tool to obtain objective indices of sleep, motor activity and circadian rhythms in primates provides an ideal opportunity to conduct cross-species studies designed to examine sex and developmental differences, and genetic and environmental influences on motor activity and related systems in controlled environments. Most human studies that have investigated genetic factors associated with actigraphy parameters have examined dozens of derived features individually. However, these findings may be misleading because of the high inter-correlations among the actigraphy metrics. Here we employ a data reduction technique for multivariate data on multiple domains entitled Joint and Individual Variance Explained (JIVE) to estimate variability attributable to the three domains derived from actigraphy, sleep, activity and circadian rhythmicity in a large sample of primates in a natural living environment.

The aims of this poster are: (1) to identify the individual and shared variability of key components of actigraphy (I.e., sleep, circadian rhythms, motor activity) and their common variance using JIVE in a study of primates; (2) to estimate the heritability of selected variables and JIVE-derived domains in the sample by type of relationships (sibs, ½ sibs; parent offspring) and sex; (3) to compare the heritability components from the primate sample to the features associated with BD in previous research of these domains.

Methods: The sample included 702 monkeys studied from 2003-2009 in the Oregon National Primate Research Center that included 30 pedigrees (i.e., 1310 parent-offspring pairs and 127 siblings and 7826 half siblings). Accelerometry in monkeys was assessed with a neck-worn Actical device for a range of 24 hrs -2 weeks. These data were analyzed for a total of 546 monkeys (320 females and 226 males) with an average age is 56.7 months (SD =48.3 months).

The features of sleep (SL), physical activity (PA) and circadian rhythmicity (CR) domains were calculated from the activity count (AC) measures. JIVE was used to extract the core features from the Sleep, Physical Activity and Circadian Rhythms domains. SL features were derived from the algorithm to determine sleep using a weighted running average of AC with a threshold. PA and CR features were derived using the 'actigraphy' package in R.

The phenotypic heritability was estimated using SOLAR software (8.4.1). Polygenic heritability estimates defined as the proportion of the total phenotypic variance explained by additive genetic (or familial) effects were calculated, adjusted for age and gender using the variance components approach implemented in the SOLAR package. Information on the covariance among relatives was used to estimate the polygenic (or additive genetic) component of variance.

Results: We found that joint variance structure explains 49.3%, 68.5% and 11.9%, and individual variation accounts for 45.7%, 20.8% and 42.9%, respectively, of the total variation in sleep, physical activity and circadian rhythm domains. Interestingly, only 11.9% of variation in circadian rhythm domain can be explained by the joint component. The first joint PC loaded most highly on physical activity (52.8%), and least on circadian rhythm features (9.2%). The heritability of this common cross-domain variance (joint PC1) was estimated as 0.354 (SE=0.088). Heritability was greater for siblings (.50, se=.240) than between parents and offspring (.19, se=.10). Half sib heritability differed significantly for same sex (.53, se=.09), than opposite sex (.30, se=.10).

Conclusions: These findings demonstrate the importance of genetic factors underlying certain features of motor activity, sleep and circadian rhythmicity in this large sample of monkeys in a natural environment. The strong heritability of the joint score in monkeys taps the same domain derived from actigraphy that discriminated people with BD from controls in our family study of mood spectrum disorder. Sex differences in heritability highlights the importance of investigating underlying genetic and environmental factors that may differ for males and females. This work demonstrates the value of cross species research that employs a common reliable objective measure of a particular domain with a well-established link to a human disorder.

Keywords: Bipolar Disorder, Sleep, Heritability, Machine Learning Classification, Motor Activity
Disclosure: Nothing to disclose.

M68. Treatment Resistant Depression in Finland (TRIFI): A Nationwide Cohort Study

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Background: Treatment resistant depression (TRD) is a common clinical problem, which causes a major burden to the patients and the society. Estimates of the prevalence of TRD vary considerably depending on the definition, data source and method used in the analysis. The objective of the present study was to investigate the prevalence of TRD in a nationwide cohort including all patients diagnosed with depression in Finland and to further explore paradigms in different treatment lines.

Methods: This population-based longitudinal cohort included all patients aged 16-65 years and diagnosed with depression (ICD10:F32-F33) between the years 2004-2016 in Finland. Data were based on the nationwide health care registers of inpatient care, specialized outpatient care, recorded sick leave and disability pensions. Patients newly started with an antidepressant were identified and followed for two years to observe the possible emergence of TRD. Patients were defined as having a TRD if they had failed two treatments of adequate duration followed by initiation of a third treatment. Exposure and non-exposure periods for medications were explored using the PRE2DUP method, which is an algorithm modeling drug use periods based on prescription drug purchases.

Results: During the years 2004-2016, 175,999 persons had their first registered depression episode and were defined as new antidepressant users. Mean age at time of diagnosis was 39.5 (SD 13.3) years and the majority were women (62.5%). In the present

cohort, 11.5% (N = 20,202) met the TRD criteria. Patients fulfilling the TRD criteria as compared to patients without TRD were of similar age (39.2 vs. 39.5 years), but more frequently men (40.4% vs. 37.1%). The median time to reach the TRD criteria was 239 (IQR 150-378) days. The most commonly prescribed antidepressants as first and second line of treatment were escitalopram (22.5%), mirtazapine (21.1%) and citalopram (18.0%). The most common third line treatments were venlafaxine (18.3%), mirtazapine (13.3%) and duloxetine (7.8%).

Conclusions: Around 11.5% of patients with depression developed TRD within an average of 7 months during their first MDD episode. This is a marked number of patients suggesting that the current treatment options for depression are insufficient, and that there is a clear need for more efficient therapies in order to prevent the prolonged suffering of depressed patients and to reduce the costs related to this disease.

Keywords: Depression, Antidepressant, Treatment Resistant Depression, Epidemiology

Disclosures: Eli Lilly, Janssen-Cilag, Lundbeck, Otsuka, Evidera, Sunovion: Honoraria (Self) Eli Lilly, Janssen-Cilag: Grant (Self)

M69. Clinical Utility of Combinatorial Pharmacogenetic Testing in Depression: Canadian Patient- and Rater-Blinded, Randomized, Controlled Trial

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Background: The pharmacological treatment of depression consists of stages of trial and error, with less than 40% of patients achieving remission during their first medication trial, and remission rates continue to decrease with each subsequent medication trial. Combinatorial pharmacogenetic (PGx) testing, a tool used to help guide the pharmacological treatment of depression, has consistently been associated with 50% relative increase in remission rate, compared to treatment-as-usual (TAU), among patients with depression who have failed at least one previous medication trial. As combinatorial PGx is unique from other PGx testing approaches, its clinical validity and utility has been assessed independently through open-label and blinded clinical trials, including the largest randomized controlled trial (RCT) (N = 1,167) of PGx in Psychiatry, the Genomics Used to Improve DEpression Decisions (GUIDED) trial. Meta-analyses by Bousman et al (2019) of GUIDED and five other trials show strong significance of PGx over TAU. Here we present results from the Canadian GAPP-MDD trial ("Pharmacogenomic Decision Support With GeneSight Psychotropic to Guide the Treatment of Major Depressive Disorder", ClinicalTrials.gov: NCT02466477), assessing the use of combinatorial PGx testing to guide depression treatment.

Methods: The GAPP-MDD trial was a 52-week, three-arm, multicentre, double-blinded (participants and raters) randomized, controlled trial evaluating clinical outcomes among patients whose treatment was guided by combinatorial PGx testing compared to TAU. This study, like the GUIDED trial, included patients who were ≥18 years old, diagnosed with MDD (QIDS-C16 ≥11 at screening and QIDS-SR16 ≥11 at screening and baseline), and had inadequate response to at least one psychotropic medication within the current depressive episode. Patient assessments were conducted at week 0 (baseline), 4, 8, 12, 24, 36, and 52. The HAM-D17 scale was the primary assessment, administered by blinded central rater. Secondary assessments included PHQ-9, QIDS-C16, QIDS-SR16, and patient reported side effects. Symptom improvement (mean % change in HAM-D17 from baseline to week 8), response (≥50% decrease in HAM-D17), and remission (HAM-D17 score of ≤7) were compared between the combinatorial PGx-guided and TAU arms. Considering the similarities in study design between the GAPP-MDD and GUIDED RCTs, patient outcomes observed in the GAPP-MDD trial were compared to those observed in the GUIDED trial.

Results: 276 patients were included in the per-protocol cohort of the GAPP-MDD study at baseline and 196 patients completed the study through week 8. 371 patients were included in the intent-to-treat cohort at baseline, with 308 completing the study through week 8. The majority of patients were female (64.5%), between 18–64 years of age (93.8%; mean = 41 years), and Caucasian (84.1%). At baseline, the mean HAM-D17 score was 21.4, with 30.4% of patients classified as having moderate, 27.5% severe, and 42.0% very severe depression. The mean number of previously failed psychiatric medications was 3.6 (SD 2.6, min 1.0, max 21.0). At week 8, patients in the combinatorial PGx-guided arm had greater symptom improvement, response, and remission rates compared to patients in the unguided TAU arm, although this did not reach statistical significance: symptom Improvement, PGx-guided 27.60, TAU 22.68, $\Delta = 4.92$, $p = 0.274$; response, PGx-guided 30.26%, TAU 22.67%, $\Delta = 7.59$, $p = 0.262$; remission, PGx-guided 15.70%, TAU 8.33%, $\Delta = 7.38$, $p = 0.131$.

Response and remission rates were the outcomes most strongly impacted by combinatorial PGx in the GUIDED trial. When comparing the current study to GUIDED, we observed even greater improvement in response and remission rates in the combinatorial PGx-guided arm compared to TAU, with a 33% relative improvement in response rate (compared to 31% in GUIDED) and an 86% relative improvement in remission rate (compared to 52% in GUIDED). Across both studies, there was an increase in the proportion of patients in the combinatorial PGx-guided arm, but not the TAU arm, who were taking medications that were consistent (i.e. congruent) with the combinatorial PGx report guidance (GAPP-MDD, increased from 83.4% to 91.1%; GUIDED, increased from 79.4% to 91.2%). Thus, treating physicians demonstrated moderate following of the PGx guidance information. The abovementioned outcomes achieved statistical significance in the GUIDED trial, which had a much larger sample of patients, but not in the smaller GAPP-MDD study. This Canadian RCT, which was designed based on effect sizes in early open-label studies of PGx testing, was stopped early, prior to meeting target recruitment, as the results of the GUIDED study demonstrated a need for a much larger sample size.

Conclusions: The GAPP-MDD RCT demonstrated improvements similar to the GUIDED RCT in clinical outcomes following combinatorial PGx testing in a smaller Canadian population of patients with depression who had failed to respond to at least one previous medication trial. Together with GUIDED, and other similar but smaller RCTs, the results from the GAPP-MDD trial, conducted in the context of the Canadian universal health care setting, indicate that combinatorial PGx testing can be an additional tool to help guide the treatment of depression.

Keywords: Pharmacogenetics, Depression, RCT

Disclosure: Nothing to disclose.

M70. Genetic vs. Stress and Mood Determinants of Sleep in the Amish

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Background: Sleep is essential to the biology of the human brain is likely tightly regulated by genetics as many core features are conserved across species. Identifying replicable genetic variants contributing to sleep may require more accurate accounting for confounding factors. We sought to examine how stress and mood disorder may independently contribute to sleep quality and impact its heritability.

Methods: Our sample included 326 Old Order Amish/Old Order Mennonite individuals (110 participants with psychiatric disorders and 216 controls). Current stress level was assessed by the Perceived Stress Scale and cumulative life stressors were evaluated by a Lifetime Stressor Inventory. Sleep quality was determined by the Pittsburgh Sleep Quality Index (PSQI). We estimated the heritability of the PSQI and examined the associations of current stress, lifetime stress, mood diagnosis, age, and sex with PSQI.

Results: Current stress, lifetime stress, mood disorders, and age were independently associated with PSQI score (all $p<0.05$) before and after adjusting for participants' relatedness. Heritability of PSQI total and subcomponent scores were low, ranging from 0 to 0.23 before and after accounting for stress and psychiatric diagnosis.

Conclusions: Sleep quality is highly heterogeneous and strongly affected by environmental stress and mental health factors even in a rural society with limited technological interference with sleep. Measuring and accounting for non-genetic and partially genetic determinants of sleep in particular stressors and mood disorders are likely important for improving the precision of genetic studies of sleep.

Keywords: Sleep, Heritability, Stress, Mood

Disclosure: Nothing to disclose.

M71. DNA Methylation, Gene and Protein Expression of FKBP5 in the Human Cortex Over the Life Course and in Severe Psychopathology

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Background: FKBP5, encoding FK501 Binding Protein 51, is a critical regulator of stress hormone signalling and is increased by several risk factors for psychiatric disorders including stress, genetic variants and aging. We hypothesised that convergence of these factors may lead to threshold effects that promote, or are observed in, psychiatric disease. In the largest and most comprehensive postmortem study to date examining brain FKBP5 across the spectrum of severe psychiatric disorders, we studied FKBP5 gene expression (gex) and DNA methylation (DNAm) patterns to understand its role in the development of psychiatric disease. Using a large collection of postmortem human brain samples from three cohorts, we report (i) the FKBP5 gex, protein and DNAm life-course trajectories; (ii) the age-related changes in FKBP5 that are moderated by psychiatric disease status and/or FKBP5 risk genotype; (iii) the FKBP5 cell-type specificity and thus cell-type contributions to the heightened FKBP5 gex phenotype.

Methods: Tissues from the dorsolateral prefrontal cortex (DLPFC; Brodmann Area 9, BA9) was assessed in 681 individuals from three postmortem brain cohorts: Series 1 (Lieber Institute for Brain Development) included a lifetime cohort of non-psychiatric controls, spanning in age from the prenatal second trimester to 85 years (CTRL; $n = 252$), and teenagers, adults, and 50+ year old subjects with schizophrenia (SZ; $n = 184$), bipolar disorder (BPD; $n = 69$) or major depressive disorder (MDD; $n = 152$). Series 2 (Neurobiobank, Ludwig-Maximilians-University) consisted of 24 CTRL subjects with no history of psychiatric or neuropathological illness. Series 3 (Victoria Brain Bank, Australia) included 60 subjects from each diagnosis (CTRL, SZ, MDD, BPD; $n = 20$/group). Methods used included expression analyses (RNA sequencing, quantitative qPCR, and RNAscope® in situ hybridisation [ISH]), protein analysis (immunoblot and immunohistochemistry [IHC]), DNAm (Illumina HumanMethylation450 BeadChip) and SNP genotyping (Illumina HumaHap650Y_V3 or Human 1M-Duo_V3 BeadChips). Statistics were performed in R; FDR corrected P values are reported unless otherwise noted.

Results: FKBP5 mRNA (V1-4: $R>0.44$, nominal $P<0.023$) and protein ($R=0.500$, $P=0.014$) levels significantly increased with age in CTRLs; this was heightened in psychiatric cases. FKBP5 mRNA was more strongly increased with age in SZ relative to CTRLs (sm.ancova analyses comparing non-parametric regression curves: $P=0.0046$). Accordingly, CpGs in the proximal enhancer and downstream conserved region of FKBP5 were significantly demethylated in cases (especially in SZ) vs CTRLs in older subjects. The aging trajectory of FKBP5 mRNA variants (all) and DNAm for 3 CpGs was weakly influenced FKBP5 risk variants rs1360780 and rs9470080 (proxy for the FKBP5 risk haplotype), with cases carrying the risk T allele having significantly higher mRNA expression at older ages, and generally lower DNAm ($P< 0.01$) in the proximal enhancer and downstream conserved regions of the gene.

Conclusions: We observed convergence of FKBP5 gene and protein expression with age, which may be related to changes in DNA methylation and genotype. Further work to delineate the cell-type specific pattern of brain FKBP5 in psychopathology is necessary to understand its role in the development of psychiatric disease.

Keywords: FKBP5, Early life Stress, Depression, Bipolar Disorder, Schizophrenia Novel Treatment

Disclosure: Nothing to disclose.

M72. Reduction of Lateral Ventricular Volume in Psychiatric Inpatients is Associated With Serotonin Reuptake Inhibitor Antidepressant Use

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Background: Patients of various neurological disorders have been found to have relatively enlarged ventricles compared to healthy controls. Aging is also associated with ventricular enlargement. We wanted to study whether psychiatric in-patient treatment affects ventricular volume.

Methods: Psychiatric inpatients ($n = 81$, of which 40 were consistently on serotonin reuptake inhibitors (SRIs) and 25 were not) at The Menninger Clinic in Houston, Tx were studied. In-patients were scanned twice (close to admission and ~ 4 weeks later) and ventricular volumes were calculated using FreeSurfer. Paired t-tests were used to compare ventricular volume before and after treatment. Data were normalized by total estimated intracranial volume (eTIV).

Results: The right lateral ventricular volume was significantly smaller after treatment: [volume/eTIV] pre-treatment, all $= 0.0049 \pm 0.00025$ SE ; [volume/eTIV] post-treatment, all $= 0.0048 \pm 0.00025$ SE ; $t(80) = -3.99$, $p = 0.00014$.

Patients who received SRIs showed a significant reduction in right lateral ventricular volume ([volume/eTIV] pre-treatment, SRI=

0.0049 ± 0.00043 SE ; [volume/eTIV] post-treatment, SRI = 0.0048 ± 0.00043 SE ; t(39) = -4.18, p = 0.00016). For the non-SRI group, no difference in ventricular volume was found.

Conclusions: Psychiatric in-patients displayed a statistically significant reduction in right lateral ventricular volume. Using SRIs (e.g., sertraline n = 7, venlafaxine n = 10, fluoxetine n = 9, duloxetine n = 2, etc.) during in-patients treatment may be associated with statistically significant reduction of right lateral ventricular volume. Despite the fact that the results are statistically robust, reproducibility studies are necessary to confirm our observations.

Keywords: Brain Ventricular Volume, SSRI, SNRI, FreeSurfer, Depression

Disclosure: Nothing to disclose.

M73. Increase in Serotonin Transporter Binding Across the Menstrual Cycle in Patients With Premenstrual Dysphoric Disorder: A Case-Control Longitudinal Positron Emission Tomography (PET) Imaging Study

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Background: With core symptoms like depressed mood, affective liability and irritability, premenstrual dysphoric disorder (PMDD) disrupts the lives of millions of women worldwide each month. Despite the clear association of symptom-onset and menstrual-cycle phase, no consistent change in ovarian hormone levels seems characteristic of this pathology. Rather than abnormal ovarian hormone levels, a heightened serotonergic sensitivity of the brain in response to menstrual cycle-dependent hormone fluctuations has been proposed to render some women vulnerable to developing PMDD symptoms. However, empiric knowledge on the pathophysiology of PMDD is still scarce. Given that (i) selective serotonin reuptake inhibitors (SSRIs) can treat PMDD symptoms successfully and that (ii) changes in serotonin transporter (5-HTT) availability in response to a pharmacological ovarian-hormone challenge in healthy women have been demonstrated, we hypothesized an increase in 5-HTT availability in midbrain and prefrontal cortex (PFC) in patients with PMDD from periovulatory (when estradiol levels peak) to premenstrual phase (when estradiol levels are low and symptoms occur). Furthermore, we hypothesized that there is an association of depressive symptom severity with an increase in midbrain 5-HTT availability in patients.

Methods: We acquired 118 positron emission tomography (PET) scans in total: in a longitudinal design, 30 patients and 29 healthy women underwent a periovulatory and a premenstrual [11C]DASB scan to measure 5-HTT binding potential (BPND), an index of 5-HTT availability. All women were medication-free and had no psychiatric or medical illnesses other than PMDD. Midbrain (highest 5-HTT expression in human brain) and PFC (based on gonadotropin challenge findings) were registered (https://osf.io/fvghx) as primary regions of interest (ROIs). To test the primary hypothesis of group differences in 5-HTT BPND across the menstrual cycle, a linear mixed model with the main effects of group (patients/healthy participants), time (periovulatory/premenstrual phase), ROI (midbrain/PFC), and their interaction as the independent variables, and with ΔBP ND value as the dependent variable, and subject as random factor was used. Significant main effects of ROI were followed up by independent t-tests to test differences between group at Bonferroni corrected α=0.025 in each ROI.

Results: [11C] DASB 5-HTT BPND change from periovulatory to premenstrual cycle phase was greater in patients with PMDD as compared to healthy participants in midbrain and PFC (primary ROIs). There was a three-way interaction (group X time X ROI) effect (comparison to 0-model: $\chi 2 = 9.92$, $p = 0.019$). Accordingly, patients with PMDD had significantly increased midbrain 5-HTT binding from periovulatory to premenstrual phase (βpremenstrual>periovulatory= 0.29, t(29)= −3.43, p = 0.0002), compared to significantly decreased midbrain BPND in healthy participants (βpremenstrual>periovulatory= −0.17, t(28)= −2.73, p = 0.011) from periovulatory to premenstrual phase. For 5-HTT BPND in PFC, patients with PMDD showed a trend towards increased 5-HTT BPND from periovulatory to premenstrual phase (βpremenstrual>periovulatory= 0.08, t(29)= -1.79, p = 0.080), compared to significantly decreased 5-HTT binding in healthy participants (βpremenstrual>periovulatory= −0.03, t(28)= −3.14, p = 0.0004).

Conclusions: We provide the first well-powered longitudinal PET imaging dataset in women with PMDD in vivo and identify elevated 5-HTT availability in midbrain as an important mechanism in the neurobiology of PMDD. Our findings advocate for clinical trials evaluating acute SSRI-administration timed to the menstrual cycle in patients with PMDD, as well as the development of preventative strategies to protect from premenstrually elevated 5-HT levels, such as a systematic testing of serotonergic precursor substances.

Keywords: PET Imaging Study, Premenstrual Dysphoric Disorder, Serotonin Transporter

Disclosure: Nothing to disclose.

M74. Gender- But Not Diagnosis-Specific Correlations of Plasma Leptin With Obesity and Insulin Resistance in Major Depressive Disorder

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Background: Leptin, a peptide hormone, encoded by the obese gene, decreases food intake and increases energy utilization via interaction with hypothalamic leptin receptors [Friedman and Halaas, 1998]. Recent animal studies revealed anti-depressant effects of leptin while clinical studies found an association of circulating leptin levels with severity of depression [Lu, 2007]. it was suggested that leptin involvement in mood regulation depends on increased mass of abdominal fatty tissue, the major source of leptin [Morris et al. 2012]. Considering co-localization of leptin and estrogen receptors in the same areas of hypothalamus [Diano et al. 1998], we aimed to assess correlations between plasma leptin levels and markers of obesity and insulin resistance (IR) in women and men patients with major depressive disorder (MDD)

Methods: Fasting plasma samples were obtained from 32 acutely ill patients with a DSM-IV diagnosis of MDD (Steiner et al., 2019). These subjects were drug-naïve or previously-treated but not medicated for ≥6 weeks. Thirty-one hospital staff members and their relatives were used as healthy controls (Steiner et al., 2019). Spearman's rank correlation coefficient test was used to assess correlations between plasma levels of leptin (ng/ml) and body mass index (BMI) (kg/m2), waist circumference/hip ratios (WC/hip) (cm/cm) and HOMA-IR (Insulin [μU/ml] x Glucose [mM/l])/22.5) scores. Study was approved by the Institutional Review Board of the University of Magdeburg. All patients signed an informed consent

Results: In healthy controls Leptin in men (n = 20) correlated with BMI [rho 0.67, p < 0.001], WC/hip [rho 0.51, p < 0.02] and HOMA-IR [rho 0.82, p < 0.0001], but not in women (n = 11): BMI [rho 0.19, p = 0.58], WC/hip [-0.31, p = 0.35] and HOMA-IR [rho 0.25, p = 0.45].

Leptin correlated in MDD men patients (n = 16) with BMI [rho 0.59, p < 0.02], WC/hip [0.66, p < 0.005] and HOMA-IR [0.65, p<0.008] but not in women patients (n = 16): BMI [0.46, p = 0.09], WC/hip [rho 0.25, p = 0.37] and HOMA-IR [-0.08, p = 0.76].

Conclusions: In MDD and healthy controls plasma leptin correlated with BMI, waist circumference/hip ratios and HOMA-IR in men but not in women. Correlations between leptin levels and obesity IR markers were gender- but not diagnosis-specific: in both MMD patients and healthy control correlations were found only in men but not in women. Our data suggest different mechanisms of leptin involvement in regulation of mood and energy homeostasis

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Keywords: Leptin, Major Depressive Disorder (MDD), Gender Differences, Body Mass Index, Insulin Resistance

Disclosure: Nothing to disclose.

M75. Response to Sertraline in Premenstrual Dysphoric Disorder is Associated With Reduction in Anxiety-Potentiated Startle, a Marker of GABA-A Receptor Function

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Background: Women with premenstrual dysphoric disorder (PMDD) appear to have altered central nervous system sensitivity to neuroactive steroid hormones, manifesting as affective symptoms and heightened arousal in the luteal phase of the menstrual cycle. In particular, women with PMDD appear less sensitive to allopregnanolone, a positive allosteric GABA-A receptor (GABA-A-R) modulator. This study evaluated psychophysiologic reactivity in women with PMDD in the follicular and luteal phases of the menstrual cycle, utilizing anxiety-potentiated startle (APS), a translational marker of GABA-A-R sensitivity. The study also assessed APS response to low-dose sertraline treatment in women with PMDD.

Methods: Female control and PMDD participants were recruited from the community, and completed prospective rating of symptoms with the Daily Record of Severity of Problems (DRSP) to confirm control or PMDD status. Participants underwent a threat of predictable and unpredictable shock task to assess anxiety-potentiated startle (APS) and fear-potentiated startle (FPS). APS and FPS were assessed during the follicular and luteal phases in a within-subject design. Women with PMDD then received 50 mg sertraline in the following luteal phase to examine its impact on APS and FPS. Treatment response was defined as having > 30% reduction in PMDD symptom severity measured by DRSP score.

Results: The sample included n = 77 participants (41 controls, 36 PMDD); 28 participants with PMDD completed sertraline treatment. There were no significant differences between controls and PMDD participants in change from follicular to luteal phases in APS nor FPS (p = 0.88, p = 0.40). However, sertraline responders showed an APS increase from the follicular to luteal phase pre-treatment, and a decrease with sertraline treatment (p = 0.026).

Conclusions: This study found that in women with PMDD who responded to sertraline, APS increased from the follicular to luteal phase pre-treatment, with a decrease post-treatment. This suggests that GABA-A-R function changes across the menstrual cycle, potentially in response to fluctuations in allopregnanolone, a GABAergic neuroactive steroid. The follicular to luteal increase in APS may serve as a biomarker predicting sertraline treatment responsiveness. Given the rapid onset of sertraline's therapeutic effect among those who responded to treatment, sertraline's impact on GABA-A-R function should be further investigated as a putative mechanism for its therapeutic action in the treatment of PMDD.

Next Steps: To further assess the dynamics of allopregnanolone interaction with GABA-A-Rs across the menstrual cycle in controls and women with PMDD. As new GABA-modulating drugs are being developed for affective disorders (e.g., Brexanolone, a synthetic ALLO FDA-approved for postpartum depression, and Sepranolone (isoallopregnanolone), GABA-A-R modulating steroid antagonists for PMDD), understanding the interaction between neuroactive steroids and the neurotransmitter systems they modulate is key in developing effective treatment targets. This may also inform a personalized medicine approach for the women with PMDD who do not respond to SSRIs.

Keywords: Premenstrual Dysphoric Disorder, Acoustic Startle Response, GABA-A Receptors

Disclosure: Nothing to disclose.

M76. Chronic Stress-Induced Epigenetic Changes of Microglia

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Background: Clinical studies have shown the association between depression and neuroinflammation. In rodents, chronic stress such as chronic mild stress and repeated social defeat stress induces microglial activation along with depressive-like behaviors. Innate immune receptors have been shown to mediate chronic stress-induced microglial activation, which causes neuronal changes and depressive-like behaviors. However, the mechanism of chronic stress-induced microglial activation remains poorly understood. Notably, stress-induced microglial activation occurs in the medial prefrontal cortex, but not in the nucleus accumbens, and is facilitated with repetition of stress. We hypothesize that epigenetic mechanisms may underlie chronic stress-induced microglial activation and the brain-region specificity.

Methods: We subjected C57BL/6N mice to single or repeated social defeat stress and isolated microglia from the medial prefrontal cortex and the nucleus accumbens. Using chromatin immunoprecipitation sequencing, we analyzed the isolated microglia for histone H3K27 acetylation, which is associated with transcriptional activation.

Results: We found that social defeat stress-induced distinct patterns of H3K27ac peaks in microglia of the medial prefrontal cortex and nucleus accumbens. Cluster analysis revealed that H3K27 acetylation peaks showed diverse brain-region specificity and responsiveness to single and repeated stress.

Conclusions: These findings show that social defeat stress induces multiple types of epigenetic changes in microglia, which may underlie brain region-specific microglial activation and priming. Further studies are needed to determine the extracellular stimuli and transcriptional programs involved in these epigenetic changes.

Keywords: Social Defeat Stress, Microglia Priming, Epigenetics

Disclosure: Nothing to disclose.

M77. Ingestion of Lactobacillus Intestinalis and Lactobacillus Reuteri Causes Depression- and Anhedonia-Like Phenotypes in Antibiotic-Treated Mice via the Vagus Nerve

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Background: The brain–gut–microbiota axis plays a role in the pathogenesis of stress-related disorders such as depression. Instead of germ-free mice, antibiotic cocktail-induced microbiome depletion has been used to investigate the role of the gastrointestinal microbiota in pathological conditions such as depression. Recently, we reported that microbiome depletion via antibiotic treatment contributed to resilience to anhedonia in mice subjected to chronic social defeat stress (CSDS)(Wang S, et al. J. Affect. Disord. 2020), suggesting that the brain–gut–microbiota axis plays a role in resilience versus susceptibility to CSDS. Furthermore, we reported that the transplantation of fecal microbiota from rats with an anhedonia-like phenotype aggravated depression- and anhedonia-like phenotypes in mice treated with an antibiotic cocktail (Yang C, et al. Transl. Psychiatry 2019). However, the precise mechanisms underlying fecal microbiota transplantation (FMT)-induced behavioral abnormalities in rodents treated with an antibiotic cocktail remain unknown. This study thus aimed to investigate the role of the brain–gut–microbiota axis in depression- and anhedonia-like phenotypes in mice.

Methods: Eight-week-old adult male C57BL/6 mice (weight, 20–25 g; Japan SLC, Inc., Hamamatsu, Japan) and male adult CD1 (ICR) mice, aged 13-15 weeks (body weight >40g, Japan SLC, Inc., Hamamatsu, Japan) were used. CSDS were performed as previously reported (Wang S, et al. J. Affect. Disord. 2020). The fecal microbiota was obtained from mice subjected to chronic social defeat stress (CSDS) and control (no CSDS) mice. FMT from these two groups was performed to antibiotic-treated mice. 16S rRNA analysis was performed to examine the composition of gut microbiota. Furthermore, the effects of subdiaphragmatic vagotomy in depression-like phenotypes after ingestion of microbes (Lactobacillus intestinalis and Lactobacillus reuteri) were examined. The data shown are the mean ± standard error of the mean. Data were analyzed using one-way analysis of variance (ANOVA) or two-way ANOVA, followed post-hoc Fisher LSD test.

Results: The ingestion of fecal microbiota from CSDS-susceptible mice resulted in an anhedonia-like phenotype, higher plasma levels of interleukin-6 (IL-6), and decreased expression of synaptic proteins in the prefrontal cortex (PFC) in antibiotic-treated mice but not in water-treated mice. 16S rRNA analysis suggested that two microbes (Lactobacillus intestinalis and Lactobacillus reuteri) may be responsible for the anhedonia-like phenotype in antibiotic-treated mice after FMT. Ingestion of these two microbes (L. intestinalis and L. reuteri) for 14 days led to depression- and anhedonia-like phenotypes, higher plasma IL-6 levels, and decreased expression of synaptic proteins in the PFC of antibiotic-treated mice. Interestingly, subdiaphragmatic vagotomy significantly blocked the development of behavioral abnormalities, elevation of plasma IL-6 levels, and downregulation of synaptic proteins in the PFC after ingestion of these two microbes.

Conclusions: These findings suggest that microbiota depletion using an antibiotic cocktail is essential for the development of FMT-induced behavioral changes, and that the vagus nerve plays a key role in behavioral abnormalities in antibiotic-treated mice after the ingestion of L. intestinalis and L. reuteri. Therefore, it is likely that the brain–gut–microbiota axis participates in the pathogenesis of depression via the vagus nerve.

Keywords: Chronic Social Defeat, Microbiota-Gut-Brain Axis, Anhedonia, Vagus Nerve, Fecal Microbiome Transportation

Disclosures: Taisho: Consultant, Grant (Self); Dainippon-Sumitomo, Otsuka: Grant (Self)

M78. Acute Effects of Electroconvulsive Therapy and Venlafaxine on Verbal Learning and Memory Dimensions in Older Adults With Major Depressive Disorder

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Background: Major depressive disorder (MDD) represents one of the most significant health challenges worldwide and is a leading cause of disability. Major depression in the geriatric population is of particular interest as it can worsen functional impairment, morbidity, and mortality. Moreover, many older patients find little benefit from typical first-line antidepressant treatment strategies such as psychotropic medications and psychotherapy. Electroconvulsive therapy (ECT) is often prescribed in such cases or for those who require expeditious treatment. We previously assessed broad neurocognitive effects of a combined regimen of ECT and venlafaxine in geriatric depression during Phase 1 of the Prolonging Remission in Depressed Elderly (PRIDE) study. While prior research and our recent findings demonstrated that ECT adversely impacts overall immediate and delayed recall of verbal information, the effects of ECT on multiple verbal learning and memory dimensions remain unclear. The purpose of this analysis was to address the limited information regarding the effects of ECT on verbal learning and memory component processes. We hypothesized that following administration of an acute course of RUL-UB ECT and venlafaxine, older adults would demonstrate changes in verbal learning and memory component processes (e.g. learning across repeated trials, semantic-cued recall).

Methods: The PRIDE study was a NIMH funded, multicenter, randomized study of an individualized continuation ECT schedule combined with pharmacotherapy to enhance long-term outcomes in older adults with MDD. In Phase 1 of the study, patients received acute ECT 3x weekly combined with venlafaxine. ECT parameters were standardized as: RUL electrode placement using a Somatics Thymatron System IV with an ultrabrief pulse width of 0.25ms and current of 0.9Amps or a MECTA SPECTRUM device with an ultrabrief pulse width of 0.3ms and current of 0.8Amps. Older adults (age > 60) with MDD, based on semi-structured diagnostic interviews, were enrolled in the study. All participants provided written informed consent for this IRB approved investigation before completing study procedures. For the purposes of this study, the neurocognitive outcome data were collected with the 2nd edition of the California Verbal Learning Test (CVLT-II). The CVLT-II is a psychometrically sound measure of verbal learning and memory that consists of learning, recalling, and recognizing 16 words that are relatable among 4 semantic categories. Both the CVLT-II Standard and Alternate forms were used in order to minimize practice effects, with form order counterbalanced across study sites, subjects, and time points (baseline, end) via a computerized algorithm. The CVLT-II was administered at Phase 1 baseline and within 72 hours following the last ECT session (end). CVLT-II raw scores were converted into

demographic-adjusted scores using the CVLT-II scoring software. Descriptive statistics were used to characterize the demographic, neurocognitive, and clinical features of the sample. Comparisons of change from baseline to end across all variables were computed with paired t-tests. All statistical tests were two-tailed with alpha=0.05.

Results: Relative to baseline following treatment with acute ECT and venlafaxine, older adults showed a statistically significant lower recall of word learning across three of the five learning trials. Specifically, word learning was lower on learning trials three ($t = 2.10$, $p = 0.04$), four ($t = 2.22$, $p = 0.03$), and five ($t = 2.99$, $p = 0.003$). Also, semantically-cued recall of words was significantly lower on both immediate ($t = 3.46p = 0.001$) and delayed recall ($t = 2.34$, $p = 0.02$) trials. Although statistically significant, for the study sample as a whole, the magnitude of change from baseline to end for most of verbal learning and memory variables was modest. There was no significant difference from baseline to end with regard to learning a second word list ($t = -0.14$, $p = 0.89$). However, compared to initial learning of the first word list, it appears that participants recalled relatively fewer words on the second list suggesting possible proactive interference. The amount of intrusion errors remained stable from baseline to end.

Conclusions: To our knowledge, this is the first study to characterize the effects of ECT on specific verbal learning and memory dimensions in older adults with MDD. We found that acute ECT plus venlafaxine affected word learning across three of the five learning trials, which suggested that learning requiring repeated exposure or rehearsal over multiple trials may be adversely affected. Also, there was a decrease in semantically cued word recall, which suggested that participants were unable to benefit from semantic-cue strategies to recall words. These findings extend on our prior research and provide new evidence of possible mechanisms of action that ECT may acutely and transiently impact memory through its actions on learning and recall component processes. Of note, for the study sample as a whole, while some of the changes in variables were statistically significant, the degree of change was relatively modest. Future research is warranted to better understand the characteristic learning and memory processes that are most affected by ECT, to assess if these effects persist or stabilize over longer periods of time, and to elucidate the underlying mechanisms.

Keywords: Electroconvulsive Therapy, Depression, Neuropsychology, Geriatric

Disclosure: Pearson: Consultant (Self)

M79. The Effect of Normobaric Hyperoxia in Depression

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Background: Normobaric hyperoxia involves breathing through nasal tubes oxygen concentrations above that of usual room air but at the usual one atmosphere of pressure. It is widely used in pulmonary diseases with decreased blood oxygen saturation. Its use in acute settings of cardiac infarction or stroke without decreased blood oxygen saturation is controversial. It is to be distinguished from hyperbaric oxygen, which requires a special chamber, can be administered only for a limited time period, is expensive, and has several inherent dangers. Several studies of normobaric hyperoxia in some neurological conditions have demonstrated clinical benefits. Oxygen enriched air may increase oxygen pressure in brain tissue and have biochemical effects such as on brain erythropoietin gene expression, even in patients without lung disease. Because of reports that mitochondrial function is reduced in brain with depression, we conducted a study of normobaric hyperoxia at 35% oxygen concentration in a convenient medium-term setting in which outpatient depressed patients received the treatment for seven hours per night for one month in the home or control treatment. The study was double-blind. The study was made possible by the technological development of portable, inexpensive oxygen generators for home use.

Methods: This pilot, randomized, double-blind study examined the efficacy of normobaric hyperoxia as a treatment for depression. Fifty-five participants aged 18-65 years with mild to moderate depression who signed an informed consent to participate and had a Hamilton Rating Scale for Depression (HRSD) score of ≥ 8 were included in the study. Participants underwent a psychiatric inclusion assessment and a clinical evaluation by a psychiatric nurse at baseline, 2 and 4 weeks after commencement of study intervention. Participants were randomly assigned to normobaric hyperoxia of 35% fraction of inspired oxygen or 21% fraction of inspired oxygen (room air), through a nasal tube, for 4 weeks, during the night. Tools were the Hamilton Rating Scale for Depression (HRSD); Clinical Global Impression (CGI) questionnaire; Sense of Coherence (SOC) 13-item questionnaire; WHO-5 Well Being Index questionnaire for the estimation of quality of life (QOL); Sheehan Disability Scale (SDS).

Results: The present study showed a significant improvement in HRSD ($p < 0.0001$), CGI ($p < 0.01$) and in SDS ($p < 0.05$) among patients with depression who were treated with oxygen-enriched air, as compared to patients who were treated with room air. In CGI, 69% of the patients who were treated with oxygen-enriched air improved compared to 23% patients who were treated with room air.

Conclusions: This study showed a beneficial effect of normobaric hyperoxia on some symptoms of depression. Limitations were small sample size and unclear biological mechanism.

Keywords: Depression, Treatment, Non Pharmacological Interventions

Disclosure: Nothing to disclose.

M80. Anxiety Symptom Response in Patients With Postpartum Depression Treated With the Neuroactive Steroid Brexanolone Injection

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Background: Postpartum depression (PPD) is one of the most common medical complications during and after pregnancy. In the United States, approximately 13.2% of mothers experience symptoms of PPD, varying by state from 9.7% to 23.5%. Women with PPD may have intense feelings of sadness, anxiety, irritability, and/or rage, and a range of cognitive, social and somatic symptoms. Anxiety is a prominent symptom of PPD, and has also been associated with more severe disease. In addition to other mechanisms, dysfunctional gamma aminobutyric acid (GABA) signaling, altered functional network connectivity, and hormonal changes during pregnancy have been implicated in the etiology of PPD. Neuroactive steroid (NAS) GABAA receptor positive allosteric modulators, which have pharmacological profiles that are distinct from benzodiazepines, activate both synaptic and extrasynaptic GABAA receptors to produce phasic and tonic inhibitory currents to enhance GABAergic inhibition. Enhancing GABAergic inhibition may restore excitatory/inhibitory balance to regulate brain network activity, which has been proposed to reduce depressive symptoms. The circulating levels of

allopregnanolone, an endogenous NAS and GABAA receptor positive allosteric modulator, rise as pregnancy progresses, and decrease rapidly after childbirth. Brexanolone injection (BRX) is a first-in-class, intravenous formulation of allopregnanolone, and is FDA-approved for the treatment of adults with PPD. In a prespecified integrated analysis of three pivotal trials of BRX in PPD, BRX met the primary endpoint of a significant reduction in depressive symptoms, assessed by change from baseline (CFB) in the 17-item Hamilton Rating Scale for Depression total score (HAMD-17) at Hour 60 (CFB ± SE: BRX90: −16.95 ± 0.74, placebo: −12.83 ± 0.71; $p < 0.0001$). In addition, CFB using the predefined Bech-6 subscale of HAMD-17, comprised of the core symptoms of depression, favored BRX90 compared with placebo beginning at Hour 24 (CFB±SE: BRX90: −30.06 ± 1.98, placebo: −24.07 ± 1.92; $p = 0.0173$) and at all measured timepoints to Day 30 (BRX90: −38.47 ± 2.11, placebo: −30.63 ± 2.02; $p = 0.0301$). AEs occurring in ≥5% of BRX and at ≥2x the rate of placebo included sedation and/or somnolence, dry mouth, loss of consciousness, and flushing/hot flush. Post hoc analyses of the integrated dataset assessed the effect of BRX on anxiety symptoms in women with PPD, using the HAMD-17 anxiety/somatization subscale (HAMD-17-A/S).

Methods: Women (N = 247) ages 18–45, ≤6 months postpartum, with PPD (defined as a major depressive episode with onset in the 3rd trimester or ≤4 weeks postpartum), and a qualifying HAMD-17 score (Studies A and B: HAMD-17 ≥26; Study C: HAMD-17 20-25) were enrolled. Patients received a 60-hour infusion of placebo or BRX titrated to 60 μg/kg/h (BRX60) or 90 μg/kg/h (BRX90), with follow-up through Day 30. Post hoc analyses included CFB in the HAMD-17-A/S (patients with a score of ≥7 considered to have "anxious depression"), as well HAMD-17-A/S response (≥50% reduction from baseline score). CFB assessments utilized mixed-effect models for repeated measures, and response assessments utilized the generalized estimating equation approach. Post hoc analyses were not adjusted for multiplicity. Safety and tolerability were assessed throughout the study by adverse events (AEs).

Results: 102 and 107 patients, regardless of baseline HAMD-17-A/S score, from the BRX90 and placebo treatment arms were included in these post hoc analyses, respectively. At baseline the mean (±SD) HAMD-17-A/S scores were high (BRX90: 7.78 ± 2.01 and placebo: 8.05 ± 2.06). Patients receiving BRX90 achieved significantly greater reductions in HAMD-17-A/S CFB compared with placebo at Hour 24 (−1.04 ± 0.35, $p = 0.0030$), Hour 48 (−1.15 ± 0.35, $p = 0.0011$), Hour 60 (−1.09 ± 0.34, $p = 0.0014$), Day 3 (−1.23 ± 0.36, $p = 0.0007$), Day 7 (−1.25 ± 0.37, $p = 0.0009$), and Day 30 (−0.79 ± 0.40, $p = 0.0490$). A significantly greater proportion of BRX90 patients achieved HAMD-17-A/S response compared with placebo at Hour 24 (54.5% versus 47.2%, $p = 0.0394$), Hour 36 (58.6% versus 50.5%, $p = 0.0461$), Hour 48 (63.6% versus 50.5%, $p = 0.0053$), Hour 60 (68.4% versus 58.5%, $p = 0.0222$), Day 3 (74.7% versus 58.9%, $p = 0.0012$), Day 7 (58.0% versus 43.4%, $p = 0.0045$), and Day 30 (64.9% versus 56.2%, $p = 0.0425$).

Conclusions: BRX treatment in women with PPD has previously been shown to provide rapid (Hour 24 for HAMD-17 total score and Bech-6 score) and sustained improvement in depressive symptoms (HAMD-17 total score and Bech-6 score at all subsequent measured time points up to Day 30) compared with placebo. In addition to its effects on core symptoms of depression in this trial, these post hoc analyses showed that BRX treatment also resulted in significantly greater reductions in anxiety-somatization symptoms and higher proportions of patients who achieved an anxiety-somatization symptom response compared with placebo (as measured by the HAMD-17-A/S subscale).

Keywords: Postpartum Depression, Postpartum Anxiety, Brexanolone Injection

Disclosure: Sage Therapeutics: Advisory Board, Consultant (Self); Asarina Pharma: Advisory Board (Self)

M81. Explorative Analysis of Cross-Correlations Between Hippocampal Subfield Volumes in Healthy Subjects After Ketamine vs. Placebo

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Background: Antidepressant doses of ketamine facilitate synaptic spine turnover towards increases of synaptic spines within several minutes to hours of administration. These neuroplastic properties were demonstrated in mice and rats, however, translational work in humans is scarce. A previous study in the Flinders Sensitive Line of depression found that ketamine reversed apical spine deficits in hippocampal CA1 pyramidal neurons 60 min after administration of ketamine. Another study in mice found rapidly occurring increases (within three hours) of functional connectivity between prefrontal neurons after chronic cortisol administration. Here, we aimed to translate ketamine's facilitation of structural plasticity by investigating cross-correlations of hippocampal subfields in healthy human subjects.

Methods: We performed a randomized, double-blind, placebo-controlled crossover MRI study in 31 healthy subjects (14 female, mean age 25.2). Two structural T1-weighted structural MRI scans were conducted on a Siemens 3T Trio Scanner with two MPRAGE sequences (TR/TE = 2300/4.21 ms, flip angle set to 9°, voxel size 1.0 × 1.0 × 1.1 mm). After the first sequence subjects received a bolus of esketamine (0.11 mg/kg) followed by a maintenance infusion of 0.12mg/kg over 20 minutes. A second MPRAGE sequence was conducted 45 min after esketamine administration. We measured hippocampal subfield volumes with FreeSurfer 6.0. In a crossover arm at least a week apart, each subject received a saline infusion (placebo). We calculated partial correlations between subfields separately for esketamine and placebo administration, adjusted for sex, age and total intracranial volume. We adjusted for multiple testing using a 5% false discovery rate (FDR), and considered alpha of $p < 0.05$, FDR-corrected, statistically significant. A quantitative comparison between all correlations of ketamine vs. placebo was done with paired t-tests.

Results: Correlations between regions were much higher after ketamine administration than placebo. ($t = 9.68$, $p < 0.001$, cohen's $d = 0.41$). There were increases of subfield cross-correlations– amongst other regions – between ipsilateral and contralateral dentate gyrus, tail and right to left CA3/4 subfield volumes.

Conclusions: In this study we demonstrated increases of hippocampal subfield volume cross-correlations within 45 minutes of ketamine vs. placebo administration. Cross-correlations of hippocampal subfield volumes calculated by automated MRI segmentations could represent a proxy measure of facilitated synaptic plasticity in hippocampal pyramidal neurons. However, in this study, there was a high number of correlation tests and low subject numbers, which demands replication in larger longitudinal datasets.

Keywords: Esketamine, Hippocampus, Longitudinal MRI

Disclosure: Nothing to disclose.

M82. Analgesia for Days With Low Dose Ketamine vs Ketorolac in Chronic Pain and PTSD Patients

Abstract not included.

M83. Effects of Discontinuation of Drugs Used for Augmentation Therapy on Treatment Outcomes in Depression: A Systematic Review and Meta-Analysis

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Background: There has been no consensus on whether and how long add-on drugs for augmentation therapy should be continued in the treatment of depression.

Methods: Double-blind randomized controlled trials that examined the effects of discontinuation of drugs used for augmentation on treatment outcomes in patients with depression were identified. Meta-analyses were performed to compare rates of study withdrawal due to any reason, study-defined relapse, and adverse events between patients who continued augmentation therapy and those who discontinued it.

Results: Seven studies were included (n = 841 for continuing augmentation therapy; n = 831 for discontinuing augmentation therapy). The rate of study withdrawal due to any reason was not significantly different between the two groups (risk ratio [RR]=0.86, 95% confidence interval [CI]=0.69–1.08, p = 0.20). Study withdrawal due to relapse was less frequent in the continuation group than in the discontinuation group (RR=0.61, 95% CI=0.40–0.92, p = 0.02); however, this statistical significance disappeared when one study using esketamine as augmentation was excluded. Analysis of the data from five studies that included a stabilization period before randomization found less frequent relapse in the continuation group than in the discontinuation group (RR=0.47, 95% CI=0.36–0.60, p<0.01). This finding was repeated when the esketamine study was excluded.

Conclusions: This meta-analysis shows that add-on drugs, other than esketamine, used for augmentation therapy for depression may be discontinued, but this may not be the case for patients who are maintained with augmentation therapy after remission.

Keywords: Depression, Antidepressant, Augmentation, Mood Disorders

Disclosure: Eisai, Otsuka Pharmaceutical, Dainippon-Sumitomo Pharma: Grant (Self); Otsuka Pharmaceutical, Dainippon-Sumitomo Pharma, Eisai: Honoraria (Self); Dainippon-Sumitomo Pharma: Advisory Board (Self)

M84. An α5-Containing Benzodiazepine Site on the GABAAR is Required for the Fast Antidepressant-Like Actions of MRK-016 on Stress-Induced Anhedonia and Weakened Synaptic Function

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Background: Major depressive disorder (MDD) is a common psychological disorder characterized by chronic depressed mood and lack of interest in once-pleasurable activities (anhedonia), and is commonly comorbid with suicidal ideation. Traditional antidepressants, like selective serotonin reuptake inhibitors (SSRIs), require six to eight weeks before remission and up to two thirds of patients remain refractory to treatment. Ketamine, in contrast, has rapid antidepressant, antianhedonic, and anti-suicidal actions in in treatment-resistant depression patients. Ketamine is believed to promote transient increases in glutamate excitation via antagonism of NMDARs on GABA-ergic interneurons. However, the presence of NMDARs throughout the brain makes it difficult to minimize ketamine's off-target effects while maximizing its antidepressant-like actions.

Negative allosteric modulators of GABAA receptors (GABAAR) containing α5 subunits (GABA-NAMs), such as MRK-016, represent a promising fast-acting antidepressant alternative to ketamine because they indirectly promote excitatory glutamatergic transmission only within reward regions of the brain. We have previously shown these drugs exhibit rapid antidepressant-like properties in preclinical models of stress-induced anhedonia and restore stress-weakened glutamatergic excitation at hippocampal temporoammonic-CA1 synapses (TA-CA1). Here we used the benzodiazepine antagonist flumazenil and mice with genetic deletion of alpha5 subunits to identify the molecular target at which GABA-NAMs mediate their rapid antidepressant-like and synaptic actions.

Methods: Eight-week old, male C57BL/6 mice were subjected to 10 days of chronic multimodal stress (CMMS), sufficient to induce deficits in reward-seeking behavior as assessed through 1% sucrose preference and female urine sniffing tests. Cohorts of stress-susceptible animals (n = 4-7) were treated with the GABA-NAM MRK-016 (3mg/kg, i.p.) with or without flumazenil (20mg/kg, i.p.), after which hedonic behavior was then reassessed. Field EPSP recordings within the synapses of hippocampal area TA-CA1 were used to quantify synaptic strength as a ratio of AMPA- vs NMDA-mediated excitation.

Male GABAAR α5 KO C57BL/6 mice were utilized to test the necessity of α5 subunits to mediate MRK-016's antidepressant-like actions. Reward-seeking behaviors were assed following the same 10-day CMMS paradigm, and AMPA:NMDA ratios were quantified across treatment groups and genotypes (n = 4-6). Additionally, qEEG transmitters were embedded above the frontal cortex and cerebellum of 12-week old male GABAAR α5 KO mice to quantify changes in high-frequency gamma oscillatory activity, a characteristic of rapid-acting antidepressant efficacy, immediately following MRK-016 administration. Where appropriate, one-, two-, and three-way repeated measure ANOVAs were employed to assess statistical significance.

Results: Treatment with a single injection of 3mg/kg MRK-016 is sufficient to significantly reverse stress-induced deficits in both sucrose preferences and female urine sniffing preferences (p<0.001), as well as TA-CA1 AMPA:NMDA ratios (p<0.05) as compared to stress-susceptible animals treated with vehicle. Pretreatment with the benzodiazepine site antagonist flumazenil is sufficient to prevent restoration both of hedonic behavior (p<0.01) and synaptic strength. MRK-016 did not reverse anhedonic behavioral deficits, nor promote synaptic strengthening, in GABAAR α5 Kos (p<0.001), though rapid reward-seeking behavioral restoration was seen in these animals following administration of 10mg/kg ketamine. Additionally, a single IP injection of MRK-016 induced significant increases in gamma-power in wild-type, but not in GABAAR α5 KO animals (p<0.05).

Conclusions: We conclude that GABA-NAMs display rapid-acting antidepressant-like properties in preclinical rodent models of anhedonia. These effects require binding at the benzodiazepine site of α5-containing GABAARs to produce a transient increase in correlated neuronal discharge at gamma frequencies, thereby inducing intrinsic activity-dependent synaptic strengthening in critical reward circuits, which may be responsible for its anti-anhedonic actions. α5 selective GABA-NAMs thus have potential as novel, fast-acting antidepressants and may yield important mechanistic information for the development of future therapies.

Keywords: Rapid Antidepressant, α5-GABAA Receptor Negative Allosteric Modulator, Reward-Seeking Behavior, Hippocampus

Disclosure: Nothing to disclose.

M85. Psilocybin, After Only a Single Treatment, has Persistent Antidepressant-Like Effects in a Rat Experimental System for the Study of Mood Disorders

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Background: Psilocybin has been granted Breakthrough Status by the FDA for its development as a treatment for major depression. Remarkably, clinical trials have shown that antidepressant effects persist for several months to years after only a single treatment. Although psilocybin's active metabolite, psilocin, activates the serotonin 5-HT2A receptor to produce its acute subjective effects, the molecular/neurological mechanisms underlying its therapeutic efficacy remain unknown. Further, whether or not the subjective "peak experience" is necessary for therapeutic efficacy is also unknown. In order to elucidate underlying molecular mechanisms and to address existential questions, we developed a translationally relevant rodent experimental system that recapitulates the long-lasting antidepressant effects observed in humans after only a single treatment. Understanding these mechanisms and issues will not only inform on the development of psilocybin as a new effective antidepressant therapeutic strategy, but will also provide insight into fundamental biological processes underlying how activation of neurotransmitter receptors just once can lead to persistent neuroadaptations and therapeutic benefits for treating psychiatric disorders.

Methods: We further developed and utilized an adolescent-based chronic restraint stress paradigm (aCRS) in combination with a cognitive behavioral outcome measure with defined neural circuitry (object pattern separation) to assess the effects of a single administration of psilocybin. Our aCRS experimental system involves restraining normal adolescent rats for one hour per day for 14 days prior to young adulthood. This stress produces persistent behavioral deficits relevant to mood disorders that last for at least several months undiminished into adulthood, a feature necessary to test the ability of a single drug treatment to have persistent antidepressant-like effects. Significantly, this paradigm is effective in females. For our experiments, we performed adolescent restraint stress in female rats for 14 days. Seven days after the final restraint stress in young adulthood, a single treatment with psilocybin (1.0 mg/kg; i.p.) was administered. Rats were allowed to remain in their home cage undisturbed for five weeks until behavioral testing. For our behavioral outcome measure we adapted and utilized a rodent object pattern separation (OPS) task. Object pattern recognition is deficient in humans with mood disorders, and this dysfunction can be normalized by treatment with antidepressants. Object pattern is mediated by the entorhinal cortex to CA3 to dentate gyrus circuit in both rodents and humans, therefore this cognitive-based outcome measure has high translational relevance. After testing of all experimental groups, brains were removed and expression levels of certain genes are currently being assessed in relevant brain regions such as the ventral hippocampus and mPFC by qPCR. Treatment groups = no stress; stress; psilocybin only; stress+psilocybin; n = 6/group. Statistical analysis was performed using appropriate tests and GraphPad Prism.

Results: We first validated the OPS test for female rats by demonstrating that they could robustly detect when the location of one object was moved with respect to the location of an identical object in a round arena. For the primary experiment, adolescent chronic restraint stress induced profound deficits in the stress-only group compared to control non-stressed as measured by the OPS test 6 weeks after the final restraint stress. The control group could detect when an object moved with sensitivity, whereas the stressed group had no measurable performance and could not detect when an object's location moved in the arena. A single administration of psilocybin given one week after the final restraint stress completely normalized pattern recognition, and results were not different from non-stressed controls. 48 hours after the OPS test, rats were assessed in the forced swim test in order to compare results to historical literature for experimental systems used to study mood disorders. Similar results were obtained, where the stressed group showed significant immobility 6 weeks after the final stress restraint, and the psilocybin treated stressed animals were not different from controls who were not stressed.

Conclusions: We are the first to demonstrate, in a translationally relevant rodent experimental system, that a single treatment with psilocybin is able to persistently normalize stress-induced cognitive behavioral deficits relevant to mood disorders. This is conserved with psilocybin's long-lasting efficacy to treat depression in humans. We are now utilizing our system to elucidate molecular/genetic mechanisms underlying psilocybin's long-lasting antidepressant-like effects. Rats are not believed to have a sense of self, and are likely incapable of having existential anxiety, fearing a reality that they as individuals do not exist, or mystical experiences. Therefore, we believe that the core antidepressant effect of psilocybin in humans is cell biological in nature, and that while correlated to antidepressant effect, ego dissolution and subjective peak/mystical experiences are not causational to antidepressant effect.

Keywords: Psychedelics, Antidepressant, Animal Models, Serotonin 5-HT2A Receptor, Pubertal Stress

Disclosure: Eleusis Therapeutics: Advisory Board, Grant (Self)

M86. A Rodent Model of Exposure Therapy to Study Adjunct Drug Treatment

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Background: Treatments for stress-related psychiatric disorders are inadequate. Behavioral therapies such as exposure therapy can be effective in ameliorating cognitive dysfunction associated with PTSD and depression. We have recently established extinction learning in rats as a behavioral intervention that models the beneficial effects of exposure on cognition. In our recent studies, we have shown that extinction used as an intervention in rats, reverses chronic stress-induced deficits in cognitive flexibility on the attentional set-shifting test (AST), a medial prefrontal cortically-mediated executive process. Extinction requires the activity of pyramidal neurons in the infralimbic cortex, and BDNF signaling mediates these effects. The combined use of psychotherapy and pharmacotherapy may be more effective than either alone. Since extinction shares mechanisms exerted by ketamine, we reasoned that extinction and ketamine used in combination will have enhanced efficacy.

Methods: In these studies, we developed a model of sub-effective extinction therapy in male and female rats (n = 5-11) that showed impairment in two readouts of prefrontal cortex function, AST and evoked local field potentials in the infralimbic cortex, following chronic unpredictable stress.

Results: We found that reducing the duration of extinction attenuated its therapeutic effects on set shifting performance and activity of the infralimbic cortex in Sprague-Dawley rats after stress (n = 6-9/group, $p<0.01$, $d=1.87$). We then established sub-effective doses of ketamine on the same measures of cognition and electrophysiology. Combining sub-effective extinction with a sub-effective dose of ketamine (1mg/kg) reversed the effects of stress on set shifting (n = 5-11/group, $p<0.01$, $d=2.27$).

Conclusions: We have developed a model to study adjunct treatment combining extinction and candidate drug therapies such as ketamine. Ongoing experiments will be conducted to determine the effects of the combined extinction plus ketamine treatment in female rats.

Keywords: Adjunctive Therapy, Fear Extinction, Stress, Ketamine

Disclosure: Nothing to disclose.

M87. A Key Requirement for Synaptic Reelin Signaling in Rapid Antidepressant Effects of Ketamine

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Background: Ketamine is a competitive N-methyl-D-aspartate (NMDA) receptor antagonist and was recently approved by the U.S. Food and Drug Administration as an antidepressant for patients with treatment-resistant depression. However, about 30 to 50% of patients with treatment-resistant depression still do not respond to ketamine. The underlying mechanism on the non-responsiveness to ketamine's antidepressant action remains unclear. Recent studies have reported a possible role for secreted glycoprotein, Reelin, in the regulation of ketamine's antidepressant effects. Here, we investigated whether the disruption of Reelin-mediated synaptic signaling alters ketamine-triggered synaptic plasticity and antidepressant effects.

Methods: To investigate the role of Reelin-dependent signaling in the antidepressant action of ketamine, we used mouse models with genetic deletion of Reelin or apolipoprotein E receptor 2 (Apoer2), as well as pharmacological inhibition of their downstream effectors, Src family kinases (SFKs) or phosphoinositide 3-kinase (PI3K). We conducted the forced swim test and novelty suppressed feeding test to analyze the antidepressant action of ketamine. We monitored ketamine-induced synaptic potentiation and NMDA receptor function in the hippocampal CA1 area with field electrophysiology recording. Over 6 male or female mice per group were used in this study. Student's t-test or two-way ANOVA was used to analyze the statistical significance.

Results: We found that disruption of Reelin, Apoer2, or SFKs impairs ketamine-driven anti-depressive behaviors and synaptic plasticity in the hippocampal CA1 area. While ketamine administration did not affect tyrosine phosphorylation of DAB1, a core adaptor protein linked to downstream signaling of Reelin, disruption of Apoer2 or SFKs impaired NMDA receptor-mediated basal neurotransmission. These results indicate that maintenance of basal NMDA receptor function by Reelin signaling is a key permissive factor required for ketamine's antidepressant effects.

Conclusions: Our results suggest that impairments in Reelin-Apoer2-SFKs pathway components may in part underlie non-responsiveness to ketamine's antidepressant action.

Keywords: Reelin, Apoer2, Src Family Kinases, Ketamine, Antidepressants

Disclosure: Nothing to disclose.

M88. The Muscarinic Antagonist Scopolamine Produces Rapid Onset of Action in a Chronic Model of Stress in Rats

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Background: Scopolamine, the non-selective muscarinic antagonist, is a rapid-acting anti-depressant. In this study, we have attempted to back-translate the efficacy of scopolamine in a chronic model of stress-induced anhedonia in rats. Concurrently, we have attempted to understand the effect of scopolamine on brain IP1 accumulation, binding to brain muscarinic receptors ex-vivo, effects on dopamine release in vivo, c-fos immunoreactivity, hippocampal LTP and sleep architecture.

Methods: The pharmacology of scopolamine was characterized in traditional calcium, cAMP and β-arrestin assays at the five muscarinic receptors. Brain autoradiography was assessed by [3H]-NMS binding on slice; brain IP1 accumulation was assessed under BQCA challenge to shift the assay sensitivity towards M1 pharmacology. Dopamine microdialysis from the rat nucleus accumbens was performed during oxotremorine-infusion within the VTA. Chronic stress in rats was introduced by random stressful stimuli for 2 weeks and during the scopolamine treatment phase.

Results: Scopolamine is a non-selective muscarinic antagonist with pIC50 of 8.5 – 9.5 for hM1 – hM5 receptor subtypes, with no apparent speciation in rats. Scopolamine occupied [3H]-NMS binding sites in rat brain in a dose (0.1 – 3 mg/kg, s.c.) dependent manner, with peak occupancy observed at 2 hours post dose. The exposure of scopolamine was dose dependent in the brain, although it was cleared in the plasma faster than in the brain compartment. In mice, scopolamine produced c-fos activation in PFC, 1-hour post dose, that was significantly reduced in M1 knockout mice. Scopolamine produced a trend towards enhancing LTP, as measured from CA1-hippocampus in-vivo; similar trends were observed from in-vitro slices as well. Likewise, scopolamine produced a dose dependent (0.025, 0.25, 2.5 mg/kg, s.c.) blockade of BQCA-stimulated IP1 accumulation in mice brain. Compared to vehicle-treated control rats, scopolamine (0.1 mg/kg, s.c.) pretreatment also completely blocked elevated dopamine release in the nucleus accumbens elicited by intra-VTA infusion of 10 uM oxotremorine-M, suggesting modulation of tonic dopamine levels by muscarinic antagonism, presumably via M5 receptor subtype. The same dose of scopolamine was then tested on rat sleep/wake cycle, with scopolamine increasing the NREM and REM latency for the first 2 hours. Finally, scopolamine (1.5 mg/kg, i.p.) was tested in the chronic model of stress-induced anhedonia in rats. Animals dosed with scopolamine that were deficient in sucrose water drinking, reversed the phenotype after 1 week of dosing, compared to traditional antidepressants that take 3-4 weeks for efficacy. In fact, when tested after 24 hours of a single dose of scopolamine, the anhedonia was partially reversed in stressed rats, indicating a fast onset of action. The effects of synaptic proteins after single and multiple doses of scopolamine are being evaluated.

Conclusions: This body of data suggests that scopolamine is a muscarinic antagonist with efficacy observed in a diverse range of CNS pharmacodynamic and efficacy assays; more importantly, it offers an important tool to back translate the clinical efficacy to rodent models of anhedonia, setting up the stage for muscarinic-selective drug discovery efforts in mood disorders.

Keywords: Scopolamine, Muscarinic Acetylcholine Receptor, Mood Disorders

Disclosure: Janssen R&D, LLC: Employee (Self)

M89. In Vitro and in Vivo Pharmacological Characterization of a Novel, Selective, Orally Available GluN2B Negative Allosteric Modulator JNJ-64300808

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Background: Blockade of GluN2B (NR2B)-containing NMDA receptors has been proposed as a therapy for a number of neurological and psychiatric diseases including mood disorders. Several GluN2B antagonists have been identified and developed over the last three decades. These compounds, however, had significant limitations in regard to their selectivity or drug-like properties (e.g. oral bioavailability). Here we characterize a novel GluN2B Negative Allosteric Modulator, JNJ-64300808, discovered at Janssen.

Methods: The pharmacology of JNJ-64300808 was explored using in-vitro assays (calcium mobilization, radioligand binding, manual patch-clamp), ex-vivo slice autoradiography, EEG/sleep analysis, and in-vivo electrophysiology. Off- target profile of JNJ-64300808 was examined using CEREP radioligand binding, functional GPCR assays and a panel of selected human kinases.

Results: JNJ-64300808 is a high affinity (pKd=8.0; human cortex,), potent (pIC50=7.6 in FLIPR, pIC50=7.3 in patch clamp; recombinant GluN1/GluN2B expressed in CHO cells) and selective negative allosteric modulator of human GluN2B receptors. Consistent with its in vitro properties, JNJ-64300808 at 3 μM blocked 54% of NMDA receptor-mediated EPSC in rat neonatal hippocampal slices similar to other GluN2B antagonists. The compound also blocked GluN2B-mediated current (48% of total NMDA current) in cultured mouse cortical interneurons.

Upon oral dosing, JNJ-64300808 occupied GluN2B receptors in rat hippocampus in time- and concentration-dependent manner, reaching 50% occupancy at 0.86 mg/kg p.o. and the plasma concentration of 407 ng/ml at 1h post dosing. At 10 mg/kg p.o., compound inhibited in vivo long-term depression measured in the CA1 of anesthetized rat hippocampus (102% of control (pre-LFS) spike amplitude after 10 mg/kg p.o. vs. 39 % for vehicle control) without significant effects on the basal synaptic transmission (93% control at 10 mg/kg s.c.). Administration of JNJ-64300808 (10 mg/kg p.o., HPMC suspension) produced minimal changes on spontaneous sleep in rat. Slight but significant reduction in the latency for non-rapid eye movement (NREM) sleep was observed, which was not associated with any alteration in the duration of NREM and rapid eye movement (REM) sleep. Electroencephalography (EEG) revealed a small reduction in power density in alpha and beta power during wake phase (-15% and -12% vs. vehicle control, respectively). Spontaneous locomotor activity was not affected after JNJ-64300808 administration.

In the selectivity assays, JNJ-64300808 showed no stimulation of inhibition of binding or function of any receptor, ion channel or transporter tested, except for minor inhibition of the endothelin 1A receptor (16% inhibition at 5 μM and 19% inhibition at 10 μM).

Conclusions: JNJ-64300808 was demonstrated to be a potent and selective GluN2B antagonist, and to functionally engage these receptors in rat hippocampus upon oral dosing indicating acceptable bioavailability and brain penetration. The compound inhibited native GluN2B-mediated signaling in vitro, including in cultured interneurons, a type of cell proposed to be crucial for the therapeutic effect of rapidly acting antidepressants. JNJ-64300808 also affected synaptic function in vivo (inhibited LTD formation, a form of plasticity).

Keywords: GluN2B, Mood Disorders, NR2B Receptor

Disclosure: Janssen Research & Development: Employee, Stock/Equity (Self & Spouse)

M90. Adolescent Fluoxetine Treatment Mediates a Persistent Anxiogenic-Like Phenotype in Female C57BL/6 Mice That is Ameliorated by Fluoxetine Re-Exposure in Adulthood

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Background: The objective of this study was to evaluate whether juvenile fluoxetine (FLX) exposure induces long-term changes in baseline responses to anxiety-inducing environments, and if so, whether its re-exposure in adulthood would ameliorate this anxiogenic-like phenotype. An additional goal was to assess the impact of adolescent FLX pre-treatment, and its re-exposure in adulthood, on serotonin transporters (5-HTT) and brain-derived-neurotrophic-factor (BDNF)-related signaling markers (TrkB-ERK1/2-CREB-proBDNF-mBDNF) within the hippocampus and prefrontal cortex.

Methods: We exposed adolescent female C57BL/6 mice to FLX in their drinking water (250 mg/l) from postnatal day [PD]-35 to PD49. After a 21-day washout period (PD70), mice were either euthanized (for tissue collection) or assessed in adulthood on responsivity to the elevated plus-maze (EPM) or the light-dark box (LDB) tests – behavioral paradigms commonly used to assess anxiety-like responses in rodents. To evaluate whether FLX re-exposure would reverse the antidepressant-induced molecular and anxiety-related alterations observed in adulthood, we reinstated FLX treatment in separate groups of mice (PD70-84). Twenty-four hours later (PD85) mice were either euthanized (for tissue collection) or evaluated on the EPM or LDB tests.

Results: Juvenile FLX history resulted in a persistent anxiogenic-like profile, along with decreases in BDNF-signaling markers, but not 5-HTTs or TrkB receptors, within both brain regions. Interestingly, FLX re-exposure in adulthood reversed the enduring FLX-induced anxiety-related responses across all behavioral tasks, while restoring ERK2-CREB-proBDNF markers to control levels and increasing mBDNF within the prefrontal cortex, but not the hippocampus.

Conclusions: Collectively, the results indicate that adolescent FLX history mediates neurobehavioral adaptations that endure into adulthood, which are indicative of a generalized anxiogenic-like phenotype, and that this persistent effect is ameliorated by later-life FLX re-exposure, in a prefrontal cortex-specific manner.

Keywords: Fluoxetine, Hippocampus, Prefrontal Cortex, ERK, BDNF

Disclosure: Nothing to disclose.

M91. Left Dorsolateral Prefrontal Cortex Repetitive Transcranial Magnetic Stimulation is Associated With Treatment-Related Decreases in Depression Symptoms

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Background: Emerging data suggest that directly observed correlates of autonomic nervous system hyperarousal may have biomarker utility in the assessment of central nervous system hyperarousal, and right-sided prefrontal rTMS has shown promise in treatment-resistant depression with anxiety or trauma-related symptoms. The current study aims to identify associations between depression improvement with and physiologic correlates of autonomic activity, including markers of heart rate variability (HRV) and galvanic skin resistance (GSR), in a cohort of patients receiving left dorsolateral prefrontal cortex (dlPFC) intermittent theta-burst (iTBS) repetitive transcranial magnetic stimulation (rTMS) for treatment-resistant depression.

Methods: Depressive symptoms were assessed and tracked with items from the Quick Inventory of Depressive Symptoms-Self-Report (QIDS-SR). Physiologic measurements were done on-line at rest and during iTBS. Statistical (root mean square of successive differences, RMSSD) and frequency spectral (very low frequency (VLF) 0.003 - 0.04Hz, low frequency (LF) 0.04 - 0.15Hz, high frequency (HF) 0.15 - 0.4Hz) components of HRV were determined.

Physiologic and psychometric responses to left dlPFC intermittent theta-burst (iTBS) stimulation were assessed over 10 consecutive treatments in two weeks. The slope of the line describing the rate of change in QIDS-SR over time was calculated and associations between the rate of QIDS change over 10 treatments and autonomic responses to iTBS during the third treatment were examined with linear regression. Patients (n = 3) that did not respond to left dlPFC iTBS were switched into a protocol with right dlPFC 1Hz stimulation.

Results: There was a highly correlated non-significant association (r2=0.82, F=8.99) between the within-individual increases in GSR during iTBS (1.86, 6.38, 1.98 μS) and the within-individual rate of change in QIDS-SR per treatment (-1.5, -1.7, -0.6), respectively. Similarly, there was a highly correlated but non-significant association (r2=0.91, F=20.01) between the change in HRV-RMSSD during iTBS (8.8, 13.62, -0.07) and the rate of change in QIDS-SR per treatment (-1.5, -1.7, -0.6), respectively. Power calculations show that for a regression model of expected effect size r2=0.8=f2=4, two and four more subjects are needed if the model contains one or two predictors, respectively.

Conclusions: Two different autonomic nervous system responses to a session of left dlPFC iTBS early in a course of 10 treatments were highly correlated. It may be the case that the pattern of sympathetic and parasympathetic nervous system responses to left dlPFC iTBS predict larger decreases in symptoms after a treatment course. In this case, increased parasympathetic input to the heart and increased sympathetic input to the skin predicted a more robust response, but these preliminary results lack the statistical power to be confident that this relationship did not occur by chance.

Keywords: Brain Based Markers for Depression, Repetitive Transcranial Magnetic Stimulation, Physiologic Biomarkers

Disclosure: Nothing to disclose.

M92. Assessment of Tryptophan Catabolism as a Biomarker of Inflammation Related Major Depressive Disorder

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Background: An association between chronic low-grade inflammation and Major Depressive Disorder (MDD) has been well established over decades of research. What is apparent from many studies is that this low-grade inflammation is only present in a subpopulation of MDD patients. There are many hypotheses as to how inflammation may be a causal factor in depressive symptomatology, rather than a mere epiphenomenon. As such, patients with inflammation related MDD may be especially well suited to a treatment that addresses this pathological pathway specifically. A challenge for drug discovery is finding this subgroup of patients for clinical trials, i.e. by using a biomarker that is sufficiently robust and could eventually be used as a companion diagnostic. Being able to match the patient to the treatment would be of great benefit to psychiatric treatment.

The premise of these studies is that tryptophan catabolism may represent the 'final common pathway' of low-grade chronic inflammation, and that it is potentially measurable in plasma. Many of the cytokines that are elevated in MDD patients are inducers of indoleamine 2,3-dioxygenase (IDO1) enzyme leading to elevated tryptophan catabolism via the kynurenine pathway. In this sense, the exact cytokine milieu may be irrelevant since numerous cytokine pathways lead to IDO1 induction. Given that downstream metabolites of this pathway are highly neuroactive, this may be the reason for altered neuronal function under chronic low-grade inflammation.

Methods: Animal studies were approved by the Regierungspraesidium Tuebingen in Germany. LPS was injected i.c.v to mice, and brains/livers were collected 24 hours later. Clinical samples were obtained from the University Clinic Ulm (plasma and CSF from 67 MDD + 47 non-psychiatric controls), Trinity College Dublin (plasma from 37 healthy controls (HC) + 39 first episode MDD + 35 recurrent MDD [REDEEM Cohort]; plasma from 57 HC + 93 MDD [EFFECT-DEP Cohort]), Goethe University Frankfurt (40 HC +40 MDD), The Netherlands Study of Depression and Anxiety (NESDA; plasma from 642 Control, 2288 mixed psychiatric diagnoses), and the Gutenberg Health Study (GHS; plasma from 4093 controls, 349 depression symptoms, 384 depression history). All sample were measured using LC-MS/MS. Animal system: Agilent 1290 Series; API 6500™ AB Sciex using a Atlantis® T3 column (3 μm, 50 × 2.1 mm; Waters). Human System: Kynurenic Acid (KA), Tryptophan (TR) and Quinolinic Acid (QA) were quantified together in one assay, while Kynurenine (KYN) is quantified separately. Both assays comprise sample clean-up by protein precipitation followed by reversed-phase chromatography and mass spectrometric detection in the positive ion multiple reaction monitoring (MRM) mode using the deuterated analogues of the analytes, namely [D5]tryptophan, [D3]3-hydroxykynurenine, [D5]kynurenic acid, [D4]kynurenine and [D3]quinolinic acid as internal standards. Plasma samples were prepared by addition of ice-cold methanol for protein precipitation. Brain samples were lipid depleted by hexane extraction.

Results: In the preclinical study, ICV LPS treatment induced a robust increase in KYN in brain (~90-fold), CSF (2-fold), and plasma (~3-fold). Taqman analysis indicated that only rna transcript levels for IDO1 increased (not IDO2 nor TDO), and was likely responsible for the increase in tryptophan catabolism. The samples from the University Clinic W2SVEVE/';.32reUlm were used to assess CSF tryptophan catabolism with plasma. In healthy controls only the downstream metabolites, KA and QA were correlated between plasma and CSF (KA: r = 0.37, p = 0.0187; QA r = 0.47, p = 0.0012). In MDD patients further correlations were found (QA r = 0.51, p = <0.0001; KYN/TR r = 0.41, p = 0.0037; QA/KA r = 0.51, p = <0.0001, QA/KYN r = 0.36, p = 0.0134). Individually and combined, the samples from 3 University clinics (Goethe, Trinity, Ulm) demonstrated a decrease in plasma KA and an increase in QA/KA compared to controls. The reduction in KA was also found in both the NESDA and GHS cohorts, and GHS also demonstrated an increase in the QA/KA. However, multiple factors were also identified that influence tryptophan catabolism (e.g., BMI, gender, smoking status). The NESDA study identified some correlation of tryptophan catabolism with inflammatory markers in 1100 subjects with current MDD ((log)TNFa correlated with KYN r = 0.17; KYN/TR r = 0.2; QA r = 0.24; QA/KA r = 0.17: (log)CRP correlated with KYN r = 0.15; QA r = 0.2; QA/KYN r = 0.18; KYN/TR r = 0.16). The depressed subjects within the GHS cohort had higher CRP levels, and higher indications of inflammatory and metabolic physiological status (e.g., arthritis, back pain, dyslipidemia).

Conclusions: Tryptophan catabolism induced by inflammation in the brain, may be reflected (albeit weakly) in the CSF and blood as demonstrated by the preclinical study. Assessment of CSF and plasma from human samples indicates the two compartments have comparable changes upon inflammatory challenge, indicating blood could be suitable for tryptophan catabolite measurements. The data from the university cohorts suggested elevated tryptophan catabolites differ between healthy control and MDD subjects, and perhaps the upper quartile could be the basis of patient identification. However, while the large epidemiological

cohorts provide some support for tryptophan catabolism being associated to inflammation-related depression, this relationship is not robust enough for real-world use as a companion diagnostic.

Keywords: Major Depressive Disorder (MDD), Depression Inflammation, Tryptophan catabolites (TRYCAT), Biomarker, Serum Levels

Disclosure: Nothing to disclose.

M93. Transcranial Magnetic Stimulation Neurophysiology of Patients With Major Depressive Disorder: A Systematic Review and Meta-Analysis

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Background: Major depressive disorder (MDD) is a mental illness with high socio-economic burden, but its pathophysiology has not been fully elucidated. Recently, cortical excitatory and inhibitory imbalance hypotheses and neuroplasticity hypotheses have been proposed for MDD. Although several studies have examined the neurophysiological profiles in MDD using transcranial magnetic stimulation (TMS), a meta-analysis of TMS neurophysiology has not been performed. The objective of this study was to compare TMS-electromyogram (TMS-EMG) findings between patients with MDD and healthy controls (HC). We tested whether patients with MDD have lower short-interval cortical inhibition (SICI) which reflects gamma-aminobutyric acid (GABA)A receptor-mediated activity, lower long-interval cortical inhibition (LICI) and cortical silent period (CSP) which represent GABAB receptor-mediated activity, higher intracortical facilitation (ICF) which reflects glutamate N-methyl-D-aspartate (NMDA) receptor-mediated activity, and lower result of paired associative stimulation paradigm (PAS) which shows the level of neuroplasticity in comparison with HC. Further, we explored the effect of clinical and demographic factors that may influence TMS neurophysiological indices.

Methods: We first searched and identified research articles that conducted single- or paired-pulse TMS-EMG on patients with MDD and HC. Subsequently, we extracted the data from the included studies and meta-analyzed the data with the Comprehensive Meta-Analysis Software.

Results: Patients with MDD were associated with lower SICI, lower CSP, higher ICF, and lower PAS compared with HC, while there was no significant difference between the groups for the LICI.

Conclusions: Our results confirmed the proposed hypotheses, suggesting the usefulness of TMS neurophysiology as potential diagnostic markers of MDD.

Keywords: Cortical Excitability, Cortical Inhibition, Excitatory and Inhibitory Imbalance Hypothesis, Major Depressive Disorder, Neuroplasticity Hypothesis

Disclosure: Nothing to disclose.

M94. Chronic Adolescent Stress Sex-Specifically Impairs Cognitive Flexibility and Alters Hippocampal Glutamatergic Activity in Adult Rats

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Background: Chronic stress is a known risk factor for development of psychiatric disease. Females that experience chronic stress during vulnerable developmental stages, such as adolescence, are the most susceptible to stress-induced psychiatric dysfunction. While considerable attention has been devoted to stress-induced manifestations of anxiety, depression, and PTSD, evidence indicates that a history of chronic stress is also a risk factor for cognitive decline and dementia – with females again at increased risk. This interplay between sex and stress history suggests sex-specific mechanisms following stress that can manifest in neural dysfunction across the lifespan. The presence of sex- and stress-steroid receptors in the hippocampus provides a point of influence for these hormones to drive changes in cognitive function. We have previously used an animal model of chronic adolescent stress (CAS) to show long-lasting consequences on affective behaviors and neuroinflammation. Here, we use this model to determine the extent to which CAS results in cognitive dysfunction across multiple domains and the role of glutamatergic signaling in these deficits in males and females.

Methods: Male and female Wistar rats born in-house remained non-stressed (NS) or were exposed to equal instances of physical restraint (60 min) and social defeat (CAS) throughout the adolescent period (PND 35-47). All experiments were conducted in adulthood (PND 80-130). Cognition was assessed in male and female rats ($n = 10$-12) using the Barnes Maze task and the Attention Set-Shift Task. A subset of female rats ($n = 5$) were ovariectomized and assessed for reversal learning to determine the interaction between stress history and sex steroids. Whole hippocampi were extracted from male and female rats ($n = 9$-10) and processed for RNA sequencing. Using the DAVID tool, enriched gene clusters (score > 1.3) were determined based on sex and stress history. Brain tissue ($n = 6$) was processed for density of glutamatergic synaptic markers (GluA1, NMDAr1a, and synaptophysin) or whole-cell patch clamping ($n = 4$) was used to determine glutamatergic activity in the hippocampus.

Results: During acquisition training on the Barnes Maze task, CAS females had shorter latencies to locate the goal box than NS controls ($F(1,141)=6.71$; $p = 0.01$) due to fewer errors committed by CAS females ($F(1,141)=4.01$; $p = 0.047$). In reversal training, CAS females showed a reduced latency to locate the new goal box compared to controls ($F(1,18)=4.9$; $p = 0.04$) as a result of higher error rates ($F(1,54)=5.28$; $p = 0.03$). This reversal deficit persisted across learning dimensions as CAS females required more trials to reach criterion during the reversal phases of the Attention Set-Shift task compared to controls ($F(1,6)=8.31$; $p = 0.03$). Ovariectomy resulted in greater performance variability overall during reversal learning ($F(1,77)=18.13$; $p<0.0001$) with CAS females showing worse performance ($F(1,77)=4.21$; $p = 0.04$). Bioinformatic prediction using gene ontology categorization indicated that in CAS females, postsynaptic membrane gene clusters, specifically genes related to glutamatergic synapse remodeling, were enriched ($ES=2.62$; $p = 3.0E-3$). Structural analysis indicated that CAS females had increased labeling of the presynaptic marker synaptophysin along basal dendrites of the CA1 region of the hippocampus when compared to controls ($t(10)=2.81$; $p = 0.02$). Functional analysis revealed that CAS females had a decreased AMPA/NMDA ratio compared to controls indicating an increase in AMPA dependent current along the Schaffer collaterals in the CA1 region of the hippocampus ($t(14) = 6.62$; $p < 0.001$). There was no effect of CAS on Barnes Maze or Attention Set Shift performance in males ($p > 0.05$). Density of NMDA1a labeling was increased along apical ($t(9) = 2.54$; $p = 0.03$) and basal ($t(8) = 2.32$; $p = 0.05$) dendritic branches of the CA3 region of the hippocampus in CAS males compared to NS controls.

Conclusions: The data observed here suggest that females are at risk for impaired cognitive flexibility following a history of stress. This cognitive deficit appears to be driven by a change in glutamatergic signaling. As both NS and CAS females displayed impairments in reversal learning following ovariectomy, with

these deficits exacerbated following CAS, it is likely that sex steroid hormones contribute to these alterations. While males displayed limited CAS-induced changes in the domains assessed here, previous work has indicated increased vulnerability to stress-induced inflammation and a dysregulated stress response. Taken together, these data indicate sex-specific consequences of chronic adolescent stress that reveal increased risk in females for development of psychiatric dysfunction and cognitive decline that are likely mediated by the impact of sex steroids on synaptic function.

Keywords: Chronic Stress, Sex Differences, Cognitive Decline, Glutamate Receptor Function

Disclosure: Nothing to disclose.

M95. Impairment of Cognitive Function Induced by Overexpression of Shati-Nat8l in the Prefrontal Cortex in Mice

Abstract not included.

M96. A Simultaneous [11C]Raclopride Positron Emission Tomography and Functional Magnetic Resonance Imaging Investigation of Striatal Dopamine Binding in Autism

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Background: The social motivation theory of autism posits that social communication symptoms in autism spectrum disorder (ASD) reflect decreased motivation to engage in reciprocal social behaviors throughout development, resulting in fewer experiences with social rewards (Chevalier et al., 2012). This framework posits that impaired mesolimbic dopamine (DA) function underlies compromised responses to social rewards in ASD, however, no study to date has investigated striatal dopamine function in response to rewards in ASD. The purpose of this study was to use simultaneous PET-MR imaging to evaluate striatal dopaminergic functioning during incentive processing in ASD, using the D2/D3 dopamine receptor antagonist [11C]raclopride. We hypothesized that the ASD group would be characterized by decreased striatal phasic DA release, indexed by the non-displaceable binding potential (BPND) of [11C]raclopride, in response to incentives. Compared to controls, we also hypothesized that the ASD group would exhibit altered functional connectivity, assessed by fMRI, between striatal seed regions that showed reduced phasic DA release and their functional targets.

Methods: Participants (n = 12 Controls, n = 10 ASD) underwent a simultaneous PET-MR scan session on a Siemens Biograph mMR scanner at the UNC Biomedical Research Imaging Center. A bolus +infusion [11C]raclopride administration protocol was used to measure background dopaminergic tone and phasic dopaminergic release, via displacement of the tracer, in response to incentives. The scan began with two resting-state fMRI runs to allow for tracer uptake. Next, participants completed a monetary incentive delay task (MID), modified for use in PET-MR studies. This modified version of the MID was developed at McLean Hospital (by DD and DAP). The incentive task included a neutral run followed by two reward runs. During the two reward runs, concatenated into one reward block for PET analyses, money could be won; during the neutral run, money could not be won. Binding potential, the ratio of selectively bound ligand to nondisplaceable ligand in the tissue at equilibrium, is thought to be negatively correlated with endogenous dopamine. [11C] Raclopride non-displaceable binding potential (BPND) was estimated voxel-wise and compared between groups between the neutral and reward runs in the striatum. Using striatal PET-derived seed regions that showed group differences in BPND in the reward relative to the neutral run, we examined group differences in functional connectivity using a general functional connectivity (GFC) approach. GFC, which aggregates resting-state and task fMRI data, offers better test-retest reliability and higher estimates of heritability than intrinsic connectivity estimates from solely resting state data of equal duration (Elliott et al., 2019). Voxel-wise whole-brain GFC was evaluated via seed-to-voxel analyses in the CONN functional connectivity toolbox.

Results: Three striatal clusters, the right and left putamen and left caudate nucleus/left putamen, demonstrated ASD>Control group differences for the contrast of (reward>neutral) BPND values, putatively reflecting decreased phasic DA release (increased BPND) in the reward relative to the neutral condition in the ASD group relative to the control group. Of these three PET-derived clusters, only one seed region, the right putamen, showed group differences in GFC. Compared to the control group, the ASD group showed significantly greater connectivity between the right putamen seed and two clusters in the precuneus and right insular cortex.

Conclusions: Using [11C]raclopride in conjunction with a reward processing task, we report evidence consistent with impaired striatal phasic DA release to rewards in ASD. We also found increased connectivity between the right putamen seed that demonstrated decreased DA release to incentives in the ASD group and the precuneus and right insular cortex. Increased striatal connectivity with the precuneus during reward processing has been associated with depressive symptom severity in anhedonic patients with major depressive disorder (MDD), and the current findings indicate a possible shared feature of ASD and MDD. Findings of increased striatal connectivity with the insular cortex, a hub for regulating large-scale brain network dynamics, align with previous literature that reports insula dysfunction in ASD. Collectively, these findings highlight a molecular mechanism that may address, in part, the pathogenesis of impaired functional brain networks in ASD and provide support for the social motivation hypothesis of ASD.

Keywords: Simultaneous PET-MR, Autism, Functional Connectivity, Raclopride, Social Motivation

Disclosure: Nothing to disclose.

M97. A Gene Associated With Herpes Zoster or Post-Herpetic Neuralgia Affects Varicella-Zoster Virus Infection or Replication

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Background: Post-herpetic neuralgia (PHN) is neuropathic pain caused by reactivation of latent varicella-zoster virus (VZV), i.e. herpes zoster (HZ). Around 20% of HZ patients are affected by PHN. The PHN pain which lasts more than three months after the onset of HZ can significantly impair quality of life. In that sense, it is important to find the way to prevent PHN and clarify the mechanisms underlying the difference between the patients with PHN and without PHN. However, the pathogenesis of PHN and HZ

has not been fully understood so far. By statistical analysis of the genome wide association study (GWAS) data of PHN or HZ affected patients and healthy subjects, we found that single nucleotide polymorphisms (SNPs) of the gene A were significantly related to PHN or HZ. In this regard, we started to investigate how the gene A impacts VZV infection or replication and severe pain. We aim to clarify whether the gene A products relate to the infection or replication of VZV in susceptible cultured cells.

Methods: We compared 96 patients of HZ or 92 patients of PHN with 282 people of healthy subjects by contingency analysis. Since the gene A SNPs were significantly associated with HZ and PHN, we established MeWo cells with or without gene A expression and investigated the difference after VZV infection. The copy number of VZV genome was analyzed by TaqMan real-time PCR.

Results: First, we investigated whether gene A affects VZV infection/replication. We developed human malignant melanoma MeWo cells with or without gene A expression. After infection with VZV, the virus genome copy number was slightly higher in the cells without the gene A than in the cells with the gene A and it was obvious from two days after infection. This result suggests that the gene A product impairs virus infection or replication to some extent. Next, we observed the cells under the microscope. The infected cells with the gene A formed larger syncytia than the infected cells without the gene A. This result suggests that the gene A induces more robust cell fusion during the virus replication. Finally, we observed the plaque phenotype. The plaque of the cells with gene A showed clearer border than that of the cells without gene A. This result implies that the infected cells with gene A may be detached easier or form clearer fusion blocks compared to the infected cells without the gene A.

Conclusions: Together with these results, the gene A would impair virus replication by enhancing cell fusion. A former report on measles virus indicated that a virus strain with robust fusion ability impair viral replication, in spite of its higher neurovirulence (Watanabe S et al., 2015, J Virol). Accordingly, the gene A might relate to higher neurovirulence of VZV replication in sensory neurons in vivo. This higher neurovirulence would lead to long-lasting pain and PHN.

Reference:

Watanabe S, et al., (2015) Measles virus mutants possessing the fusion protein with enhanced fusion activity spread effectively in neuronal cells without causing strong cytopathology, but not in other cells. J Virol 89(5):2710-2717.

Keywords: Neuropathic Pain, Herpesvirus, Human Genetics

Disclosure: Nothing to disclose.

M98. Quetiapine Ameliorated Psychotic-Like Hyperactivity in Dopamine-Deficient Mice Partially via 5-HT1A Receptor

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Background: Parkinson's disease (PD) is a neurodegenerative disorder. The primary mechanism is the degeneration of dopamine (DA) neurons in the substantia nigra. The main symptoms of PD are motor symptoms. However, more than half of PD patients experience psychosis, including hallucinations and delusions, in the later stages of the disease. Although the mechanism of PD psychosis is unclear, progressive loss of DA and environmental change could be one of the triggers of PD psychosis. Clozapine (multi acting receptor targeted antipsychotics (MARTA)), donepezil (cholinesterase inhibitor), pimavanserine (5-HT2A/2C inverse agonist) and quetiapine (MARTA) are known to be effective in PD psychosis. Dopamine-deficient (DD) mice are knockout mice that are unable to synthesize dopamine (DA) due to lack of tyrosine hydroxylase (TH) gene. To prevent loss of noradrenaline and adrenaline, TH expression is rescued under control of dopamine beta hydroxylase (DBH) promoter. DD mice can move normally under L-3, 4-dihydroxyphenylalanine (L-DOPA) treatment, but show bradykinesia when DA in the brain is decreased as seen in PD. We previously found that DD mice showed hyperactivity by a novel environment exposure when DA in the brain was almost completely depleted. Such hyperactivity was ameliorated by clozapine and donepezil. Locomotor hyperactivity in mice can be regarded as psychotic-like behavior. Therefore, we can hypothesize that the hyperactivity of DD mice may reflect some aspects of PD psychosis. Indeed, it is conceivable that DD mice meet validation criteria of animal models for PD psychosis. First, PD psychosis and DD mice show psychotic-like behavior (face validity). Second, psychotic-like behavior appears during dopamine deficiency (construct validity). Third, both PD psychosis and hyperactivity in DD mice can be treated with clozapine, donepezil (predictive validity). In the present study, we investigated the effects of drugs those used to treat PD psychosis on hyperactive DD mice as a putative model of PD psychosis. We especially focused on serotonergic drugs to understand the relationship between psychotic-like behavior and the serotonin system.

Methods: DD mice were maintained on a paste diet containing L-dopa. DD mice were received intraperitoneal L-dopa injection (50 mg/kg) and then L-dopa was removed from a diet 72 hours prior to experiment to deplete DA in the brain. Then, we conducted an "Open-Field-Test (OFT)" in which mice were placed in a novel environment (35 × 40 × 25 cm) and their locomotion was monitored using supermex apparatus. Saline, tandspirone (5HT1A agonist; 3, 6mg/kg), paroxetine (selective serotonin reuptake inhibitor; 8mg/kg), pimavanserin (5-HT2A/2C inverse agonist; 5mg/kg), and quetiapine (20mg/kg) were injected into DD and WT mice after 3 hours of exposure to the novel environment. For each mouse, locomotion in the novel environment was monitored for an additional 3 hours. Experiments were conducted using 15 DD mice and 16 WT mice.

Results: DD mice maintained hyperlocomotion after saline injection. Tandospirone, paroxetine and pimavanserin inhibited locomotor activity in WT mice. However, DD mice remained hyperactive during OFT after administration of these drugs, suggesting that these drugs were not effective in reducing hyperactivity in DD mice. In contrast, quetiapine ameliorated locomotion in both DD and WT mice. Because quetiapine acts on multiple receptors, we next used receptor-specific agonists and antagonists to determine which receptors mediate the effects of quetiapine. 8-OH-DPAT (5-HT1A agonist) but not EMD 281014 (5-HT2A antagonist) ameliorated hyperactivity in DD mice. To confirm whether 5-HT1A receptor mediated the effect of quetiapine, mice were received WAY100635 (5-HT1A antagonist) injection 30 minutes prior to quetiapine administration and investigated locomotor activity. WAY100635 partially blockade the hyperactive inhibitory effect of Quetiapine.

Conclusions: Hyperactivity in DD mice was ameliorated by Quetiapine and 8-OH-DPAT administration. Since effect of Quetiapine was partially blocked by WAY100635, Quetiapine might ameliorate hyperactivity in DD mice via 5-HT1A receptor.

Keywords: Quetiapine, Parkinson's Disease, Psychotic-Like Experiences, Dopamine-Deficient Mice, 5-HT1A Receptors

Disclosure: Nothing to disclose.

M99. Central Amygdala Inflammation Drives Pain Hypersensitivity and Impaired Opioid Signaling in a Sex-Specific Manner in an Animal Model of Multiple Sclerosis

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Background: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that is characterized by chronic inflammation and demyelinating lesions within the CNS. Chronic pain is a highly prevalent symptom associated with MS. Unfortunately, classical analgesics provide inadequate relief for MS-related pain, contributing to overall disease burden and reduced quality of life. Despite the high incidence of chronic pain in MS, the underlying mechanisms remain poorly understood. The amygdala is a small nucleus within the limbic brain that plays a critical role in pain processing and regulation. Chronic pain alters amygdala processing of noxious stimuli and mu-opioid function in this brain region. These changes contribute to pathological pain hypersensitivity and unpleasantness. Microglia, the resident macrophages of the brain, drive the transition from acute to chronic pain. Chronic pain has been shown to induce microglial activation within the amygdala, and local ablation of microglia improves pain hypersensitivity. Here, we describe how activated microglia within the central nucleus of the amygdala (CeA) disrupt nociceptive sensory processing and contribute to pain hypersensitivity in experimental autoimmune encephalomyelitis (EAE), the most frequently used animal model of MS.

Methods: We employed the myelin oligodendrocyte glycoprotein (MOG 35-55)-induced EAE model in male and female C57BL/6 mice. Baseline thermal pain thresholds and analgesic responses to escalating doses of morphine (0.1mg/kg, 3mg/kg, 10mg/kg, 30mg/kg, i.p.) were assessed in the tail withdrawal assay (49oC water) prior to the first sign of clinical onset (flaccid tail or >10% reduction in body weight). The reinforcing properties of morphine (0.5mg/kg, 10mg/kg, i.p.) were assessed with the conditioned place preference (CPP) paradigm. At the onset of clinical symptoms, EAE and control animals were euthanized, or injected with morphine (5mg/kg i.p.) or saline (0.9% NaCl, i.p.) one hour prior to euthanasia. Microglial activation and morphine-evoked neuronal activity within the CeA were assessed using immunocytochemical markers Iba-1 and c-FOS, respectively. To examine the contribution of activated microglia within the CeA to pain behaviours, naïve male and female C57BL/6 mice received a bilateral stereotactic injection of vehicle (saline; 100nL per side) or lipopolysaccharide (LPS; 1µg dissolved in 100nL saline per side) into the CeA (coordinates from bregma: AP -1.06, ML ± 2.25, DV -4.50) to induce focal inflammation. Animals were allowed to recover for 72 hours following surgery, at which point thermal pain hypersensitivity and morphine antinociception were assessed in the tail withdrawal assay.

Results: Prior to the onset of clinical symptoms, male and female mice with EAE displayed reduced thermal nociceptive thresholds compared to controls ($p<0.05$, 2-way ANOVA, $n = 9$-20). Morphine injection produced a similar degree of analgesia in both male control and EAE mice in the thermal tail withdrawal assay ($p>0.05$, 2-way ANOVA, $n = 5$), whereas morphine analgesia was diminished in female mice with EAE ($p<0.0001$, 2-way ANOVA, $n = 5$). Systemic morphine administration induced robust place preference in control animals but failed to elicit a place preference in EAE animals of either sex ($p<0.05$, 2-way ANOVA, $n = 8$-10). Based on Iba-1 immunolabeling, microglial density ($p<0.05$, unpaired t-test, $n = 8$-16) and activation ($p<0.05$, unpaired t-test, $n = 8$-20) in the CeA were greater in EAE mice at onset of the disease compared to controls. Following morphine injection, control animals exhibited a marked increase in c-FOS+ cells within the CeA in comparison to basal expression levels ($p<0.0001$, 2-way ANOVA, $n = 8$-10). Morphine administration did not evoke an increase in CeA c-FOS expression in male or female mice with EAE ($p<0.9999$, 2-way ANOVA, $n = 9$-16). Intra-CeA injection of LPS induced profound focal microglial activation in both sexes, as evidenced by enlarged cell body size ($p<0.0001$, unpaired t-test, $n = 14$-15) and increased Iba-1 staining density surrounding the injection site compared to saline-treated controls ($p<0.001$, unpaired t-test, $n = 14$-15). Strikingly, intra-CeA LPS-injection led to blunted morphine analgesia in the thermal tail withdrawal assay in female mice ($p<0.05$, 2-way ANOVA, $n = 7$-8), but did not alter morphine analgesia in males ($p>0.05$, 2-way ANOVA, $n = 7$-8).

Conclusions: We demonstrate that the analgesic efficacy of morphine on thermal nociceptive thresholds in both EAE and LPS-treated mice is sex-dependent, with females exhibiting a considerably blunted analgesic response. Our data suggest that activated microglia within the central nucleus of the amygdala contribute to the sexually dimorphic effects of morphine and might drive neuronal adaptations that lead to pain hypersensitivity in EAE. Our results provide insight into why opioids are less effective in treating MS-related pain and suggest that inhibiting microglial activation might be a viable target to improve analgesic efficacy for this patient population.

Keywords: Chronic Pain, Neuroinflammation, Opioid, Pain Analgesia

Disclosure: Nothing to disclose.

M100. Prefontal-Lateral Hypothalamic Neurons Encode Social Dominance in Mice

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Background: Both humans and mice live in groups organized by social hierarchies. By adjusting behavior based on their social rank, animals decrease unnecessary aggression and save energy. Although hierarchies are central to successful group dynamics, the neural basis of dominance behaviors remains poorly understood. Cross-species evidence suggests that the medial prefrontal cortex (mPFC) is crucial for social dominance behaviors (Wang et al., 2011, Zhou et al., 2017, Zink et al., 2008). However, it remains unknown if mPFC encodes social rank and which subpopulations of mPFC play a role in social dominance. Given the role of the lateral hypothalamus (LH) in modulating reward and social interactions, and its connectivity with the mPFC, it is well-positioned to help modulate social behaviors in a rank-dependent manner.

Methods: Considering that dominant animals typically have priority access to resources, we designed a novel behavioral task: the "reward competition assay". This assay utilizes a trial structure to facilitate statistical comparisons wherein pairs of mice compete for a reward signaled by a tone. After male mice learned to associate an Ensure reward with the tone, they were paired with a cage mate to compete for the rewards. To validate this task, we first ranked mice using the tube test (Wang et al., 2011) to establish a social hierarchy in the cage. Across the session, mice that were dominant in the tube test won more rewards than subordinates (paired t-test; % rewards subordinate vs dominant $p<0.01$; $n = 12$). During this novel reward competition assay we recorded from 998 mPFC single units and used supervised machine learning to determine if social rank was encoded in mPFC. We then used optogenetics to photo-identify and manipulate mPFC-LH projectors during social competition. In addition, to analyze behavior we developed a Deep Learning tool, AlphaTracker, that allows to track multiple identical mice.

Results: We analyzed individual cell responses to the tone for winning and losing trials and to reward port entries done by the self and the competitor (other animal) during the tone and in the intertrial intervals (ITI). We found that Dominant mice had

stronger inhibition to winning (Wilcoxon rank-sum, p = 0.0094) and more cells that were responsive to port entries of self and other (Fisher test for self p = 0.01; Fisher test for other p = 0.012), while subordinate mice had stronger responses to port entries done by the competitor during the tone (Wilcoxon rank-sum, p = 0.0013). Overall, individual mPFC cells showed patterns of activity that differed with rank. To explore rank differences at the population level, we analyzed mPFC population dynamics in a lower dimension state-space using principle component analysis. Winning and losing neural trajectories (population firing rates in the PC space) were highly separated in the neural state-space even before the cue onset. In addition, the length of the neural trajectories for winning and losing trials was higher for subordinates compared to dominants, suggesting faster changes in the mPFC population dynamics for subordinates (2way ANOVA main effect of rank F(1,50)=399 p = 1.6x10-25, trial type F(1,50)=99 p = 1.7x10-13 and interaction F(1,50)=54 p = 1.5x10-9). These large rank differences in population dynamics suggest that social rank is encoded at the population level in mPFC, and we confirmed this by showing that a support vector machine classifier was able to decode social rank using data from a single trial. Next, to identify potential mPFC circuits involved in social rank encoding, we used phototagging to record two subpopulations of mPFC cells (mPFC->LH and mPFC->BLA). Removing mPFC->LH cells, but not mPFC->BLA cells, from the population data decreased the accuracy of social rank decoding (mPFC->LH p = 0.004; mPFC->BLA p = 0.091). This result suggested that mPFC->LH cells are part of the mPFC circuit that encodes social rank. To directly test the hypothesis that mPFC-LH neurons modulate social dominance, we injected a cohort of mice with either channelrhodopsin (ChR2) or a fluorophore (eYFP) in mPFC-LH neurons and implanted an optic fiber in the mPFC. After mice learned the reward association, mice performed the reward competition two days in a row: the first day with no light (as normal) and on the second day the relative subordinate mouse received optogenetic manipulation (5 min epochs of 4 5 ms pulses at 100 Hz every 200 ms). The mPFC-LH stimulation increased trials won (chR2 n = 9, eYFP n = 6; 2way RM ANOVA interaction of virus and light F(1, 14) = 5.82 p = 0.03; Bonferroni corrected t-test ChR2 p = 0.01) and increased the proportion of time in the reward port (ChR2 n = 9, eYFP n = 6; 2way RM ANOVA interaction of virus and light F(1, 14) = 6.73 p = 0.02; Bonferroni corrected t-test ChR2 p = 0.02), but did not affect general effort as measured in the effort based T-maze (ChR2 n = 8, eYFP n = 9; 2way RM ANOVA no significant effect of light, virus or interaction).

Conclusions: Altogether we present a novel behavioral paradigm and tool to study social dominance behaviors. We demonstrated that mPFC encodes social rank and competitive success at the population level during social competition. Finally, we identified the mPFC-LH circuit as a pathway that contributes to the social rank encoding and modulates social dominance behaviors.

Keywords: Machine Learning, Optogenetics, Social Behavior, Circuit-function, mPFC

Disclosure: Nothing to disclose.

M101. OCD Candidate Gene SLC1A1/EAAT3 Impacts Dopamine Neuron Firing and Behavioral Flexibility

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Background: Obsessive compulsive disorder (OCD) is a severe condition that affects 2-3% of the population. Genetic findings have pointed to SLC1A1, encoding the neuronal glutamate transporter EAAT3/EAAC1. Localized to peri-synaptic regions, EAAT3 modulates glutamate concentrations around glutamate receptors. In dopamine (DA) neurons, amphetamine (AMPH) triggers EAAT3 internalization. We previously reported that a loss of Slc1a1 in mice, based on an insertion of an excisable STOP cassette, leads to 1) decreased dorsal striatum DA levels, 2) diminished AMPH-induced locomotion and stereotypic behavior, and 3) attenuated SKF-38393 (D1-agonist)-induced grooming behavior. Viral rescue of midbrain Slc1a1 expression partially restored the behavioral response to AMPH. Here, in order to examine more directly the impact of Slc1a1 loss on DA neuron activity, we used in vivo single-unit recordings in Slc1a1-STOP mice to assess DA neuron spike firing patterns, at baseline and in response to AMPH. Based on diminished baseline and AMPH-induced DA release, we hypothesized diminished baseline spike firing and a diminished modulation by AMPH. Second, we used a novel operant counting-based task that separates rigid/inflexible behavior from reward learning and motivation to examine behavioral flexibility in STOP mice. Finally, to test more directly the developmental impact of DA neuron EAAT3 overexpression on compulsive-like behaviors, we generated mice with DA neuron-specific overexpression of EAAT3.

Methods: Adult (male and female, 8 weeks old) mice were used for all studies. Single unit recordings: Adult STOP and littermate control mice (n = 8/group) were used for in vivo recordings in anesthetized mice. Using glass electrodes (impedance 4-10 MΩ), DA neurons were sampled in six recording tracks within the midbrain (AP -3.0, ML 0.8, and DV 3.5 mm). During the recordings, mice received i.p. injections of 3.0 mg/kg AMPH. Counting-based operant task: Food-restricted STOP mice and WT littermates (n = 10/group) were trained on a response chain with a requirement of 20 presses on the 'count' lever, following which 1 press on the 'reward' lever would produce a milk reward. Micro-analysis of response structure was performed to examine differences in Inter-Response Times (IRT) and 'burst' responses. Overexpressor mice: We used the Flexible Accelerated STOP Tetracycline Operator-knockin (FAST) system to generate tTA-mediated overexpression of EAAT3 in DA neurons using a tyrosine hydroxylase promoter driver (TH-tTA) mouse line. Doxycline-supplemented chow was used to regulate tetO-driven EAAT3 expression. Mice were evaluated for AMPH-induced locomotion and for AMPH and SKF-38393-induced preservative behaviors (n = 10-11).

Results: In vivo recordings: A 2-way repeated-measures (RM) ANOVA did not show a significant interaction between genotype and midbrain subregion in baseline measures. However, STOP mice showed reduced variability in inter-spike interval (ISI) [2-way RM ANOVA; genotype x region, F(2,41) =0.057, p = 0.9447; genotype F(1,41)=4.508, p = 0.0398, n = 8/group] and diminished AMPH-induced modulation of DA spike firing [3 mg/kg, non-linear regression, p<0.001]. Counting-based operant task: EAAT3 ablation did not affect performance on continuous reinforcement, progressive ratio or extinction schedules [2-way RM ANOVA; time x genotype, p>0.1, n = 10/group]. Micro-analysis of response structure revealed that STOP mice showed reduced variability in Inter-Response Time (IRT) [Quartile-Coefficient of Variation: unpaired t test, t=2.183, p = 0.0425], and diminished burst responses [2-way RM ANOVA; short IRT-duration x genotype, F(2, 36) =3.478, p = 0.0416; genotype F(1,18)=8.417, p<0.01] in the counting task. Overexpressor mice: TH-tTA+//tetO-Slc1a1 mice displayed increased AMPH-induced locomotion [3.0 mg/kg: third order polynomial, least squares regression analysis, F (4, 370) = 16.33, P < 0.0001, n = 10-11], and stereotypic behavior [8.0 mg/kg: 2-way RM ANOVA; genotype F (1, 19) = 7.088, p = 0.0154]. No significant effect on SKF-38393-induced grooming behavior was observed. Normalizing DA neuron EAAT3 expression in the same mice following 4 weeks of doxycline treatment diminished the

genotypic differences in AMPH-induced locomotion [3.0 mg/kg: third order polynomial, least squares regression analysis, F (4, 370) = 2.105, P = 0.0796] and stereotypic response [8.0 mg/kg: 2-way RM ANOVA; genotype F (1, 19) = 0.02466, P = 0.8769].

Conclusions: Our findings indicate that changes in EAAT3 expression can alter neurotransmission in DA neurons and modulates behavioral rigidity and compulsive-like behavior. Specifically, we found that EAAT3 ablation diminishes phasic DA transmission, suggesting that deficits in phasic DA activity may mediate AMPH-induced compulsive-like stereotypies observed in STOP mice. Further, our operant data uncovered a novel role for EAAT3-mediated DA transmission in behavioral flexibility, independent of motivation for reward. Together with the in vivo recordings, these data suggest that phasic striatal DA transmission may facilitate flexibility in operant behavior. Finally, our overexpressor data suggests that changes in EAAT3 expression can dynamically tune DA transmission to modulate drug-induced compulsive-like behaviors. Taken together, these findings support a key role for SLC1A1 in DA neurons in OCD-relevant behavior.

Keywords: Glutamate Transporter (EAAT3), Basal Ganglia, Dopamine, Obsessive-Compulsive Disorder (OCD), Amphetamine

Disclosure: Nothing to disclose.

M102. Assessing the Role of Direct and Indirect Pathway Projecting Spiny Projection Neurons in Compulsive Behavior

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Background: Compulsive behaviors are hallmark symptoms of Obsessive Compulsive Disorder (OCD), and are often present in other severe neuropsychiatric illnesses. Aberrant striatal activity has been linked to compulsive behavior in both correlative studies in humans and causal studies in rodents. Despite this evidence, it is not currently understood how the major opposing cell-types in the striatum, D1- and D2-spiny projection neurons (SPNs), contribute to striatal hyperactivity to drive compulsive behavior. A prevailing theory suggests that excessive activation of the D1-associated direct pathway or decreased inhibition of the D2-associated indirect pathway may result in compulsive behavior, though little direct experimental evidence exists to support this idea. Using head-mounted miniature microscopes for in vivo calcium imaging (Inscopix), we sought to determine the role of D1- and D2-SPNs in mediating compulsive behavior in mice with a highly penetrant compulsive grooming phenotype (Sapap3-KO mice).

Methods: Cohort 1: Male and female Sapap3 KOs (n = 9) and WT littermate controls (n = 11) were injected with AAV-GCaMP6m and implanted with GRIN lenses in CS to visualize striatal calcium activity during spontaneous grooming behavior in both D1 and D2-SPNs. Cohort 2: D1-cre/Sapap3-KOs (n = 12) and WTs (n = 11) were injected with cre-dependent AAV-GCaMP6m to selectively image D1-SPN activity during spontaneous grooming behavior. Cohort 3: A2A-cre/Sapap3-KOs (n = 7) and WT (n = 8) mice were injected with cre-dependent AAV-GCaMP6m to selectively image D2-SPN activity during spontaneous grooming behavior. Raw calcium signals were segmented using the CNMFe algorithm and converted to a Z-score using the mean and SD for a single cell across the entire session. To determine if cells were activated/inhibited by grooming onset, Ca2+ event rates were shuffled (1000 iterations) to create a null-distribution, and event rate difference >1SD from null distribution was considered significant. For each cell Z-scored calcium activity was averaged across all peri-grooming periods (-3s to 10) and trial averaged activity was clustered using spectral clustering. To compare across genotypes and genetically defined cell types, spectral clustering was performed on all cohorts simultaneously.

Results: For all cohorts, Sapap3-KOs displayed increased grooming time ($p < 0.01$) and increased grooming bouts ($p<.05$) compared to WT mice. Spectral clustering revealed 8 distinct grooming-onset associated functional clusters across all cohorts. Cohort 1: Assessing all SPN subtypes together, Sapap3-KO mice displayed increased grooming onset-associated calcium activity relative to WTs ($p < .01$). This increase was associated with an increase in the percentage of individual SPNs activated at the onset of grooming in KO mice ($p < .001$). Spectral clustering identified significant increases in the proportion of cells participating in functional clusters associated with the onset of grooming (cluster 2 $p < 05$; cluster 3 $p < .01$) as well as reductions in the proportion of cells found in functional clusters associated with inhibition at grooming onset ($p < .01$). Cohort 2: D1-SPN activity was elevated during grooming ($p < .05$), but there was no elevation was at grooming onset. No increase in grooming onset-activated neurons were detected in D1-SPNs ($p > .05$) Spectral clustering identified a reduction in an increase in proportion of SPNs participating in a functional cluster that slowly increases its activity during grooming ($p < .05$) and a reduction in the proportion of SPNs found in a cluster with late-onset activation. Cohort 3: Activity of D2-SPNs were elevated immediately at the onset of grooming in a similar fashion as in Cohort 1 ($p < .05$). A significant increase in the percentage D2-SPNs activated at grooming onset in KOs compared to WTs was identified ($p < .05$). Spectral clustering revealed increases in the proportion of D2-SPNs from KO mice participating in functional clusters associated with grooming onset (cluster 3 $p < .01$; cluster 4 $p < .05$) as well as a reduction in the proportion of grooming-onset inhibited cells ($p < .001$).

Conclusions: Using in vivo microscopy in freely moving Sapap3-KO and WT mice, we demonstrate that KO mice have increased grooming-associated striatal activity manifested through alterations in the makeup of functional clusters of striatal SPNs. Surprisingly, D1-SPN activity was not increased at grooming onset but rather was increased later on during grooming, which was reflected in no significant changes in the proportion of functional clusters associated with grooming onset, but with alterations in clusters whose activity occurred during the grooming bout. Unexpectedly, D2-SPN activity was increased in KOs at the onset of grooming and associated with increases in grooming-onset associated functional clusters and reduction in grooming inhibited functional clusters. These data point to a novel model in which D2-SPN hyperactivity may promote compulsive grooming behavior. Ongoing work is testing how activity of these specific cell types is modulated by SSRIs, whether inhibition of D2-SPNs is sufficient to prevent or reduce compulsive behavior, and if compulsive behavior can be decoded by the activity of specific functional clusters of striatal SPNs.

Keywords: Striatum, Obsessive Compulsive Disorder, In Vivo Microscopy, Calcium Imaging

Disclosure: Nothing to disclose.

M103. Effects of Visual Attention Modulation on Dynamic Effective Connectivity and Visual Fixation During Own-Face Viewing in Body Dysmorphic Disorder

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Background: Body dysmorphic disorder (BDD) is marked by preoccupation with misperceived appearance flaws, which they

believe render them ugly and disfigured. The consequences are profound, with a high prevalence of suicide attempts and hospitalization. In contrast to its impact on quality of life, it is understudied, and the neurobiological models to explain vulnerability to BDD are scarce. Further, the study of brain functional or effective connectivity in BDD has been even less explored. This is the first study to employ dynamic effective connectivity (DEC) analysis to explore the effects of visual attention modulation during own-face viewing (i.e. modulated viewing, ModV) compared to unconstrained "natural" viewing (NatV) in BDD and healthy controls (HC).

Methods: 37 adults with BDD and 30 HC aged 18-40 were included in this study. The BDD participants met DSM-5 criteria for BDD with face concerns. Symptom severity was quantified with Yale-Brown Obsessive-Compulsive Scale Modified for BDD (BDD-YBOCS), and Brown Assessment of Beliefs Scale (BABS), assessing BDD symptoms and insight, respectively.

There were two sets of stimuli for the NatV condition: photos of the participant's own face and scrambled face, and two sets of stimuli for the ModV condition: the same photos with a semi-transparent crosshair between the eyes, and the same scrambled face with a crosshair. The fMRI data, with eye-tracking monitoring, were acquired while the participants underwent the two conditions. During NatV, they viewed the faces without restrictions. During ModV, they viewed the same images while maintaining attention on a central crosshair. The rationale was that fixating visual gaze on the crosshair would enhance global visual processing and reduce local processing. They performed 3 fMRI runs and were randomly assigned to one of the two groups: NatV-NatV-ModV (NNM) or NatV-ModV-NatV (NMN).

Data preprocessing was done using fMRIPrep 1.4.0. Fourteen ROIs in dorsal and ventral visual streams were selected: 2 ROIs in primary visual cortex (V1) [bilateral calcarine], 6 ROIs in ventral visual stream (VVS) [bilateral inferior occipital gyrus (IOG), fusiform gyrus (FG), and inferior temporal gyrus (ITG)], and 6 ROIs in dorsal visual stream (DVS) [bilateral superior occipital gyrus (SOG), inferior parietal gyrus (IPG), and superior parietal gyrus (SPG)]. Hemodynamic deconvolution was then performed on the time-series extracted from these ROIs to minimize intra-subject HRF variability, and to improve estimation of effective connectivity. DEC, a dynamic measure of directional connectivity between pairs of ROIs, was computed at each time point using Kalman-filter based time-varying Granger causality. 12 intra-hemispheric connections were chosen and divided into 4 categories: 1) VVS Lower (Calcarine to IOG), 2) VVS Higher (IOG to FG; IOG to ITG), 3) DVS Lower (Calcarine to SOG), and 4) DVS Higher (SOG to IPG; SOG to SPG). Linear mixed model was used to analyze the data (fixed factors: group [BDD or HC], order [NNM or NMN], run [1st or 2nd or 3rd run], level [Lower or Higher]; random factor: participant). Pearson correlation was used to find the associations between DEC, symptom severity measures of BDD-YBOCS and BABS (in BDD participants), and mean fixation duration from eye-tracking data.

Results: In DVS, BDD and HC exhibited greater DEC for DVS Lower compared to DVS Higher during 1st and 3rd runs. For DVS Lower, the participants with NNM order exhibited greater DEC during 1st NatV compared to 2nd NatV and ModV, while the participants with NMN order showed greater DEC during 2nd NatV compared to 1st NatV and ModV. For DVS Higher, the BDD with NMN order showed greater DEC during ModV and 2nd NatV compared to 1st NatV, while the BDD with NNM order only showed greater DEC during 2nd NatV compared to 1st NatV. However, the HC with NNM order showed greater DEC during 2nd NatV and ModV compared to 1st NatV, while the HC with NMN order only showed greater DEC during ModV compared to 1st NatV.

In VVS, both BDD and HC exhibited greater DEC for VVS Higher compared to VVS Lower across all the 3 runs. For both VVS Lower and VVS Higher, the participants with NNM order showed greater DEC during 1st NatV compared to 2nd NatV. There was no common pattern between BDD and HC with NMN order. All these differences were significant ($p < 0.05$).

From the correlation results, there was a negative correlation between BDD-YBOCS and DEC during 1st NatV for DVS Higher ($p<0.01$), and a positive correlation between BABS and DEC during 2nd NatV for DVS Higher ($p = 0.02$), and negative correlation between BABS and DEC during ModV for VVS Lower ($p = 0.05$). The mean fixation duration was also found to positively correlate with the DEC during 2nd NatV for DVS Lower ($p = 0.04$), and negatively correlate with the DEC during ModV for VVS Lower ($p = 0.03$). Moreover, there were negative trends between BDD-YBOCS and mean fixation duration during 1st and 2nd NatV.

Conclusions: These results provide evidence of greater connectivity in BDD within the DVSLower during attention modulation and when viewing their faces naturalistically afterwards. This suggests that visual attention modulation may facilitate enhanced global and configural visual processing and may have a subsequent carry-over effect when viewing faces naturalistically. The clinical relevance is underscored by the observation that those with more severe BDD symptoms have lower DVS connectivity when viewing their face naturalistically. Visual attention modulation may thus hold promise for future studies of perceptual retraining.

Keywords: Body Dysmorphic Disorder, Visual Processing, fMRI Faces Paradigm, Dynamic Connectivity, fMRI Effective Connectivity

Disclosure: NOCD, LLC, Google, LLC: Consultant (Self)

M104. Influence of Fluoxetine on Perseverative Behavior-Related Activity in Lateral Orbitofrontal Cortex

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Background: In patients with obsessive-compulsive disorder (OCD), abnormal orbitofrontal cortex (OFC) activity normalizes after fluoxetine treatment. However, little is known about how fluoxetine affects encoding of compulsive behaviors in OFC neurons. Here, using the Sapap3-KO mouse as a model for OCD-relevant perseverative behaviors, we measured self-grooming related neural activity with in vivo calcium imaging in lateral OFC (LOFC) before and after fluoxetine treatment.

Methods: Sapap3-KO mice and WT littermates ($n = 8$ KO, 4 female; 7 WT, 3 female) were injected with virus encoding fluorescent calcium indicator (AAV5-hysn-GCaMP6f) and implanted with gradient-index lenses in lateral OFC (LOFC) to visualize neural activity using Inscopix microscopes. Grooming and neural activity were measure pre- and post-fluoxetine treatment (18mg/kg, 4 weeks).

Results: Before fluoxetine treatment, Sapap3-KOs engaged in more grooming bouts than WTs (3.8 vs. 1.1 bouts/min; $p = 0.002$). Fluoxetine decreased the number of grooming bouts in KOs compared to baseline ($p = 0.003$). Using linear decoder analysis, LOFC population activity in KOs predicts grooming behavior better than population activity in WTs ($p<10\text{-}6$) for two reasons. First, KOs show an increased percentage of grooming-inhibited neurons compared with WTs (23% vs 13%; $p = 10\text{-}7$). Second, in grooming-inhibited neurons, neural activity was more strongly correlated with grooming ($p = 0.0001$). Following fluoxetine, LOFC populations in KOs predict grooming behavior more poorly compared to their pre-fluoxetine baseline ($p<10\text{-}3$) due to a decrease in percentage of grooming-inhibited neurons (23% vs. 17%; $p = 10\text{-}5$).

Conclusions: Fluoxetine-associated decreases in the Sapap3-KO perseverative grooming phenotype may be caused by weakened

LOFC representations of grooming due to a reduction in the number of grooming-associated inhibited neurons. Ongoing experiments are monitoring activity in the exact same cells during a reversal learning paradigm to determine whether overlapping neural ensembles are disrupted in both behavioral paradigms.

Keywords: Compulsion, Lateral Orbitofrontal Cortex, In Vivo Calcium Imaging

Disclosure: Nothing to disclose.

M105. Single-Dose Effects of Citalopram on Neural Responses to Emotional Stimuli in Borderline Personality Disorder Assessed With Functional Magnetic Resonance Imaging: A Randomized, Crossover Trial

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Background: Psychiatric medication that has a soothing effect on the limbic response to emotional stimuli could improve affective instability symptoms as observed in Borderline Personality Disorder (BPD).

Methods: In this Placebo-controlled, double-blind clinical trial, we assessed the immediate effect of Citalopram on brain function. After compound intake, patients with BPD viewed affective pictures while undergoing functional Magnetic Resonance Imaging (fMRI). The Blood Oxygenation Level Dependent (BOLD) response was compared to a Placebo condition in regions of interest encompassing prefrontal brain areas and the amygdala.

Results: Citalopram reduced the amygdala response to pictures of faces with negative affective expressions ($P<0.05$). The neural response to pictures showing negative affective scenes was not significantly affected. We also observed no significant effects of Citalopram on prefrontal brain regions.

Conclusions: The results confirm the assumption that Citalopram can alter neural responding to emotional stimuli in BPD, although more research is necessary to evidence whether results are robust. The study lends limited support to Citalopram altering disordered emotion processing. Corroboration by further studies is necessary to evaluate the utility of this medication to treat BPD. In face of the lack of effective medication to treat BPD, pharmacological fMRI studies can reveal neural target engagement and identify candidates for clinical trials.

Keywords: Borderline Personality Disorder, Emotion Modulation, pharmacoBOLD

Disclosure: Boehringer Ingelheim International GmbH: Consultant (Self)

M106. Altered Gene Expression Rhythms in the Prefrontal Cortex of Subjects With Schizophrenia

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Background: Schizophrenia (SZ) is a chronic neuropsychiatric illness associated with cognitive dysfunction and disrupted circadian behaviors. Consistent with circadian rhythm dysfunction, studies have demonstrated abnormal peripheral gene expression of circadian clock genes and changes in rhythmic expression of hormones in individuals with SZ. Molecular rhythm patterns, however, have only just begun to be characterized in the human brain. Our lab has recently observed a distinct set of diurnally rhythmic transcripts in SZ subjects relative to a cohort with no history of psychiatric illness (non-psychiatric; NP) using rhythmic analysis of RNA-sequencing (RNA-seq) data from the dorsolateral prefrontal cortex (DLPFC), a region associated with executive dysfunction and heavily implicated in SZ. Pathway analysis demonstrated that genes that gained 24 h rhythmicity in SZ were associated with mitochondria (MT) dysfunction and GABA-ergic signaling, which is consistent with previously observed abnormal expression of transcripts associated with MT and GABA-ergic signaling. A growing body of literature, however, has demonstrated that gene expression can also be regulated by ultradian rhythms (rhythms with a period < 24 h). 12 h rhythms are observed in various aspects of human behavior (sleep patterns, cognitive performance) and biology (body temperature, blood pressure, migraine onset, circulating hormone levels). 12 h transcript expression rhythms are enriched for MT-associated proteins across species and tissues, suggesting 12 h rhythmicity is a conserved component of MT gene expression and may have a potential role in MT rhythmic disruptions in SZ DLPFC.

Methods: In the current study, we utilized multiple approaches to investigate ultradian rhythms in gene expression from RNA-seq data obtained by the CommonMind Consortium through the NIMH Repository & Genomics Resource, a centralized national biorepository for genetic studies of psychiatric disorders, in both NP ($n = 104$) subjects and subjects with schizophrenia (SZ; $n = 46$). Prior to either analysis, time of death (TOD) was determined for each subject and normalized to a zeitgeiber time (ZT) scale. We then applied the eigenvaluepencil method, which assumes gene expression is the result of multiple superimposed oscillations and identifies the combination of rhythmic components that best explains the data without any constraints on the period, amplitude, or phase of the rhythm. In the second analysis, samples were ordered by TOD and expression for each gene was fit to a sinusoidal curve with a fixed frequency of 12 h using a nonlinear least-squares method.

Results: The eigenvaluepencil analysis found that transcripts in the DLPFC of both NP and SZ subjects were enriched for rhythmic components with 12 and 24 h periods. Ingenuity pathway analysis (IPA) indicated that transcripts with a 24 h rhythmic component were associated with the circadian rhythm signaling pathway and various intercellular communication pathways in NP subjects, but MT and GABA receptor signaling pathways in SZ subjects, consistent with previous findings from our lab. In both NP and SZ cohorts, transcripts with 12 h rhythmic components were associated with MT and EIF2 signaling pathways. In the NP cohort, these transcripts peak in expression at ZT 2-3 (~9 AM/PM), but in SZ we observe an anti-phasic shift and found that these transcripts peak in expression at ZT 9 (~3 PM/AM). Using the nonlinear regression analysis we observed significant 12 h rhythms in transcript expression and confirmed that these are associated with MT and EIF2 signaling pathways in both NP and SZ cohorts, peak at two distinct timepoints (ZT 2-3 and ZT 9) and have an anti-phasic shift in timing in SZ.

Conclusions: Overall, in the DLPFC of SZ subjects, transcripts associated with MT function gain a 24 h rhythmic component and shift timing of a 12 h rhythmic component. Intriguingly, in the NP cohort, transcripts associated with MT peak in expression during transition periods (~9 AM/PM), in which people are likely switching from states of wake/sleep and/or fasting/eating and may require increased energy. However, in the SZ cohort these genes have an anti-phasic shift and peak during static periods (~3 PM/AM), implicating a deficit in MT expression during transition periods in SZ. These data suggest alterations at multiple levels in the rhythmic regulation of MT-associated genes in schizophrenia. Future work will be necessary to determine whether these changes underlie disruptions in energetic homeostasis and neuronal dysfunction in the DLPFC that could potentially contribute to symptoms of cognitive dysfunction in schizophrenia.

Keywords: Schizophrenia (SCZ), Circadian, Human Postmortem Brain Tissue

Disclosure: Nothing to disclose.

M107. OMG! Enhanced NGR/p75/KAL9 Signaling Leads to Dendritic Regression During Adolescence

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Background: Onset of gray matter reductions during adolescence, coupled with postmortem findings of smaller dendrites, have consistently been shown in schizophrenia. Once formed, dendrites are largely stable structures due to competing growth and regression signals. Kalirin (KAL) is a Rho GEF that activates RhoA downstream of NGR1/p75 to restrict dendritic growth. Myelin-associated inhibitors (MAIs) are ligands for NGR1. We hypothesized that the normative increase in the MAI OMGp during adolescence paired with a genetic enhancement in NGR1/p75/KAL9 signaling would cause regression of the dendritic arbor.

Methods: A combination of dissociated cortical culture and hippocampal slice culture was used to evaluate the NGR1/p75/KAL9 signaling pathway. CRISPR/Cas9 was used to insert the Kalrn-PT mutation in C57/Bl6J mice. Full dendritic reconstructions of primary auditory cortex Layer 3 pyramidal cells were performed in 4- and 12-week old mice.

Results: Kalrn-PT confers enhanced RhoA activity. Knockdown of KAL9 rescues the dendritic deficits seen with NGR1 overexpression. L3 PCs from A1 in Kalrn-PT mice demonstrate dendritic regression across adolescence, with significant impairments at 12 weeks. A reduction in dendritic spine number per neuron and dendritic spine tissue density was seen in 12-week Kalrn-PT mice. OMGp treatment activates the NGR1/p75/KAL9 pathway and causes dendritic regression in vitro. Studies of the differential effect of OMGp on Kalrn-WT versus Kalrn-PT are pending.

Conclusions: The increased RhoA activity arising from the PT mutation results in increased NGR1/p75/KAL9 signaling and subsequently leads to a regression of dendritic length and complexity across adolescence, coupled with a reduction in spine number per neuron and spine tissue density. OMGp acts as a ligand for this pathway.

Keywords: Schizophrenia (SCZ), Dendrites, Adolescence

Disclosure: Nothing to disclose.

M108. Effects of SEP-363856, a Novel TAAR1 Agonist, on Negative Symptoms in Schizophrenia: Results of a 6-Month, Open-Label Treatment Study

Abstract not included.

M109. A Randomized Controlled Trial of SMS Text Messaging to Improve Clinical Engagement in Early Psychosis

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Background: Clinical disengagement of youth in early psychosis clinics continues to be a significant barrier to recovery as evidenced by high rates of treatment non-adherence, appointment non-attendance, and premature drop-out from clinical service, with the first one to two years of treatment representing a critical risk period. In addition to phone and email-based outreach efforts as part of early psychosis services, efforts to improve engagement in clinical services have explored augmentation with mobile technology interventions. One such mobile technology intervention, SMS text messaging, has shown promising early findings for individuals experiencing psychotic disorders, although with limited investigations in the context of early psychosis populations. To address this, we conducted a randomized clinical trial to evaluate the efficacy of a weekly SMS intervention delivered over nine months to improve clinical engagement for individuals experiencing early psychosis in the initial stages of treatment (ClinicalTrials.gov registration No. NCT04379349). The weekly SMS text messages consisted of questions and recommendations related to wellbeing and distress, appointment attendance, and medication adherence. We hypothesized that the active SMS intervention would lead to improvements in clinical engagement including clinic attendance rates, attitudes towards medication treatment, and clinician and self-report ratings of treatment engagement, as well as secondary improvements in clinical symptoms and functioning.

Methods: Participants between the ages of 16 and 29 presenting with a first episode psychosis were randomized to either an active or sham SMS intervention, delivered weekly for nine months, in addition to their usual early psychosis care. Participants were blinded to group assignment, and underwent assessments to evaluate clinical engagement, psychopathology, neurocognition, and community functioning at baseline, months 1, 3, 6, and 9, with the exception of neurocognition and functioning that were repeated only at month 9. Intent-to-treat and pre-specified per-protocol analyses were conducted using linear mixed models to evaluate changes in our primary (clinical engagement) and secondary (clinical, neurocognition, functioning) outcomes over time between intervention groups.

Results: 61 participants (Active SMS $n = 32$; Sham SMS $n = 29$) were recruited and enrolled in this trial, with 44 participants ($n = 22$ per group) completing the trial. Participants in the active SMS group exhibited a significant improvement over time in attitude towards medication ($\beta = 2.88$, $p = 0.044$) and clinician-rated availability ($\beta = -2.12$, $p = 0.02$), compared to the sham group. Paradoxically, the active SMS group also exhibited a significantly reduced clinic attendance rate over time compared to the sham group ($\beta = -0.21$, $p = 0.007$). In clinical symptoms, the active SMS group exhibited reductions over time in positive symptoms ($\beta = -2.33$, $p = 0.022$) and avolition-apathy ($\beta = -3.67$, $p = 0.046$) compared to the sham group, although the sham group exhibited a significant improvement in social functioning compared to the active SMS group ($\beta = -14.65$, $p = 0.031$). Notably, clinic attendance rate was inversely correlated with change in social functioning specifically in the active SMS group ($r = -0.74$, $p = 0.01$). Per-protocol analyses of study completers revealed findings consistent with the intent-to-treat analyses.

Conclusions: This trial sought to investigate a weekly SMS text messaging intervention as a means to improve clinical engagement for young people with early psychosis, as well as secondary effects such an intervention may have on clinical, cognitive, and functional outcomes. Over the course of the intervention, we found that weekly active SMS text messaging resulted in improvements in participants' attitude towards medication and clinical engagement (specifically availability), as well as in positive and some negative symptoms, although with a reduction in clinic attendance rates over time that appeared to be related to improvements in social functioning. Overall, these findings suggest that SMS text messaging may help support at least some aspects of clinical engagement, including attitudes towards medication treatment, and this may contribute to improvements in symptoms for individuals experiencing early psychosis. These findings build on existing literature to support the potential utility of asynchronous digital

interventions to augment early psychosis care, and inform future refinements for clinical trials to evaluate the effectiveness of such interventions to improvement engagement and treatment outcomes in early psychosis populations.

Keywords: Treatment Adherence, Early Psychosis, SMS Text Messaging

Disclosure: Nothing to disclose.

M110. The M1/M4 Agonist Xanomeline, in Combination With the Peripheral Anticholinergic Trospium, is Effective for Acute Treatment of Schizophrenia: Results of a Phase 2 RCT Comparing KarXT vs Placebo

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Background: Xanomeline is an investigational non-dopaminergic antipsychotic believed to have antipsychotic properties due to selective M1 and M4 agonist properties. In clinical trials dating back to the 1990s, xanomeline showed antipsychotic efficacy for both Alzheimer's disease and schizophrenia. Despite promising antipsychotic efficacy, development stopped due to tolerability problems associated with unopposed pro-cholinergic adverse events such as nausea or vomiting. To mitigate this tolerability problem without jeopardizing CNS mode of action, the peripheral anticholinergic trospium has been combined into a combination oral formulation known as KarXT. Phase 1 studies have shown that KarXT, relative to xanomeline, has fewer pro-cholinergic AEs. Here we present the results of the efficacy and safety of KarXT in a phase 2 study of acute psychosis in subjects with schizophrenia.

Methods: This was a double-blind, placebo-controlled, monotherapy (non-adjunctive), 5-week inpatient trial conducted at 12 US sites using a flexible dose design. Subjects generally did not reach their maximal dose until Day 8. The primary endpoint was the least-squares mean (LSM) change in PANSS total (PANSS-T) score from baseline to week 5. Secondary endpoints were LSM change from baseline to week 5 on the PANSS positive and negative symptom subscales, PANSS Marder negative factor score, and achieving a CGI-S score of 1 or 2 by last visit. Additional post hoc efficacy analyses to be presented include predefined categorical responses (e.g., proportion of KarXT vs placebo groups achieving ≥ 30% improvement from baseline PANSS-T over the course of the trial).

Safety and tolerability assessments included standard AE and SAE rates, labs, and EPS rating scales. Additional post hoc safety analyses include duration of AEs (e.g., nausea or vomiting) associated with pro-cholinergic effects of muscarinic agonist xanomeline and peripheral anticholinergic AEs associated with trospium.

Results: 182 subjects were randomized (KarXT N = 90; placebo N = 92). The study met the primary endpoint, with the PANSS total score showing a 11.6-point improvement compared to placebo at endpoint (-17.4 KarXT vs. -5.9 placebo $p<0.0001$) at week 5. In a post hoc analyses, statistically significant differences (all $p<.01$) were seen in predefined ≥ 30% PANSS response criteria at every assessment time point for KarXT compared to placebo (weeks 2, 4, and 5). By the end of study (week 5), 44.4% (32 out of 72) of the KarXT group met the ≥30% PANSS improvement criteria compared to 12.3% (9 out of 73) of the placebo group (mITT population; OR 5.79 [95% CI 2.5-13.5], $p < .0001$).

KarXT was well tolerated. Over 90% (91%) of KarXT subjects were able to tolerate the highest dose of 125mg/30mg BID. The number of AE related discontinuations was low and equal in the KarXT and placebo groups (N = 2 per group). AEs consistent with pro-cholinergic MOA (e.g., nausea [n = 15; 16.9% KarXT vs n = 4; 4.4% placebo] or vomiting [n = 8; 9.0% KarXT vs 4; 4.4% placebo]) and peripheral anticholinergic properties (e.g., constipation [n = 15; 16.9% KarXT vs n = 3; 3.3% placebo] and dry mouth[n = 8; 9.0% KarXT vs n = 1; 1.1% placebo] were more common in the KarXT group. None of these were severe, and none were associated with discontinuation. Most of the pro-cholinergic AEs started within the first 2 weeks of KarXT treatment and were self-limited in resolving before study endpoint. Most of the nausea reported in KarXT-treated patients was rated mild (n = 13/15; 87%), 14% moderate, and none were severe. The mean onset of nausea for the KarXT group was 9.1 days after initiation and lasted a mean of 10.7 days. For the 4 patients in the placebo-arm with nausea as an AE, 3 (75%) were mild, one (25%) moderate and none severe. Onset and duration for nausea reported in placebo group had a similar pattern as KarXT, with a mean onset at 11 days and lasting a mean of 14.3 days. Vomiting was reported in 8 KarXT patients with 5 (62.5%) rated mild, 3 (37.7%) moderate and none severe. Mean onset of vomiting was 13.25 days from initial KarXT exposure, and mean duration was 1.5 days. Vomiting was also reported in 4 placebo-treated subjects, 3 (75%) mild, 1 (25%) moderate and none severe. The mean time to onset of vomiting AE for placebo group was 15.5 days after randomization and lasted a mean of 1.3 days. Rates of other AEs associated with current antipsychotics (e.g., somnolence, weight gain, and extrapyramidal symptoms/akathisia) were similar across KarXT and placebo arms.

Conclusions: KarXT treatment, relative to placebo, showed statistically and clinically meaningful efficacy for the treatment of acute symptoms of schizophrenia. New categorical data analysis shows clinically meaningful differences in response rates. As anticipated, KarXT subjects had greater likelihood of AEs consistent with pro-cholinergic MOA of xanomeline (e.g., nausea or vomiting), but the pro-cholinergic AEs were self-limited, mild or moderate in severity, and were not associated with discontinuations. Relative to AEs observed in xanomeline monotherapy, these findings support the concept of trospium mitigating pro-cholinergic AEs. These data support Phase 3 development of KarXT for the treatment of schizophrenia and suggest that M1/M4-preferring muscarinic agonists may represent a promising new class of antipsychotic drug that avoids AEs commonly associated with currently available antipsychotics.

Keywords: Schizophrenia, Antipsychotics, M1 and M4 Muscarinic Receptors, Clinical Development, Efficacy and Safety

Disclosure: Karuna Therapeutics: Employee (Self)

M111. Phase 3 Safety and Tolerability Results of the Combination Olanzapine and Samidorphan in Patients With Schizophrenia: The 1 Year ENLIGHTEN-2-Extension

Abstract not included.

M112. White Matter Microstructural Organizations in Patients With Severe Treatment-Resistant Schizophrenia: A Diffusion Tensor Imaging Study

Abstract not included.

M113. Contributions of Autonomic Arousal-Related and Task Activity to Cognitive Performance in Med-Naïve First Episode Psychosis Patients and Healthy Controls

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Background: Cognitive impairment is integral to the pathophysiology of psychosis. Recent findings implicate autonomic arousal-related activity in both momentary fluctuations and individual differences in cognitive performance. Although altered autonomic arousal is common in First Episode Psychosis (FEP) patients, its contribution to cognitive performance is unknown.

Methods: 24 FEP patients (46% male, age = 24.31 (4.27) years) and 24 healthy controls (42% male, age = 27.06 (3.44) years) performed the Multi-Source Interference Task in-scanner with simultaneous pulse oximetry.

First-level models included the cardiac-BOLD regressor, which reflected autonomic arousal-related activity and was created by convolving HR at each heartbeat with the hemodynamic response function, in addition to task (congruent, interference, and error) and nuisance (motion and aCompCor physiology) regressors. Group models examined the effect of cognitive performance (reaction times * error rate) on arousal-related and task activity, while controlling for group, sex, age, and Framewise Displacement. Analyses were thresholded at a voxelwise and clusterwise p = 0.01.

Results: Autonomic arousal-related activity was significantly related to cognitive performance across a broad bilateral region of occipital cortex. This relationship was stronger in healthy controls than FEP patients. Greater arousal-related activity in the bilateral prefrontal cortex (BA 9) was related to better performance in healthy controls, but not FEP patients. Greater task activity, on the other hand, was related to worse performance within the occipital cortex and thalamus.

Conclusions: Autonomic arousal systems contribute to cognitive performance and the pathophysiology of FEP. Increased arousal-related activity in visual regions may improve attention and reduce the need for task-related recruitment of these regions.

Keywords: Psychosis, Cognition, Autonomic

Disclosure: Nothing to disclose.

M114. Better Functional Capacity and Cognitive Performance in Clozapine Responders Compared to Non-Responders: A Cross-Sectional Study

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Background: Treatment resistant schizophrenia is associated with considerable everyday disability. Disability in schizophrenia is commonly found to be correlated with impairments in cognitive performance and with impairments in performance on measures of functional capacity. However, what has not been well examined is the association between symptom status, cognitive functioning, and functional capacity in treatment refractory patients comparing good and poor response to clozapine treatment (TRS vs UTRS). In this study, we examine clozapine treated patients and compare cognitive and functional capacity performance across response status using a computerized functional capacity assessment battery, termed the University of Miami Computer-Based Functional Assessment System (CFAS). The CFAS assesses performance on a variety of everyday activities including: ATM Banking/ Financial Management, Prescription Refill via Telephone/Voice Menu System, and purchasing a transportation ticket.

Methods: Twenty-seven clozapine treated patients were tested with the Brief Assessment of Cognition in Schizophrenia (BACS), were clinically rated with the PANSS, and performed three computerized tests of functional capacity (FC): ATM Banking, Ticket Purchase, and Prescription Refill with a telephone voice menu. Prior to treatment all patients had been designated as treatment refractory (failed at least two full trials of antipsychotic medications) and at least moderately ill based on current overall clinical state. Patients were divided on the basis of having moderate or more severe PANSS symptoms (Total>58). Patients were considered responders or TRS if they had a PANSS score less than 58 and non-responders or UTRS if greater than 58.

Results: Time to completion and efficiency scores for the FC measures were correlated with BACS composite scores in the group as a whole, all Pearson r>.47, all p<.02. In comparisons of global symptom severity, patients with mild symptom severity (n = 18) performed faster and more efficiently than those who were not on all FC measures, all t>2.07, all p<.05, all d>1.1. Cognitive performance was better in the less symptomatic group, t=3.34, p = .003, d=1.25. Additionally, PANSS negative scores were generally significantly correlated with CFAS performance and in all cases negative subscale scores were more strongly correlated with CFAS performance than positive scores. For the BACS, again, there was a significant correlation negative symptoms and BACS total scores, but the correlation with PANSS positive symptoms was not significant.

Conclusions: Our study suggests several important factors related to clozapine and everyday functional outcomes. Clozapine response is correlated with significantly better cognitive performance and functional capacity, as well as lower symptoms. Clozapine response may lead to improved functioning and quality of life and the potential for more independent living. This further supports the use of clozapine in patients with TRS not only for greater symptom relief but also for improved functioning.

Keywords: Schizophrenia, Clozapine, Functional Capacity, Antipsychotics, Cognitive Functioning

Disclosure: Nothing to disclose.

M115. Insula Structure in Psychotic Disorders and Psychosis Spectrum Youth

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Background: Smaller insula volume is consistently reported in psychosis-spectrum disorders and is hypothesized to result, in part, from abnormal neurodevelopment. Insula structure can also be characterized through surface-based morphology measures (thickness, gyrification, sulcal depth) which have distinct developmental trajectories. Moreover, the insula is heterogeneous, comprised of three cytoarchitecturally distinct sub-regions (agranular, dysgranular, and granular), which traverse the anterior-posterior axis and are differentially implicated in affective, cognitive, and somatosensory processing. Consequently, comprehensive examination of sub-regional volume and morphology may inform the etiology of psychosis.

Methods: We characterized insula sub-regional volume, morphology, and phenotypic correlations in a large cohort of healthy adults (n = 196) and people with a psychotic disorder (n = 303), and 1,368 individuals from the Philadelphia Neurodevelopmental Cohort (PNC), including typically developing individuals (n = 381) and youth with psychosis-spectrum symptoms (n = 381) and other psychopathology (n = 606). Group differences were assessed first using ANCOVA of region-of-interest data, and then validated through voxel-wise and vertex-based analyses. Partial correlations within the psychosis and psychosis-spectrum youth samples were used to determine a-priori associated with clinical phenotypes.

Results: Insula volume was significantly lower in psychotic disorders (in both early and chronic stages) (p<.001) and psychosis-spectrum youth (p = .015). In both cohorts, lower

volume followed an anterior-posterior gradient. Morphological abnormalities were limited to lower gyrification in psychotic disorders ($p = .01$). Considering diagnosis, insula abnormalities were specific to schizophrenia-spectrum, not psychotic bipolar disorder. Insula structure was associated with cognition, and positive and negative symptoms of psychosis.

Conclusions: These findings are the first to demonstrate an anterior-posterior gradient of insula structural abnormalities in psychotic disorders, particularly schizophrenia, which is also present in youth with psychosis-spectrum symptoms, and associated with clinical features. Lower gyrification in psychotic disorders suggests very early abnormal neurodevelopment. Neurodevelopment of insula granular regions may be important for understanding the pathophysiology of psychosis-spectrum disorders.

Keywords: Psychosis, Insula, Brain Structure, Morphometry

Disclosure: Nothing to disclose.

M116. BNST Hyperconnectivity in Schizophrenia Patients With Comorbid Anxiety

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Background: Models of schizophrenia propose alterations in fear and anxiety circuitry. Studies of fear processing provide evidence for altered amygdala function and connectivity. However, studies of individuals with schizophrenia have yet to examine the bed nucleus of the stria terminalis (BNST), a brain region that is critical for anxiety. In the present study, we examined BNST function and connectivity to threat in schizophrenia.

Methods: Participants included healthy control subjects (HC, $n = 15$), individuals with schizophrenia and anxiety (SZ+Anx, $n = 15$), and individuals with schizophrenia without anxiety (SZ-Anx, $n = 16$). Group differences (SZ vs HC and SZ+Anx vs SZ-Anx) in BNST function and connectivity (gPPI) were measured during a threat anticipation task.

Results: In response to unpredictable threat, the SZ+Anx group demonstrated BNST hyperconnectivity with the salience network (insula/dorsal anterior cingulate cortex) compared to the SZ-Anx group ($p < .05$, cluster corrected). In response to predictable threat, the SZ+Anx group had BNST hyperconnectivity with the fear network (sublenticular extended amygdala/prefrontal cortex/anterior insula). BNST activation did not differ across groups.

Conclusions: Importantly, BNST connectivity differed for patients with or without anxiety disorders, highlighting the importance of considering comorbid anxiety in studies of emotion processing in schizophrenia.

Keywords: BNST, Stress and Anxiety Disorders, Schizophrenia Subtypes

Disclosure: Nothing to disclose.

M117. Persistent Neural Habituation Deficits in Early Psychosis: A 2-Year Follow-Up

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Background: Habituation—the decrease in response to repeated information—is a fundamental, highly conserved mechanism for learning. Convergent evidence from cognitive, electrophysiological, and genetic studies indicates neural habituation is disrupted in schizophrenia, with disruptions co-occurring in brain regions that show inhibitory dysfunction. Although inhibitory deficits have been proposed to contribute to the onset and progression of illness, little is known about the progression of habituation deficits.

Methods: We measured neural habituation in 138 participants (75% male, including 70 early psychosis patients with < 2 years of illness and 68 healthy controls), with 108 participants assessed longitudinally at both baseline and two-year follow-up. Habituation slopes (i.e. rate of fMRI signal change) to repeated images were computed for the anterior hippocampus, FFA, and occipital pole. Habituation slopes were entered into a linear mixed model to test for effects of group, time, and region.

Results: Early psychosis patients habituated 11-16% more slowly than healthy control participants across regions ($p < 0.002$). The FFA showed a trend effect of time ($p = 0.055$) with both groups habituating less at follow-up compared to baseline. No time effects were detected in the anterior hippocampus or occipital pole, and none of the regions showed a group by time interaction ($p > 0.11$). Together, these findings indicate habituation deficits in early psychosis patients remained stable over two-year follow-up.

Conclusions: These results demonstrate that neural habituation remains impaired but does not further decline over the early course of illness, suggesting a period when interventions targeted at ameliorating inhibitory deficits may be effective.

Keywords: Hippocampus, Occipital Pole, FFA, Schizophrenia, Longitudinal Analysis

Disclosure: Nothing to disclose.

M118. D-Serine Acting at NMDA Receptors Controls Cortical Parvalbumin Interneuron Development

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Background: Fast spiking parvalbumin interneurons (PV-IN), derived from the medial GE progenitor, are dysregulated in various neurodevelopmental disorders including schizophrenia, and evidence exist for the contribution of NMDAR hypofunction in PV-IN to the pathophysiology of schizophrenia. Cortical PV IN (PV-CIN) development includes spatial and temporal control of proliferative progenitor cells that originate from the ganglionic eminence, as well as their subsequent differentiation and migration to the neocortex, where they ultimately form connections with excitatory cortical pyramidal neurons. Embryonic and early postnatal neocortical neurons express highly functional $Ca2+$ permeable N-methyl-D-aspartate receptors (NMDARs) before they form synapses. NMDAR activation modulates progenitor proliferation, radial migration, and differentiation of excitatory neurons, however evidence for its role on CIN migration is limited and the role of its co-agonist, such as D-serine, is unknown. NMDARs are unique compared to other glutamate receptors, because in addition to the binding of glutamate, NMDAR activation requires the binding of a co-agonist, either glycine or D-serine. D-serine is racemized from L-serine by the enzyme serine racemase (SR). Previous studies have shown that mice lacking D-serine due to genetic deletion of SR display forebrain NMDAR hypofunction. Here, we investigated the role of D-serine mediated NMDAR activation in PV CIN development.

Methods: Wild-type and SR knockout (SR−/−) mice were generated from heterozygous SR+/- breeding pairs, and both male and female littermates were used for all experiments. Brains were harvested either from embryos (E15) and then fixed overnight in 4% PFA or from postnatal mice (P16) that were perfused with saline followed by 4% PFA. Immunofluorescent labeling or in situ hybridization was performed on cryostat (10-20uM) or microtome (30uM) sections, respectively. Sections were either probed for Srr or incubated in primary antibody (mouse anti-SR, anti-phospho-H3, Nkx2.1, MAP2, Ki67, GABA) and appropriate secondary antibodies. Fresh E15 forebrains were used for immunoblotting and high-performance liquid chromatography (HPLC). (N = 3-4/group). The McLean Hospital Institutional Animal Care and Use Committee approved all animal care and experimental procedures.

Results: At E15, we detected the presence of SR mRNA in various regions including the cortical plate and MGE. Both SR and the D-serine transporter Slc1a4 were expressed in the forebrain, and D-serine levels were similar to early postnatal expression. In the absence of SR, we observed an abnormal distribution of migrating INs at the VZ and a reduction in the levels of INs that reach the neocortex. We also observed a reduction in the activity of signaling molecules in SR−/− embryos, such as Src kinases, which are involved in neuronal migration. Furthermore, we observed an increase in proliferation, but a reduction in differentiation. At P16, there was a significant reduction in PV interneurons and perineuronal nets ensheathing these neurons in the somatosensory cortex and hippocampus of SR−/− mice.

Conclusions: Our results show that embryonic D-serine mediated NMDAR activation controls the distribution, differentiation and proliferation of GABAergic progenitors, which is important for regulating the number of postnatal PV-CINs. In summary, our results lay the groundwork for elucidating novel roles that the NMDAR co-agonist D-serine, which is produced by inhibitory neuron progenitors, plays in PV-CIN development.

Keywords: NMDA Receptor, Parvalbumin Fast-Spiking GABAergic Interneurons, D-serine, NMDAR Hypofunction, Schizophrenia

Disclosure: Nothing to disclose.

M119. An Adolescent Sensitive Period of Heightened Local Inputs Onto Frontal Top-Down Projection Promotes Adult Attentional Behavior

Abstract not included.

M120. An Investigation of Short Tandem Repeat Variation in Schizophrenia Post-Mortem Brain

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Background: A pathogenic role of short tandem repeat (STR) variation has been established for neurological disorders (i.e. trinucleotide repeat disorders); however, a role in schizophrenia (SCZ) remains to be established. STR variation of rare or common frequency may contribute to SCZ genetic risk.

Methods: The next-generation sequencing based STR calling algorithm, 'STRetch', profiled STRs in whole genome sequenced DNA, extracted from post-mortem cerebellum and dura matter (n = 54 SCZ, n = 60 controls (CONT). STRetch is advantageous since it enables detection of repeats greater than the 150bp read length.

Results: The STRetch outlier approach identified SCZ singleton repeat expansions, the most significant, a 142bp expansion of the (AACAT)n motif in CYFIP1 intron 1 (Reference STR Length: 13bp, Allele Statistics: z score: 10.2, p-adjusted: 3.1.x10-20). CYFIP1 is located in the 15q11.2 region and encodes a protein that binds FMR1; notably, expansions within the FMR1 5'UTR underlie Fragile X syndrome. In addition, repeat expansions across multiple samples were identified in STRs within reported SCZ-GWAS positive loci. For example, 2 samples (SCZ, CONT) contained repeat expansions of the (AGC)n motif in TCF4 intron 1, of 177bp and 174bp length (Reference STR Length: 25bp, Allele Statistics: z scores: 10.2, 10.2; p-adjusted: 1.9.x10-22, 6.4x10-22)

Conclusions: STR variation in SCZ/CONT post-mortem brain was identified across the genome, but overall validity and SCZ-association is limited by sample size. Future analyses will include additional STR calling methods, and additional samples from post-mortem brain and a hospital-biobank. The linkage disequilibrium of STR variation with SCZ GWAS index variants will also be investigated.

Keywords: Short Tandem Repeat, DNA, Whole-Genome, Sequencing, Schizophrenia, Human Post-Mortem Brain

Disclosure: Nothing to disclose.

M121. Integration of Brain-Specific Imputed Transcriptomes and Epigenomes Across Neuropsychiatric Traits Identifies Disease-Specific and Shared Dysregulation of Cre-Transcript Units

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Background: Machine learning approaches allow us to leverage reference -omics datasets to train predictive models for quantitative traits. These models can then be integrated with GWAS summary statistics to identify disease-specific changes. Here we predict brain-specific transcriptomes, chromatin accessibility, and histone modifications for 40 neuropsychiatric traits to study the effects of risk loci on transcriptional regulation.

Methods: We generate brain-specific predictive models for (1) transcription at the gene and isoform level from dorso-lateral prefrontal cortex (DLPFC, n = 924), (2) chromatin accessibility (DLPFC homogenate, n = 164), FANS-sorted neuronal (n~100) and FANS-sorted glial nuclei (n~100) from 2 brain regions, and (3) histone modification as assayed by ChIP-seq from DLPFC homogenate for H3K27ac (n = 122 and 191) and H3K4me3 (n = 163). Then, we impute the transcriptomes and chromatin accessibility for 40 neuropsychiatric traits and linked genes with proximal (distance to TSS and data-driven modeling) and distal (by leveraging in-house brain-specific Hi-C data) cis-regulatory elements (CRE) such as promoters and enhancers. Finally, we perform correlation-aware meta-analysis of the gene expression and epigenetic features at the gene level to impute transcriptional dysregulation for each neuropsychiatric trait.

Results: Our approach allows the modeling of the joint effects of risk variants on both expression and functional epigenomic features (e.g. chromatin accessibility and histone modifications) greatly increasing power to detect gene-trait associations and explaining a higher percentage of the genetic heritability via mechanisms of transcriptional dysregulation. We identify unique, shared and converging mechanisms of transcriptional regulation across neuropsychiatric diseases.

Conclusions: Imputing disease-specific patterns of transcriptional dysregulation via integration of transcriptome and

epigenome imputation can help us formulate hypotheses for the functional roles of risk loci and enables the study of converging trans-disease processes.

Keywords: Genomics, Epigenetics, Heritability, Machine Learning

Disclosure: Nothing to disclose.

M122. Dynamic Resting fMRI Connectivity Across the Psychosis Spectrum

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Background: Psychiatric diagnosis using current clinical practice and categorization is not biologically based. The heterogeneity among DSM categories and the overlap of symptoms between different groups presents a challenge to categorizing individuals by disorders and developing predictive modeling approaches. There are multiple ongoing efforts to try to address this, one of which is the bipolar–schizophrenia network for intermediate phenotypes (B-SNIP) study, which is a multisite consortium of investigators study that collected multiple brain imaging data and assessment measures within three psychotic disorders and controls. In the B-SNIP study, subject categories are based on distinctive biological phenotypes, called Biotypes. Biotype categories are defined as cases with impaired cognitive control and poor sensorimotor function (Biotype1), cases with impaired cognitive control but overstated sensorimotor response (Biotype2), and cases with cognitive and sensorimotor functions close to normal (Biotype3). The purpose of this preliminary study is to examine differences across psychosis categories and normal control using functional magnetic resonance imaging (fMRI)-based dynamic functional network connectivity and to compare between the DSM and Biotype categories.

Methods: The data that we worked with includes 615 subjects (36.74 +-12.43 years; 323 women and 292 men) including individuals with schizophrenia (n = 156), schizoaffective disorder (n = 104), bipolar disorder with psychosis (n = 132) and controls (n = 223). These individuasl were also categorized as Biotype1 (n = 102), Biotype2 (n = 139) and Biotype3 (n = 151). Brain extraction, slice-timing, motion correction, and spatial normalization was performed using SPM. Data was smoothed using a Gaussian kernel with a 6 mm full-width at half-maximum, and voxel time courses were z-scored. Intrinsic connectivity networks (ICNs) were extracted using the Neuromark pipeline which adopts spatially constrained ICA to estimate individual-subject brain network features. 53 ICN spatial priors were used to estimate subject-specific ICs followed by dynamic functional network connectivity (dFNC). dFNC was estimated via tapered sliding window approach and functional connectivity calculated for each time point between ICNs from windowed data. Exemplar clustering was applied to the windowed dFNC data to obtain brain connectivity patterns (states) over time and resulted in 5 states. Then for each state and each subject, we computed the median of the state that lead to 5 averaged dFNC for each subject. We then performed analysis of variance (ANOVA) statistical tests to obtain group differences between groups of subjects. We implemented a cross-validated classifier within each framework.

Results: Result suggest several findings regarding the occupancy across different groups among five states. Healthy controls showed significantly more occupancy of state 1 versus all patient and Biotypes groups. In addition, schizophrenia showed higher occupancy of state 2, Biotype1, Biotype2 and schizoaffective disorder follow same property as well, but Biotype1, Biotype2 and Biotype3 occupancy levels were less distinctive compared to SZP, SADP and bipolar disorder. We also found group differences in resting-state dynamic functional connectivity within the 146 pairs of ICNs for DSM categories and within 155 pairs of ICNs for Biotypes in five states in the level of significant 0.05. Among those differences, Biotype shows more cell-wise differences, predominantly in state 4; while DSM categories differed the most in state 5. In addition, group differences were found between thalamus and postcentral gyrus in 4 states out of 5 states in both DSM and Biotype such that normal group had lower functional connectivity. DSM and biotype classification were both well above chance (25% for 4 way classification) with DSM at 61% and biotype at 44%.

Conclusions: Results suggest interesting differences in DSM and biotype categorizations. In all cases, healthy controls showed more occupancy in highly modularized states 1 in comparison with patient groups. Biotype showed slightly more cellwise differences but less distinctiveness in occupancy levels. Mutually exclusive cell-wise differences were found across groups for DSM and Biotype. DSM categories classified more accurately using the dFNC data (61% vs 44%). Results suggest we can learn by comparing newer categories to DSM categories in the context of biological changes, there is still much more work to be done including incorporating additional data types.

Keywords: fMRI, Schizophrenia, Connectivity, Dynamics, Psychosis Spectrum

Disclosure: Nothing to disclose.

M123. Investigating Cross-Network Connectivity in Early Stage Psychosis and the Modulatory Effects of Diagnosis and Substance Abuse

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Background: Psychotic disorders are among the most debilitating of mental illnesses and are associated with significant functional and structural impairments. The early stage of psychosis (ESP) - the initial years following the first episode of psychosis (FEP)- is considered a critical period and window of opportunity during which effective intervention/treatment may help slow functional decline and promote favorable outcomes. However, the factors that render sub-groups of patients vulnerable to exacerbation of symptoms and poor recovery outcomes are not well understood. A greater understanding of such key influencing factors and biological markers during the critical ESP period would aid the identification of vulnerable subgroups of patients and potentially lead to improved outcomes.

The Triple Network Model of psychopathology identifies the salience network (SN), central executive network (CEN), and default mode network (DMN) as key networks underlying the pathophysiology of psychiatric disorders. In particular, abnormal SN-initiated network switching impacts the engagement and disengagement of the CEN and DMN, and is proposed to lead to the generation of psychosis symptoms. Cross-network connectivity has been shown to be abnormal in both substance abuse disorders (SAD) and psychosis. However, none have studied how substance abuse affects cross-network connectivity in early stage psychosis (ESP) patients. In this study, we used resting state functional MRI (rs-fMRI) data to investigate the effect of substance abuse on cross-network connectivity. In addition, we aim to perform sub-group analysis by exploring whether current substance abuse, or diagnosis (affective vs non-affective psychosis) would have an interaction effect. A better understanding of how substance abuse affects cross-network connectivity could

potentially identify vulnerable patient sub-populations and guide better treatment.

Methods: Data were collected from 55 participants (20 controls, 35 FEP patients) of both sexes. Resting state functional magnetic resonance imaging (rs-fMRI) data were acquired on a 3T Siemens scanner in 2 runs of 6.2 minutes (124 time-points) each with a gradient-echo echoplanar imaging sequence sensitive to blood oxygenation level-dependent (BOLD) contrast. Pre-processing was performed with the fMRIPrep pipeline, and resting state networks were determined with group independent component analysis (GICA) performed with FSL's melodic. Reliable measures of cross-network connectivity were obtained with FSLNets. Linear regression models were run in Stata where cross-network connectivity was the outcome, with group (ESP patients or controls), diagnosis (affective or non-affective psychosis), and current substance abuse as the predictors.

Results: Preliminary analysis revealed that ESP patients had significantly lower functional connectivity compared to HCs between the Right CEN and Salience CON (mean contrast: -1.230, p-FDR = 0.0416, unadjusted 95% CI = -2.139 to -0.321). There was also a trend of higher functional connectivity compared to HCs between the Posterior DMN and Salience CON (mean contrast: 1.187, p = FDR = 0.072, unadjusted 95% CI = 0.221 to 2.153). For CEN-Sal connectivity, there was a significant main effect of diagnosis (휒2 (df = 2) = 6.88, p = 0.032). Post-hoc analysis suggested that the effect of diagnosis was significant, and largely driven by the difference between non-affective psychosis and controls (-1.690, p-Bonferroni = 0.022, 95% CI = -3.195 to -0.184). There was also a significant main effect of current substance abuse on CEN-Sal connectivity (휒2 (df = 2) = 10.03, p = 0.0066). Post-hoc analysis suggested that the effect of substance abuse was significant, and largely driven by the difference between patients with substance abuse and controls (-2.292, p-Bonferroni = 0.002, 95% CI = -3.896 to -0.689).

Conclusions: We have provided evidence supporting the Triple Network Model, where ESP patients had lower cross-network connectivity between the CEN and Salience networks compared to controls, indicating a functional decoupling within these two key networks in ESP patients. We also found that diagnosis and co-morbidity with current substance abuse has modulatory effects on cross-network connectivity in ESP patients. The reduced CEN-Salience FC was exacerbated in patients diagnosed with non-affective psychosis, and patients with current substance abuse, highlighting that patients in these sub-groups could be more vulnerable to the cross-network connectivity abnormalities, and have a greater need for intervention.

Keywords: Resting State Functional Connectivity, Early Psychosis, Substance Abuse

Disclosure: Nothing to disclose.

M124. Increased Glutamate in the Right Superior Temporal Gyrus in Antipsychotic-Naive Psychosis: A Whole Brain 1H-MRS Study in Schizophrenia and Bipolar-I

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Background: Proton magnetic resonance spectroscopy (1H-MRS) studies have examined glutamatergic abnormalities in psychosis, mostly in single voxels and this literature suggests increases in brain glutamate in some brain regions eg: medial frontal, striatum, hippocampus). Though the critical brain nodes remain unknown, psychosis involves networks with broad, subtle abnormalities. Hence like with other neuroimaging modalities, an unbiased approach is advantageous. We used a whole brain 1H-MRS approach to examine glutamine-plus-glutamate (Glx), as well as other standard metabolites in early schizophrenia and bipolar-I disorders.

Methods: Whole-brain proton spectroscopic imaging was acquired at 3T using echo-planar acquisition with spin-echo excitation EPSI sequence [TE = 17.6 ms, TR = 1551 ms, TR = 511 ms, non-selective lipid inversion nulling with TI = 198 ms, FOV = 280×280×180mm, voxel size of 5.6×5.6×10mm, echo train length of 1000 points, bandwidth of 2500 Hz, reduced k-space sampling (acceleration factor = 0.7), and a nominal voxel volume of 0.31cm3. Young psychosis subjects (n = 76, mean age=?; Sz=48, BP-I=2119 antipsychotic-naïve and 17 antipsychotic-treated) and healthy controls (HC, mean age=23; n = 51) were studied. Glx (glutamate+glutamine), N-acetylaspartate (NAA), choline, creatine and myo-inositol were fitted with MIDAS, referenced to water and partial volume corrected for CSF. Voxels were filtered for spectral resolution between 2-12Hz, CRLB fitting of 1-20% and CSF <30%. Group contrasts for each metabolite (adjusted for gray/white matter voxel tissue proportion and age) from all individual voxels that met spectral quality, were analyzed in common brain space with AFNI. Only voxels that were in face to face contact and had significant group differences (p<0.001) in the same direction were included in clusters (cluster-level alpha-value -CCLAV ≤0.05).

Results: Compared to HC, the psychosis group had increased Glx in one cluster (20 voxels) centered in the right posterior cingulate gyrus (CCLAV=0.02). Antipsychotic-naïve patients had increased Glx in right superior temporal areas compared to HC (32 voxels, CCLAV >0.01) and compared to medicated psychotic patients (18 voxels, CCLAV =0.04). Compared to BP-I, the Sz group had increased Glx in the right posterior cingulate gyrus (19 voxels, CCLAV=0.05).

Compared to HC, the psychosis group had increased creatine in two clusters: one large cluster (91 voxels) involving left insula, temporal, parietal and occipital regions (CCLAV=0.02); and a smaller cluster (16 voxels) in bilateral occipital cortex (CCLAV=0.05). The Sz vs HC group had increased creatine in two large clusters: one (185 voxels) involving bilateral occipital regions (CCLAV >0.01); and another (152 voxels) in left insula, temporal, parietal and occipital regions (CCLAV >0.01). Compared to BP-I, the Sz group had increased creatine in two clusters: one large cluster (91 voxels) involving left insula, temporal, parietal and occipital regions (CCLAV=0.02); and a smaller cluster (16 voxels) in bilateral occipital regions (CCLAV=0.05). Compared to HC, the antipsychotic-treated Sz group had increased creatine in four clusters: in the left insula, frontal, parietal and temporal regions (251 voxels, CCLAV >0.01); bilateral occipital (218 voxels, CCLAV >0.01); right insula (86 voxels, CCLAV >0.01); and left frontal (24 voxels, CCLAV =0.05). Finally, compared to antipsychotic-naïve, medicated Sz had increased creatine in one cluster (21 voxels) in the left middle frontal area (CCLAV =0.04).

Conclusions: Unbiased spectroscopic brain examination supports that elevations in Glx in the right STG and posterior cingulate may be critical to the pathophysiology of schizophrenia. Much broader increases in creatine are related to antipsychotic treatment consistent with reduced cortical glucose metabolism described with exposure to these medications. The Glx results supports a model of NMDA hypofunction in areas critical for sound discrimination, early in the illness, regardless of antipsychotic therapy. Postmortem and neuromodulation schizophrenia studies focusing on right STG, may provide critical mechanistic and therapeutic advancements, respectively.

Keywords: Disorders of Glutamate, Schizophrenia, Bipolar, Creatine

Disclosure: UptoDate: Royalties (Self)

M125. Handwriting Kinematics in Schizophrenia Patients Treated With Long-Acting Injectable Atypical Antipsychotics: Results From the Alpine Study

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Background: There remains a clinical need to develop biomarkers of response to antipsychotic therapy. State- and trait-related markers of dopamine activity have been studied, but their utility is often limited by available resources or assessment complexity (eg, neuroimaging). Handwriting movements, which are mediated by striatal dopaminergic mechanisms, may provide insight into the therapeutic response to antipsychotics. Baseline and follow-up standardized, tablet-based handwriting kinematic assessments were administered in the ALPINE study (NCT03345979). ALPINE was a blinded prospective randomized controlled trial of 1 of 2 long-acting injectable (LAI) antipsychotics started during an acute psychotic episode of schizophrenia. The primary efficacy outcome measure of the study was the change in Positive and Negative Syndrome Scale (PANSS) total score from baseline to week 4 within each treatment group. This study provided a platform to assess whether blinded baseline handwriting kinematics may serve as a biomarker for future therapeutic response to acute antipsychotic treatment.

Methods: Adults (aged 18−65 years) with an acute exacerbation of schizophrenia were enrolled as inpatients and randomly assigned to LAI treatment (aripiprazole lauroxil or paliperidone palmitate; combined for this analysis). Patients were discharged 2 weeks later if clinically stable, and then followed for an additional 23 weeks. The primary clinical outcome of interest for this post hoc analysis was the relationship between baseline handwriting kinematics and treatment response at week 4. Treatment response was defined 2 ways: (1) ≥20% reduction from baseline in PANSS total score or (2) ≥2-point improvement from baseline in Clinical Global Impression–Severity (CGI-S) score. Handwriting kinematics were assessed as an exploratory measure at screening (defined as baseline for this analysis) and at several visits over the treatment period. Here, only the baseline handwriting kinematic assessment was analyzed as a potential predictor of PANSS total or CGI-S response at 4 weeks.

Handwriting kinematics were assessed using a non-inking pen/tablet system to capture and record information from 4 handwriting tasks (complex loops, maximum speed circle drawing, overlay circles, and left-right loops) found in prior studies to be proxies of dopamine dysfunction. Two kinematic measures were considered for this exploratory study: peak velocity (lower score corresponds with greater dysfunction) and percentage of non-ballistic movements (%NBM; higher percentage corresponds with greater dysfunction). Because the overlay circles and left-right loops tasks were similar, their scores were averaged for each parameter. Baseline scores for peak velocity and %NBM in the complex loops, maximum speed circle drawing, and combined overlay circles and left-right loops tasks were compared between treatment responders and nonresponders at week 4.

Results: Of the 195 randomized patients, 167 had baseline handwriting kinematic assessments; 143 patients also had a week 4 efficacy assessment and were included in this post hoc analysis. These patients (N = 143) had a mean age of 43 years, and 76% were men; mean PANSS total score at baseline was 94.47. At week 4, PANSS response ≥20% was observed in 47% and CGI-S response ≥2-point improvement was observed in 29% of patients. For either response criterion, responders were similar to nonresponders on severity of baseline psychopathology or prior exposure to anticholinergics. PANSS responders had a lower baseline mean peak velocity on all handwriting kinematic tasks, indicating slower pen movements during completion of tasks at baseline, compared with nonresponders. Specifically, PANSS responders (n = 67) had lower peak velocities compared with nonresponders (n = 76) on the complex loop (8.80 vs 12.15 cm/s) and combined loop (11.86 vs 15.80 cm/s) tasks at baseline. PANSS responders had a greater %NBM compared with nonresponders on the complex loop (57% vs 47%) and combined loop (45% vs 36%) tasks. Similarly, CGI-S responders (n = 41) had lower peak velocities compared with nonresponders (n = 101) on the complex loop (8.99 vs 11.21 cm/s) and combined loop (11.57 vs 14.91 cm/s) tasks at baseline. CGI-S responders had a greater %NBM compared with nonresponders on the complex loop (60% vs 49%) and combined loop (48% vs 37%) tasks. PANSS response did not appear to vary according to other baseline clinical or demographic characteristics.

Conclusions: This post hoc analysis characterized the relationship between baseline handwriting kinematic measures and subsequent therapeutic response to atypical long-acting antipsychotics in patients with acute exacerbation of schizophrenia in the ALPINE study. Patients who achieved either PANSS or CGI-S response after 4 weeks of LAI antipsychotic treatment had lower movement velocities and a greater %NBM in handwriting tasks at baseline than those who did not. The pattern of these findings suggests that patients who will have better PANSS or CGI-S response to antipsychotic therapy at 4 weeks show greater baseline striatal dopamine dysfunction relative to those who have a poorer PANSS or CGI-S response, indicating that handwriting kinematics may be a useful predictor of therapeutic response to antipsychotic treatments.

Keywords: Schizophrenia, Long-Acting Injectable, Handwriting, Extrapyramidal Symptoms

Disclosure: Alkermes, Inc.: Consultant (Self)

M126. BI 425809 Once Daily in Patients With Schizophrenia: Feasibility of Novel Endpoints to Assess Motivation

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Background: Currently, no pharmacotherapy is approved for cognitive impairment associated with schizophrenia (CIAS). N-methyl-D-aspartate (NMDA) receptor hypofunction is believed to be the underlying pathophysiology leading to CIAS. BI 425809, a glycine transporter-1 inhibitor, in development for treatment of CIAS, elevates the concentration of glycine (NMDA co-activator) in the synaptic cleft, and may improve cognitive deficits. Preclinical and clinical studies suggest that this mechanism of action may also enhance motivational deficits that often accompany CIAS. To evaluate this possibility, the BI 425809 Phase II trial (NCT02832037) assessed two aspects of motivation, effort valuation, and reward learning, using the balloon effort task (BET) and probabilistic reversal learning task (PRLT), which are rarely used outside of academic research. The aim of the analyses presented here was to explore the feasibility and validity of the BET and PRLT in the context of a large multicenter trial in patients with schizophrenia.

Methods: This Phase II, double-blind, parallel-group study randomized patients (1:1:1:1:2) with schizophrenia to oral BI 425809 (2, 5, 10, and 25 mg) or placebo, once daily for 12 weeks. In addition to clinical endpoints of cognition, computerized BET and PRLT were evaluated (US sites only). Academic versions of the BET and PRLT were used, with some adaptation of the BET. Patients completing the BET chose whether to complete an easy or hard task for a low- or high-potential reward, respectively. Patients were shown the potential reward associated with each task at the start of each trial and chose which to complete. Patients completing the PRLT chose between two stimuli (one commonly and one rarely rewarded). Once the patients learned the more frequently rewarded stimulus, the reward contingencies reversed, and patients had to modify their value representations through feedback.

Results: Overall, 509 patients were randomized across 11 countries. Among the US population, 194 and 200 patients

completed the BET and PRLT, respectively.Both tasks were well tolerated. Mean proportions of difficult choices across the three reward values in the BET were comparable to prior studies. Patients selected difficult choices more frequently for 100% reward probability trials than for 50% reward probability trials. The task did not demonstrate a ceiling effect. There were no significant clinical symptom differences between patients who selected all difficult tasks compared with those who varied their choice. Patients who completed 0 initial discriminations (non-learners) in the PRLT demonstrated worse cognition than those who completed ≥1 discriminations (learners). There were no differences in clinical symptom severity between subgroups.

All data are presented as mean (SD).

CGI-S, Clinical Global Impressions-Severity; MCCB, Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery; PANSS, Positive and Negative Syndrome Scale; SCoRS, Schizophrenia Cognition Rating Scale; SD, standard deviation.

Conclusions: The BET shows promise for clinical trial use though the difficulty level of the PRLT raises some concerns about this measure. Inclusion of these novel measures in the BI 425809 Phase II trial will enable us to further evaluate their reliabilities and sensitivity to treatment.

Funding: Boehringer Ingelheim International GmbH (NCT02832037; 1346-0009).

Keywords: Balloon Effort Task, Probabilistic Reversal Learning, Schizophrenia

Disclosure: Verasci: Employee (Self)

M127. Central Nervous System Depressant Drugs Induce Paradoxical Hyperactivity in Dopamine Deficient Mice

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Background: Central nervous system (CNS) depressants such as barbiturates, alcohol, and benzodiazepines are drugs that lowers brain activity by increasing activity of gamma aminobutyric acid (GABA) A receptor. CNS depressants can be used as an anesthetic, sedative and sleeping pill. However, it is known that CNS depressants could induce paradoxical reaction which is characterized by acute excitement and excessive movement. Although paradoxical reactions are relatively rare phenomena, mechanisms of paradoxical reactions are unknown, and it is not possible to predict occurrence of the reactions in advance. Interestingly, we found that dopamine deficient (DD) mice became hyperactive immediately after injection of sedative dose of pentobarbital for wild type mice. DD mice are tyrosine hydroxylase (TH) knockout mice with the concomitant restoration of TH expression under control of the dopamine beta-hydroxylase (DBH) promoter to prevent the loss of epinephrine and norepinephrine. This finding raised the possibility that DD mice might show paradoxical reactions by CNS depressants and be able to use as an animal model for paradoxical reactions. Therefore, in the present study, we investigated the effect of CNS depressants in DD mice. Furthermore, we explored the mechanisms of paradoxical reactions in DD mice.

Methods: DD mice were maintained with paste diets with L-DOPA and benserazide (DOPA decarboxylase inhibitor). L-DOPA and benserazide were removed from diets 72 hours prior to experiments. Wild type (WT) mice ($n = 7$) and DD mice ($n = 7$) received pentobarbital (30 mg/kg), ethanol (1.5 g/kg), diazepam (3 mg/kg) or vehicle by intraperitoneal injection and then change of locomotor activity was monitored for 30 minutes using supermex apparatus. For immunohistochemistry, WT and DD mice were treated with diazepam or vehicle and stayed in supermex apparatus for 1 hour. Then brains were collected after perfusion with 4% paraformaldehyde (PFA) and post-fixed overnight in 4% PFA solution. Fixed brains were embedded into paraffin and cut into 7.5 um sections. The immunohistochemical analysis was performed using rabbit polyclonal c-Fos antibody.

Results: Pentobarbital, ethanol, and diazepam inhibited locomotor activity in WT mice, indicating that CNS depressants could induce sedative effects as expected. However, DD mice became hyperactive immediately after treatment of those CNS depressants contrary to WT mice. These results suggested that CNS depressants induced paradoxical reactions in DD mice. Since diazepam brought most obvious hyperactivity in DD mice among the drugs we investigated, we analyzed c-Fos expression in WT and DD mice brains as a marker of neuronal activity 1 hour after diazepam and vehicle treatment. Diazepam treatment decreased c-Fos immunopositive neurons in WT type mice especially in the cortical regions including motor cortex, somatosensory barrel cortex, and visual cortex compared to vehicle treatment. On the contrary, diazepam treatment increased c-Fos immunoposive neurons compared to vehicle treatment in those regions. These results indicated that diazepam could not lower neuronal activity in DD mice.

Conclusions: DD mice became hyperactive by CNS depressants treatment. CNS depressants normally suppressed neuronal activities as seen in WT mice. However, neuronal activity was upregulated in DD mice by diazepam treatment, indicating that CNS depressants did not inhibit but excite neuronal activity in DD mice. Although the mechanisms of opposite effects of CNS depressants in DD mice have not been determined yet, it is possible that GABA might change inhibitory to excitatory, or excitation and inhibition balance might be disturbed in DD mice. It is also possible that to determine the mechanisms of hyperactivity in DD mice induced by CNS depressants might lead to elucidation of cause of paradoxical reactions.

Keywords: Dopamine, CNS Depressant Drugs, Mouse Behavior, Hyperlocomotion

Disclosure: Nothing to disclose.

M128. Antipsychotic Efficacy and Associated Changes in Prefrontal Neurotransmitter Levels: Preliminary Results From a 7-Tesla Neuroimaging Study of Early Phase Schizophrenia

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Background: Antipsychotic (AP) drugs are the mainstay for the treatment of psychosis, yet the neural and molecular processes associated with their efficacy remain largely unknown. Previous work has shown that increases in functional interactions between the prefrontal cortex and the striatum underlie successful AP treatment. However, it remains unknown whether efficacious treatment alters prefrontal neurotransmitter systems. The following study examined whether changes in a ratio between excitatory glutamate (GLU) and inhibitory γ-aminobutyric acid (GABA) are associated with antipsychotic treatment response in patients with early phase schizophrenia (SZ).

Methods: Patients with the following inclusion criteria were examined: DSM defined diagnosis of schizophrenia, schizophreniform or schizoaffective disorder; age 18-40 years; current positive symptoms rated ≥4 (moderate) on one or more of psychosis-relevant Brief Psychiatric Rating Scale; antipsychotic medication use for a cumulative lifetime period of ≤2 years. Patients underwent 7 Tesla (7T) MRI scanning during initiation of treatment with an antipsychotic drug as per routine care, underwent biweekly clinical evaluations for symptomatic changes and medication adherence, and were rescanned after 6 weeks of

treatment. Scans were acquired at the University of Pittsburgh on a Siemens 7T human imaging/spectroscopy optimized scanner. A magnetic resonance spectroscopic imaging oblique slice was acquired positioned to include portions of the anterior cingulate cortex (ACC) based on our previous work (Sarpal et al., 2015). GLU and GABA data were processed by in-house scripts that calculate their concentrations versus creatine. GLU/GABA ratios were calculated for each scan and compared with psychotic symptoms trajectories. A linear mixed-model analyses was used to calculate slopes of the trajectories of positive symptoms for each participant. These slopes were then compared with percent change in our GLU/GABA ratios: (% change = (pretreatment GLU/GABA – posttreatment GLU/GABA)/(pretreatment GLU/GABA)).

Results: A total of 13 patients in this preliminary cohort had GABA and GLU measurements with superior quality and were included in our analysis. Spectra with Cramér-Rao lower bound (CRLB) values<10 for GLU and <15 for GABA were included. A negative relationship was observed between slope of positive symptom reduction and percent change in GLU/GABA ratio in the ACC ($r = -0.63$; $p = 0.02$). Thus, patients with a better response to treatment (a more negative slope) demonstrated decreases in GLU and increases in GABA resulting in a greater reduction in GLU/GABA ratio. The CRLB for this group was 4.1 for GLU and 10 for GABA.

Conclusions: Our finding of a treatment-related decrease in GLU/GABA ratio could be driven by both increased GABA and decreases in GLU and may reflect an engagement of GABAergic neurons in the ACC, which has been associated with negative symptoms. To our knowledge, these results are the first to examine changes in prefrontal GABA concentrations across antipsychotic treatment with the high degree of accuracy provided by 7T imaging. Overall, results from this work may lead to prognostic signatures of treatment response versus non-response, and quantitatively address our imprecise and empirical approach to treatment of early phase schizophrenia.

Keywords: Schizophrenia, Antipsychotic Response, GABA, Glutamate, MRSI

Disclosure: Nothing to disclose.

M129. Relationship Between Pimavanserin Exposure and Psychosis Relapse in Patients With Dementia-Related Psychosis: Clinical Results and Modeling Analysis From the Phase 3 HARMONY Study

Abstract not included.

M130. A Ketamine Model of Thalamic Dysconnectivity for Characterizing Clinical Features of Schizophrenia

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Background: N-methyl-D-aspartate receptor (NMDAR) hypofunction may account for many of the cognitive and clinical symptoms of schizophrenia (SZ). Resting state fMRI (rsFMRI) studies have repeatedly demonstrated a pattern of thalamic dysconnectivity in SZ involving excessive connectivity with sensory regions and deficient connectivity with frontal and cerebellar regions. NMDAR hypofunction in SZ may underlie thalamic dysconnectivity and its clinical sequelae, but direct evidence is lacking. The NMDAR antagonist ketamine, when administered at sub-anesthetic doses to healthy volunteers, induces transient SZ-like symptoms and has recently been shown to disrupt rsFMRI thalamic connectivity. However, the extent to which the pattern of ketamine thalamic dysconnectivity resembles the SZ pattern has not been directly tested. The current study first characterized ketamine's effect on rsFMRI thalamic connectivity and examined whether ketamine's effects were mediated by excess glutamate release. We then tested the hypothesis that the thalamic dysconnectivity brain map representing ketamine's acute NMDAR hypofunction effect would have higher, and more positive correlations with individual subject rsFMRI thalamic dysconnectivity maps from patients with SZ, compared to healthy controls. Further, we examined whether subject correlations with ketamine's thalamic dysconnectivity pattern were associated with cognitive function and clinical symptoms.

Methods: In a double-blind placebo-controlled crossover design implemented across three randomly ordered test days, intravenous ketamine (0.23 mg/kg bolus followed by 0.58mg/kg/hour infusion) or placebo saline was administered to healthy male volunteers ($n = 18$) while undergoing rsFMRI. Using a seed-based connectivity approach, we examined the effects of ketamine on thalamic connectivity during rsFMRI. To test if ketamine's effects were mediated by proximal NMDAR blockade or downstream increases in glutamate release, we examined whether pre-treatment with oral lamotrigine (200 mg) a glutamate release inhibitor, blocked or attenuated ketamine-induced changes in thalamic connectivity. We then conducted a series of analyses to evaluate the extent to which the pattern of ketamine thalamic dysconnectivity was relevant to clinical features of SZ. To this end, we created an fMRI template map representing ketamine's thalamic dysconnectivity effects (i.e., z-score deviations from placebo condition). Individual subject rsfMRI thalamic connectivity maps from our previously published FBIRN study comprising patients with SZ ($n = 183$; 75% male) and healthy controls (HC; $n = 178$; 71% male) were z-transformed (i.e., deviations from HC mean) and then correlated with the ketamine effect map. This yielded a single correlation for each subject reflecting the similarity of their map with ketamine's effect map. These "ketamine similarity coefficients" were then compared between SZ and HC, and were correlated with cognitive measures (CMINDS working memory and attention scores) and clinical symptom ratings.

Results: Compared to saline, ketamine produced hyper-connectivity between the thalamus and 15 non-contiguous clusters in motor cortex, temporal cortex, occipital cortex, and other sensory regions (voxel-wise cluster defining threshold of $p < .001$, corrected-cluster significance threshold of $p < .05$). Hyper-connectivity in these clusters was not significantly attenuated by lamotrigine pre-treatment. Ketamine similarity coefficients were significantly higher and more positive in SZ than in HC ($t = 8.06$, $p < .001$). Further, larger and more positive ketamine similarity coefficients were associated with worse attention in HC ($\beta = -.31$, $p = .005$) but not in SZ (supported by a significant slopes difference test, $F = 8.57$, $p = .004$), whereas no associations with working memory were observed in either group. In SZ, higher positive ketamine similarity coefficients correlated with more severe hallucinatory symptoms (Spearman rho = .25, $p = .007$), but were unrelated to negative symptoms.

Conclusions: We observed a strong overlap between ketamine-induced thalamic changes and the thalamic dysconnectivity pattern observed in SZ, including hyper-connectivity with motor and sensory regions. Direct correlation of ketamine's thalamic dysconnectivity brain map with the maps from individual subjects provided quantitative evidence to support this qualitative observation. This result supports the hypothesized role of NMDA receptor hypofunction as a pathophysiological mechanism contributing to thalamic dysconnectivity in SZ. In SZ patients, greater similarity to the ketamine thalamic dysconnectivity pattern was associated with more severe hallucinations. While similarity to the ketamine pattern was associated with poorer attention in healthy controls, no associations with cognition were observed in SZ

patients. Ketamine-induced sensory and motor hyper-connectivity was not attenuated by pre-treatment with lamotrigine, suggesting that its effect resulted from primary NMDA antagonism and not from downstream hyperglutamatergia at non-NMDA sites. Taken together, NMDA antagonism via ketamine induces a thalamic dysconnectivity pattern that successfully models the hypothesized role of NMDA hypofunction in the pathophysiology of SZ thalamic dysconnectivity and associated clinical characteristics.

Keywords: Ketamine, Thalamus, Resting State Functional Connectivity, Hallucinations, NMDA Antagonists

Disclosure: Nothing to disclose.

M131. Abnormal Anterior Cingulate Activation Revealed by a Novel Ankle-Shock Stress Task in Schizophrenia and Association With Depression and Psychosis

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Background: Etiologically, both depression and schizophrenia (SZ) have substantial environmental contributions, including exposure to stress, although it remains unclear how they interact with relevant brain circuitry to lead to psychopathology. Brain areas responsible for assessment of and response to stress have been implicated in both depressive and psychotic disorders. We hypothesized that dysfunctional activation in key stress-sensitive frontal and limbic brain areas in response to stress underlies a component of both psychotic and depressive symptoms in SZ.

Methods: We developed a highly translational stress induction paradigm that is human MRI compatible and ethical. In animal studies, foot shock using electric current is a classic approach to induce stress. We developed an analogous ankle shock threat (AST) paradigm in which a small amount of current is delivered to the ankle to generate a shock, generating anticipatory stress in response to a potential threat. Eighteen schizophrenia spectrum disorder patients (SZ, 10/18 male) and 12 adult community controls (CC, 9/12 male) were included in this study. Structured Clinical Interview for DSM-IV was completed to verify psychiatric diagnoses. Symptoms were measured by the Brief Psychiatric Rating Scale (BPRS) and the Maryland Trait and State Depression scale (MTSD). fMRI data was collected using a 3-T Siemens Prisma scanner and 64-channel coil, with an electrode attached to one ankle of each participant. A pre-determined voltage was applied for 0.1s during the task (similar to the experience of touching a surface with static electricity, using the Transcutaneous Aversive Stimulator, Coulborne Instruments). There are 3 conditions in this paradigm: (1) a shock condition in which a few random shocks are delivered while a color sign on the screen indicates shocks are possible; (2) a threat condition in which the same color is present but no shock is given; and (3) a safe condition in which no shock is given and the color indicates safety. All image preprocessing includes slice timing corrections and were volume co-registered. Images were linearly detrended, normalized into MNI standard space, and spatially smoothed (FWHM=8mm) using SPM12. First level models were developed for each subject by entering all the volumes into a single analysis regressing the "shock", "threat" and "safe" conditions. The contrast of interest was the threat - safe condition to study how the brain is processing the threat of the unpleasant shock but without the interference of the actual shock. All experimental protocols were approved by the University of Maryland Baltimore IRB.

Results: Nominally significant group differences were found in multiple regions from the threat - safe contrast, with left anterior cingulate cortex (LACC) found to have the strongest group differences after correction for family-wise error ($p<0.05$). The strength of this stress-induced ACC activation was significantly correlated with trait depression scores measured by MTSD and psychosis scores from the BPRS in SZ (both $p<0.01$).

Conclusions: This novel translational stress-based task can reliably engage and reveal deficient activation in SZ in the ACC, a region known to be associated with error-monitoring, social and emotional evaluation, and other reward and affective processing. These findings suggest that aberrant ACC activation in evaluation of an anticipated stressful threat may increase the vulnerability to more severe depressive and psychotic symptoms in SZ.

Keywords: Stress Reactivity, Psychosis, Depression, Anterior Cingulate Cortex (ACC), Functional MRI (fMRI)

Disclosure: Nothing to disclose.

M132. High Gamma Activity During NREM Sleep in Clinical High Risk for Psychosis Individuals

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Background: Youth at Clinical High Risk (CHR) are a unique population enriched for precursors of major psychiatric disorders. Sleep disturbances have been reported extensively in CHR individuals. However, there is a dearth of studies investigating the neurophysiological underpinning of these disturbances.

Methods: The present study examined differences in sleep EEG spectral power between CHR and healthy control (HC) groups, as well as their relationship with sleep disturbances and clinical symptoms in CHR individuals.

Whole night sleep high density (hd)-EEG recordings were collected in twenty-two CHR and twenty HC. Sleep architecture was computed to assess for sleep disturbances in CHR relative to HC groups. Welch's modified periodogram method in 2-s Hamming windows (with 50% overlap) was utilized to decompose the EEG time series data during NREM sleep into the frequency domain in the 0.5–40 Hz range. Sleep EEG power spectra in five frequency bands: Delta (1-4.5 Hz); Theta (4.5-8 Hz); Alpha (8-12 Hz); Beta (12-25 Hz); and Gamma (25-40 Hz) was computed and compared between CHR and HC groups. Furthermore, we performed correlation analyses between sleep EEG power spectra and sleep architecture parameters as well as clinical symptoms, assessed with the Scale of Prodromal Symptoms (SOPS), in CHR patients.

Results: CHR individuals had significantly higher Wakefulness After Sleep Onset (WASO) compared to HC participants ($p = 0.04$). Furthermore, in CHR NREM sleep gamma EEG power was significantly increased in a large fronto-parieto-occipital area ($p = 0.01$) relative to HC. Additionally, increased NREM gamma power in medial fronto/parietal areas correlated with worse SOPS negative symptoms, whereas higher NREM gamma activity in lateral fronto-occipital regions was associated to more WASO.

Conclusions: Altogether, these findings indicate that increased EEG gamma activity during NREM sleep may represent a neurophysiological biomarker for some of the objectively assessed sleep disturbances and clinical symptoms of CHR individuals.

Keywords: Sleep Architecture, NREM EEG Gamma Power, Clinical High-Risk of Psychosis, Sleep Disturbance

Disclosure: Nothing to disclose.

M133. Involvement of Presynaptic Grin2d-Containing Nmda Receptors in Tonic GABA Release From Immature Fast-Spiking Interneurons

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Background: Due to the challenges of identifying precursors of parvalbumin (PV)-expressing GABAergic fast-spiking (FS) interneurons in early postnatal development, roles of N-methyl-D-aspartate (NMDA) receptors (NMDARs) in immature FS neurons are poorly understood. Hablitz JJ et al (PLosOne, 2011) reported that NMDARs are present on GABAergic cells' presynaptic terminals between postnatal day 12 (P12) to P15, but not between P21 to P25, in rat frontal cortex, mediating miniature-IPSC potentiation at postsynaptic pyramidal neurons. Lack of IPSC potentiation or tonic facilitation of GABA release from PV-positive FS neurons has also been reported in P27 and after (Pafundo DE et al, Biol Psychiatry, 2018). However, significance of tonically active presynaptic NMDARs in FS neurons and their subunit composition has been unexplored. A hint of specific involvement of Grin2d subunit of NMDARs was suggested from a study that pharmacological blockade of Grin2c/2d-containing NMDARs in P7-9 by Grin2c/2d subunit antagonist DQP-1105 resulted in deficits in GABA release ex vivo and hyperexcitability at P21 in vivo (Hanson E et al, J. Neurosci. 2019). The Grin2d subunit is highly expressed in PV neurons and somatostatin (SST)-positive interneurons after P4 and peaking at the second postnatal week, but not in pyramidal cells in the cortex (Monyer H et al, Neuron 1994; Standaert DG et al, Brain Res Mol Brain Res. 1996), whereas Grin2c subunit is expressed in glial cells in the cortex (Alsaad HA et al, Neurochem Res. 2019). We hypothesize that Grin2d-containing presynaptic NMDARs in immature FS neurons are involved in tonic facilitation of GABA release.

Methods: ANIMAL Ppp1r2cre(+/−)/Grin1(f/f) KO mice (namely, Grin1 mutant mice) were generated as previously described (Belforte et al, Nat Neurosci. 2010), displaying several schizophrenia-like phenotypes (Nakazawa K et al, 2017). In this mutant mice, Grin1 gene was disrupted in ~50% of cortical and hippocampal interneurons, ~70% of which are PV containing, from the second postnatal week using mice expressing cre recombinase under control of the Ppp1r2 promoter. Importantly, 84% of cortical PV neurons received Cre recombination (i.e., Grin1 deletion), whereas frequency of Cre recombination in any other non-PV-type interneurons are at most 15% in the cortex. Ppp1r2cre(+/-)/Grin2d KO mice were also generated by crossing a floxed-Grin2d mice (Shelkar GP et al, Sci Rep. 2019) to Ppp1r2cre line.

EX VIVO ELECTROPHYSIOLOGY: We conducted spontaneous IPSC (sIPSC) recording from auditory cortex L2/3 pyramidal neurons at P11-P14 in the presence of NMDAR blocker MK-801 in the recording pipette.

Results: First, to confirm the tonic facilitation of GABA release from GABA neurons at P11-14 in mice, we recorded the sIPSC events from auditory cortex L2/3 pyramidal neurons in the presence of NMDAR blocker MK-801 (2 mM) in the recording pipette. We found in the control mice that bath application of NMDA (30 μM; $p<0.05$, t-test) and AP5 (50 μM; $p<0.01$, t-test) increase and decreases, respectively, the sIPSC frequency, but not sIPSC amplitudes (cell number n≥5 from 2-3 animals, both sexes), suggesting that tonic NMDARs are present in immature FS neurons at P11-P14, tonically releasing the GABA. This effect disappeared in Ppp1r2cre/Grin1 KO mutant mice at the same ages (cell number n≥5 from 2-3 animals, both sexes). Next, bath application of Grin2c/2d antagonist DQP-1105 (20 μM) significantly decreased the sIPSC frequency in the control mice at P11-P14 ($p<0.05$, t-test). In contrast, Ppp1r2cre/Grin2d KO mouse line, which was generated by crossing a floxed-Grin2d mice to Ppp1r2cre line, exhibited a minimal reduction in sIPSC frequency by DQP-1105 These results suggest that (1) Grin2d is deleted in the majority of FS neurons in the Grin2d KO mice, and that (2) Grin2d-containing NMDARs are involved in tonic facilitation of GABA release in controls.

Conclusions: Our results suggest that Grin2d subunit of NMDARs in immature FS neurons is crucial for tonic GABA release.

Keywords: GABA Neuron, NMDA Receptor, Transgenic Mice

Disclosure: Nothing to disclose.

M134. Gamma Oscillations Predict Pro-Cognitive and Clinical Response to Auditory-Based Cognitive Training in Schizophrenia

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Background: Cognitive impairments are pervasive and disabling features of schizophrenia. Targeted Cognitive Training (TCT) is a promising, evidence-based intervention designed to stimulate auditory-based learning and plasticity in healthy neural systems mediating higher-order cognition. While TCT is efficacious at the group level, individual responses to this resource- and time-intensive intervention vary considerably. Gamma oscillatory biomarkers can potentially be used to identify TCT-sensitive individuals and develop biomarker-guided treatment approaches that may help parse the heterogeneity of TCT responses.

Methods: Forty-two schizophrenia patients were recruited from a long-term residential treatment facility. All participants received medication management, individual and group therapy, and engaged in structured social activities per standard of care rehabilitative programming. Schizophrenia patients were randomized to receive either 1-h of TCT ($n = 21$) or 1-h of computer games (TAU; $n = 21$). The TCT group additionally completed 30-h of cognitive training exercises over 3 months. The auditory steady state response paradigm was used to elicit gamma-evoked responses at baseline (T0) and after participants underwent either 1-h (T1) of TCT or 1-h of or computer games (TAU). MATRICS Consensus Cognitive Battery was used to assess cognition. Clinical symptoms were assessed using the Scale for the Assessment of Positive (SAPS) and Negative (SANS) Symptoms.

Results: Evoked gamma power measured at baseline predicted cognitive gains after a full course of TCT (MCCB $R2=0.31$). Change in evoked gamma power after 1-h TCT exposure predicted improvement in both positive (SAPS $R2=0.40$) and negative (SANS $R2=0.30$) symptoms. These relationships were not observed in the TAU group (MCCB, SAPS, SANS all $R2<0.06$).

Conclusions: The capacity to support gamma oscillations, as well as the plasticity or 'capacity for change' of the underlying circuitry, likely reflect neural mechanisms underlying the effectiveness of TCT and may be used to predict individualized treatment outcomes. These findings suggest that gamma oscillatory biomarkers applied within the context of experimental medicine designs can be used to personalize individual treatment strategies for pro-cognitive interventions in patients with schizophrenia and add to the growing body of evidence that neurophysiologic biomarkers may advance the sensitivity of predictive algorithms used to detect therapeutic response in heterogeneous clinical samples.

Keywords: Schizophrenia (SCZ), Auditory Steady-State Response, Cognitive Remediation

Disclosure: Nothing to disclose.

M135. Neural Compensation Between Prefrontal Cortex Regions in a Novel Operant Devaluation Task in Rats

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Background: The reinforcer devaluation task is often used to model flexible goal-directed action, the ability to adaptively modify behavior when the value of a reinforcer changes. Deficits in goal-directed action are reported in multiple neuropsychiatric conditions, including schizophrenia. However, dysfunction is not always apparent in early stages of schizophrenia, possibly due to neural compensation. We designed a novel devaluation task in which goal-directed action could be guided by stimulus-outcome (S-O) [presumably orbitofrontal cortex (OFC)-mediated] or response-outcome (R-O) associations [presumably prelimbic cortex (PL)-mediated] to maintain. Therefore, if our task is able to model neural compensation, damage to either PL or OFC should not impair devaluation because the non-damaged region can compensate for the loss using the alternate strategy.

Methods: In Experiment 1, male and female rats (n = 44) received bilateral OFC, PL, combined OFC+PL, or sham lesions and then completed our devaluation task. In Experiment 2, male rats (n = 31) received bilateral PL or sham lesions and then completed behavioral training. During devaluation testing, rats were divided into Cue Normal or Cue Switch test conditions. In Cue Switch test conditions, the cuelight and spatial lever location predicted conflicting outcomes so we can determine whether rats are devaluing using an S-O or R-O strategy. For both experiments, we used mixed-effects modeling to analyze the results.

Results: In Experiment 1, Sham, OFC, and PL lesioned rats showed intact devaluation, whereas the OFC+PL lesion group exhibited impaired devaluation. In Experiment 2, Sham rats exhibited normal devaluation performance (based on the lever-location) in both Cue Normal and Cue Switch conditions. PL lesioned rats showed normal devaluation performance in the Cue Normal condition but a reverse devaluation effect in the Cue Switch condition suggesting the PL-lesioned rats were devaluing based on the outcome predicted by the cuelight.

Conclusions: Our results suggest that our devaluation task can successfully model neural compensation between OFC and PL. This research demonstrates a method to study how functional neural circuitry is subtly altered like in early stages of schizophrenia.

Keywords: Devaluation, Lateral Orbitofrontal Cortex, Prelimbic Cortex, Prefrontal Cortex, Goal-Directed Behaviors

Disclosure: Nothing to disclose.

M136. Contribution of Hippocampal Projections to Discrete Symptoms of Schizophrenia

Abstract not included.

M137. Daridorexant is Efficacious in Improving Sleep as Well as Daytime Functioning in Insomnia Patients

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Background: Insomnia disorder is characterized by nighttime sleep disturbance and daytime functioning impairment; current medications do not address the latter. To do both, pharmacotherapy should ideally induce restorative sleep of sufficient continuity and duration, and drug action should rapidly dissipate to avoid next-morning effects. To address unmet need, 2 large confirmatory double-blind, placebo-controlled, parallel-group phase 3 trials of the efficacy and safety of daridorexant at 3 doses were conducted.

Methods: Adult (≥18-64 y) and elderly (≥65 y) patients with insomnia disorder, as defined per DSM-5 criteria were randomized 1:1:1 in study 1 (NCT03545191, 930 patients) to daridorexant 25 mg, 50 mg or placebo (PBO) and in study 2 (NCT03575104, 924 patients) to daridorexant 10 mg, 25 mg, or PBO. After a week single-blind PBO run-in, double-blind treatment was administered nightly for 3 months (M), followed by a week single-blind PBO run-out period. Primary endpoints were change from baseline (BL) in wake time after sleep onset (WASO; min) and latency to persistent sleep (LPS; min) measured by polysomnography at M1 and M3. Secondary endpoints were change from BL in subjective total sleep time (sTST; min) and daytime functioning at M1 and M3 using the sleepiness domain score (0 to 40) of the new Insomnia Daytime Symptoms and Impacts Questionnaire (IDSIQ) developed according to FDA guidance.

A Bonferroni-based gatekeeping procedure was used to control the study-wise type I error rate (5%) in daridorexant vs PBO comparisons for the primary and secondary endpoints at M1 & M3. Null hypothesis testing followed a hierarchical gatekeeping strategy moving from M1 to M3 for higher dose daridorexant vs PBO and then from M1 to M3 for lower dose daridorexant vs PBO. Based on a two-sample z-test, 900 subjects gave 98.9% power to detect an effect size of 0.37 for single hypothesis testing, and 90% power to detect an effect size of 0.37 for testing 9 independent null hypotheses. Sample size accounts for the initial Bonferroni correction, and assumes standard deviations per treatment group for change from BL in WASO, LPS, sTST and IDSIQ sleepiness domain score of 40, 40, and 54 mins and 5, respectively.

Other endpoints included the IDSIQ total score (0 to 140 points), Alert/Cognition and Mood domains (scores 0 to 60 and 40 resp.). Safety assessments included morning sleepiness by daily visual analog scale - (VAS), rebound and withdrawal.

Results: Demographic and BL characteristics were similar in both studies (~40% ≥65 y, ~60% female). In study 1, change from BL was significant vs PBO for daridorexant 25 and 50 mg in WASO at M1 (18.4, -29.0 vs -6.2) and M3 (23.0, -29.4 vs -11.1) and in LPS at M1 (-28.2, -31.2 vs -19.9) and M3 (-30.7, -34.8 vs -23.1) (all $p<0.002$).

In study 2, WASO change from BL was significant vs PBO for daridorexant 25 mg at M1 (-24.4 vs -12.6; $p = 0.0001$) and M3 (-24.3 vs -14.0; $p = 0.0028$) but not for 10 mg (M1: -15.3 vs -12.6; M3: -16.0 vs -14.0). Daridorexant 10 mg and 25 mg non-significantly reduced LPS at M1 (-22.6, -26.5 vs -20.0) and M3 (23.1, -28.9 vs -19.9).

In study 1, change from BL in sTST for 25 and 50 mg was significant vs PBO at M1 (34.2, 43.6, vs 21.6; p-values ≤0.0013) and M3 (47.9, 57.7, vs 37.9; p-values ≤0.0334). In study 2, improvements from BL in sTST were significant vs PBO for 25 mg at both time points (M1: 43.8 vs 27.6, M3: 56.2 vs 37.1, both $p<0.0001$) but not for 10 mg (M1: 41.0 vs 27.6; M3: 50.7 vs 37.1).

IDSIQ sleepiness domain scores in study 1, significantly improved (decreased) from BL vs PBO for daridorexant 50 mg at M1 (-3.8 vs -2.0; $p<0.0001$) and M3 (-5.7 vs -3.8; $p = 0.0002$); 25 mg improvements were not significant vs PBO at M1 (-2.8 vs -2.0) or M3 (-4.8 vs -3.8). In study 2, IDSIQ sleepiness domain score reductions from BL for 10 and 25 mg were not significant vs PBO (M1: -3.2, -3.5 vs -2.8; M3: -4.8, -5.3 vs -4.0).

Daridorexant 25 mg, 50 mg and PBO decreased scores for IDSIQ total (BL: 73.1, 74.5, 73.6; M1: 9.2, -13.5, -6.2; M3: -15.6, -19.3, -12.1), IDSIQ mood domain (BL: 19.2, 19.8, 19.1; M1: -2.7, -3.9, -1.2; M3: -4.2, -5.4, -2.6), and IDSIQ alert/cognition domain (BL: 31.7, 32.3, 32.2; M1: -3.8, -5.8, -3.1; M3: -6.6, -8.2, -5.7) more than PBO in study 1 (NS).

In study 2, daridorexant 10 mg 25 mg also decreased IDSIQ total (BL: 75.1, 73.1, 74.5; M1: 10.3, -11.9,-8.8; M3: -15.7, -17.3, -13.1), IDSIQ mood domain (BL: 19.8, 19.2, 19.7; M1: -2.8, -3.5, -2.3; M3: -4.3, -4.8,-3.5), and IDSIQ alert/cognition domain (BL: 32.5, 31.7, 32.2; M1: -4.3, -4.9, -3.8; M3; -6.7, -7.2, -5.6) scores more than PBO (NS).

In both studies adverse events (AEs) were similar in all treatment groups; most frequent were nasopharyngitis and headache. Morning VAS did not show signs of morning sleepiness. There were no AEs indicative of withdrawal. Mean WASO, LPS, and sTST remained improved at run-out vs BL, indicating an absence of rebound. In Study 1, a fatal outcome (cardiac arrest, 25 mg) not related to treatment was observed. In both studies, few AEs of special interest were observed; complex sleep behavior (study 1: 2 on 25 mg, 1 on 50 mg; study 2; 3 on 25 mg), AEs related to excessive daytime sleepiness (study 1: 1 on PBO, 2 on 25 mg, 1 on 50 mg; study 2: 1 on PBO, 2 on 10 mg, 7 on 25 mg), and suicidal ideation/self-injury (study 2: 1 on 10 mg, 1 on 25 mg).

Conclusions: Daridorexant significantly improved LPS (50 mg, 25 mg study 1), WASO (50 mg, 25 mg both studies) and sTST (50 mg and 25 mg both studies), and daytime functioning (50 mg). Overall, 50 mg daridorexant appeared to provide more robust efficacy than the lower doses without affecting the safety profile.

Keywords: Insomnia, Daridorexant, Dual Orexin Receptor Antagonist, Daytime Functioning, Patient Reported Outcomes

Disclosure: Idorsia Pharmaceuticals Ltd.: Employee, Stock/Equity (Self)

M138. Effects of the Stress Peptides PACAP and CRF on Sleep in Male and Female Mice

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Background: Problems with sleep are characteristic of many psychiatric disorders, including major depressive disorder (MDD), generalized anxiety disorder (GAD), and post-traumatic stress disorder (PTSD). Previous work from our lab demonstrated that chronic social defeat stress in mice produces effects on sleep that resemble those seen in depression, including increases in REM sleep and decreases time awake. Some of these effects, including REM sleep fragmentation, persist well beyond the termination of stress exposure. While stress is known to alter patterns of sleep, the contribution of stress-related peptides pituitary adenylate cyclase-activating polypeptide (PACAP) and corticotropin-releasing factor (CRF) on alterations of sleep after stress has not been fully characterized. Administration of either PACAP or CRF produces stress-like effects on behavior (e.g., enhanced acoustic startle, reduced social interaction and motivation, disrupted attention), although the effects of PACAP tend to be longer-lasting than those of CRF. Here, we examined the effects of PACAP and CRF on sleep architecture using a wireless telemetry system that enables long periods of continuous data collection in freely moving mice.

Methods: We used male and female C57BL/6J mice to observe the effects of PACAP and CRF on sleep and diurnal rhythms. Mice were implanted with ICV cannula and wireless telemetry transmitters (DSI) that allowed untethered, continuous recordings of EEG, EMG, body temperature and locomotor activity. PACAP (0.25 µg), CRF (1.0 µg) or vehicle (aCSF) were administered by intracerebroventricular (ICV) infusion in 1.0 µl over 4 min. These doses were selected because they have effects on other behaviors (startle, elevated plus maze). Data were collected for 4 days prior to treatment (Baseline) and for another 10 days after infusion (Test). Effects of PACAP and CRF on three vigilance states—active/wake, slow wave sleep, and REM sleep—were quantified. For the initial analyses, duration and bouts of each vigilance state were calculated as percent change from Baseline at 2 time points (24 hr and 1 week after infusion), although data for this entire time period were collected and stored. Changes in vigilance state by Condition (Vehicle, PACAP or CRF) and Timepoint (Baseline, 24 Hours, and 1 Week) were compared with two-way ANOVAs. Significant effects were further examined with Tukey's post-hoc comparisons.

Results: Across the entire 24 hr period after ICV infusion, mice that received PACAP spent significantly more time in slow wave and REM sleep compared with their own Baseline ($P<0.01$) as well as with Vehicle- and CRF-treated animals ($P's< 0.05$). PACAP-treated animals also spend less time awake compared with their own Baseline ($P< 0.01$), as well as with Vehicle- and CRF-treated mice ($P's< 0.01$). At 1 Week after administration, PACAP treated mice continued to spend more time in REM sleep than at Baseline ($P< 0.05$). In contrast, CRF did not appear to affect time spent in each vigilance state across these 24-hr periods, with no significant changes from Baseline or compared with Vehicle animals. However, both PACAP and CRF caused alterations in sleep patterns specifically during the dark phase of the light cycle, when mice are typically active and awake: compared with Baseline, Dark-phase REM was increased in both PACAP- ($P< 0.01$) and CRF-treated mice ($P< 0.05$).

Conclusions: The effects of PACAP on sleep have broad similarities with those produced by chronic social defeat stress. Specifically, PACAP produces persistent effects on REM sleep that remain evident for at least 1 week after administration. CRF, on the other hand, does not produce the same types of disruptions in sleep patterns after stress at doses that produce stress-like effects on other behaviors, although effects are detectable during certain periods of the light/dark cycle. Further work will investigate potential differences between males and females on these endpoints. Understanding the impact that these stress-related peptides have on sleep in both males and females will improve the development and assessment of novel therapeutics for stress-related psychiatric disorders.

Keywords: Sleep, REM Sleep, Corticotropin-Releasing Factor (CRF), PACAP

Disclosure: Nothing to disclose.

M139. Assessment of the Abuse Potential of the Dual Orexin Receptor Antagonist Lemborexant Compared With Suvorexant and Zolpidem

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Background: Lemborexant (LEM) is a dual orexin receptor antagonist approved in the United States and Japan at doses up to 10 mg for the treatment of insomnia in adults. Previous studies have demonstrated that LEM is not associated with physical dependence or reinforcing effects in animal models. Here, the drug abuse potential of LEM was examined in a phase 1 clinical trial according to guidance from the US Food and Drug Administration.

Methods: This was a single-center, single-dose, randomized, double-blind, 6-way crossover study (NCT03158025; E2006-A001-103). Abuse potential was assessed for oral doses of LEM (10mg [LEM10]; 20mg [LEM20]; 30mg [LEM30]) vs placebo (PBO) and 2 active comparators with known drug liking, zolpidem immediate release 30mg (ZOL) and suvorexant 40mg (SUV). Subjects were healthy, nondependent, recreational sedative users who were able to discriminate/like the effects of both SUV and ZOL from PBO during a qualification phase. During the treatment phase, study drug was administered following an overnight fast, with each treatment separated by ≥14 days. Abuse potential was assessed by the peak maximum effect (Emax) on the 100-point bipolar "at this moment" Drug Liking Visual Analog Scale (VAS) (primary

endpoint). Key secondary endpoints included Emax on the bipolar Overall Drug Liking VAS, bipolar Take Drug Again VAS, and assessment of subjective drug value (maximum = $50.00). On a bipolar VAS, a score of 0 corresponds with a negative response, a score of 50 with a neutral response, and a score of 100 with a positive response. Endpoints were analyzed using a mixed-effect model, which included treatment, period, treatment sequence, and first-order carryover effect (where applicable) as fixed effects, baseline (predose) measurements as covariate (where applicable), and subject nested within treatment sequence as a random effect. In the assessments of "at this moment" Drug Liking, Overall Drug Liking, and Take Drug Again, for comparisons of LEM vs PBO, a P-value >0.05 indicates that LEM and PBO are not similar. For all other comparisons and for the assessment of subjective drug value, a P-value <0.05 indicates a statistically significant difference.

Results: Of 225 screened subjects, 107 were randomized to the qualification phase, and 39 met qualification criteria and were randomized to the treatment phase. A total of 20/107 (18.7%) qualification failures could not discriminate SUV from PBO, 7/107 (6.5%) could not discriminate ZOL from PBO, 15/107 (14.0%) could not discriminate either SUV or ZOL from PBO, and 1/107 (0.9%) had a PBO response out the acceptable range. Abuse potential endpoints were analyzed in the group of subjects who received and completed all treatments (n = 32), and safety outcomes were assessed in the group of subjects who received ≥1 dose of study drug during the treatment phase and had ≥1 postdose safety assessment (n = 39). For "at this moment" Drug Liking, mean Emax values for ZOL (78.3) and SUV (76.1) were significantly greater vs PBO (57.8; both P<0.05), confirming study validity with a validation margin of 11. All doses of LEM (LEM10, 78.4; LEM20, 80.5; LEM30, 83.6) were not similar to PBO (all P>0.05), but were not different from SUV or ZOL. In the assessment of Overall Drug Liking, mean Emax values for all doses of LEM (LEM10, 76.6; LEM20, 78.2; LEM30, 77.3) were different from PBO (54.7, all P>0.05) but not ZOL (75.6) or SUV (79.0). For Take Drug Again, mean Emax scores for all LEM doses (LEM10, 78.2; LEM20, 79.8; LEM30, 78.2) were also not similar to PBO (55.5, all P>0.05) but not different from ZOL (78.7) or SUV (79.3). In addition, subjects assigned a significantly greater mean hypothetical subjective drug value to LEM (LEM10, $14.44; LEM20, $16.92; LEM30, $14.88), ZOL ($16.55), and SUV ($13.74) compared with PBO ($2.65, all P<0.05). The incidence of treatment-emergent adverse events (TEAEs) was higher with LEM10 (94.6% [35/37]), LEM20 (97.1% [33/34]), LEM30 (97.1% [34/35]), ZOL (97.1% [34/35]), and SUV (91.2% [31/34]) vs PBO (38.9% [14/36]). The most common TEAE was somnolence (LEM10, 91.9% [34/37]; LEM20, 88.2% [30/34]; LEM30, 97.1% [34/35]; ZOL, 85.7% [30/35]; SUV, 85.3% [29/34]; PBO, 16.7% [6/36]), which was expected as LEM, SUV, and ZOL are sleep-promoting drugs that were administered in the morning. There were no serious TEAEs or deaths.

Conclusions: All doses of LEM demonstrated abuse potential vs PBO and all LEM doses appeared to have a similar abuse potential profile in this subject population. Drug effects for LEM were not significantly different from ZOL or SUV. LEM was well tolerated. LEM has been placed in schedule IV, the same drug schedule as ZOL and SUV.

Keywords: Lemborexant, Insomnia, Abuse Potential

Disclosure: Eisai Inc.: Employee (Self)

M140. Psilocybin and Ketamine Acutely Promote Wakefulness, Suppress REM Sleep but Differentially Modulate High Frequency EEG Oscillatory Power in Wistar Kyoto Rats – a Preliminary Analysis

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Background: Psilocybin is a classical serotonergic psychedelic gaining scientific interest for its potential ability to treat a variety of psychiatric disorders, such as depression. A topic of current interest is the extent to which psilocybin may share commonalities in mechanism of action with other agents such as ketamine, a known rapid-acting antidepressant. While sleep disturbance is an essential feature of depressive illnesses, the effects of psilocybin and ketamine on sleep-wake behaviour and the electroencephalogram (EEG) have not been directly studied in animal models of depression. Aspects of depression can be modelled in Wistar–Kyoto (WKY) rats, which exhibit abnormal behavioural, physiological and sleep-wake characteristics, and show decreased sensitivity to conventional monoamine-based antidepressants. The aim of this study was to determine how psilocybin and ketamine at different doses affect sleep-wake behaviour and EEG oscillations in WKY rats, both acutely and long-term.

Methods: Studies were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986. Adult male WKY rats (n = 6) were surgically implanted with EEG and electromyography (EMG) electrodes under general anaesthesia. The EEG comprised a frontoparietal difference signal (frontal: 2 mm anterior and 1 mm lateral from bregma; parietal: 1 mm lateral from lambda) transmitted through intraperitoneal telemetry. Animals were housed in recording boxes with free access to food and water on a 12/12-hour light/dark cycle. Animals were first dosed with a saline vehicle, followed by a drug treatment 24 h later: psilocybin (1, 3 or 10 mg/kg, intraperitoneal) or ketamine (5 or 10 mg/kg, subcutaneous). Treatments were administered 2 hours (h) after light onset. All animals received all treatment conditions by escalating the doses on a weekly basis, with 6 days washout until the next vehicle treatment. EEG and EMG were recorded for 0.5 h before and 24 h after each dosing using Spike2 software (CED, Cambridge UK). EEG/EMG signals were amplified, analogue filtered (0.5-100 Hz), digitized (256 Hz), and then digitally filtered (EEG: 0.5-100 Hz and EMG: 5-100 Hz). The subsequent EEG/EMG recordings were automatically scored as wake, non-rapid eye movement (NREM) sleep, or REM sleep in 10 s epochs using SleepSign (Kissei Comtec, Japan). Power spectral analysis was performed on EEG data recorded over the 0-1 h and 1-7 h periods post-treatment separately for wake, NREM and REM sleep. EEG power spectra were computed for consecutive 2 s epochs by fast Fourier transformation (Hanning window, 0.5 Hz resolution) between 0.5-100 Hz. Epochs with artefacts (5xSTD of RMS) were discarded. Repeated measures ANOVA followed by Dunnett's post-test was used to compare the different treatment groups to vehicle.

Results: All doses of psilocybin and 10 mg/kg ketamine were acutely wake-promoting, increasing time spent awake during the 4 h after treatment (Vehicle: 33.9%; Psilocybin 1 mg/kg: 46.2%, p = 0.035; 3 mg/kg: 53.8%, p = 2.7x10-4; 10 mg/kg: 56.1%, p = 5.8x10-5; Ketamine 5 mg/kg: 33.9%, p = 1; 10mg/kg: 52.1%, p = 8.5x10-4). This increased wake came mainly at the expense of REM sleep, which was significantly decreased at higher doses across 7 h after treatment (Vehicle: 7.9%; Psilocybin 3 mg/kg: 3.6%, p = 0.013; 10 mg/kg: 1.7%, p = 2.5x10-4; Ketamine 10mg/kg: 4.2%, p = 0.042; others p > 0.05). NREM sleep amounts were more modestly decreased in this time period, only significantly so with 3 mg/kg psilocybin and 10 mg/kg ketamine (Vehicle: 64.0%; Psilocybin 3 mg/kg: 56.2%, p = 0.014; Ketamine 10 mg/kg: 56.0%, p = 0.010; all others p > 0.05). Wake, NREM and REM sleep amounts were not different from vehicle in the subsequent dark period (all p > 0.05) and neither were they different 6 days after treatment (all p > 0.05).

In the EEG, psilocybin decreased high-frequency gamma (50-100 Hz) oscillatory power during wakefulness in the first hour post-treatment (Psilocybin 1 mg/kg: -0.9 dB, p = 0.002; 3 mg/kg:

-1.4 dB, p = 3.4x10-4; 10 mg/kg: -1.7 dB, p = 5.2x10-4), whereas high frequency oscillations (30-100 Hz) were increased by ketamine (Ketamine 5 mg/kg: +2.5 dB, p = 0.0014; 10mg/kg: +5.2 dB, p = 1.7x10-5). These changes in gamma power persisted during the light period significantly for the higher doses of psilocybin only, but were present in all vigilance states (Psilocybin 3 mg/kg Wake: -0.87 dB, p = 0.0085; NREM: -1.9 dB, p = 0.020; REM: -0.81, p = 0.011; 10 mg/kg: Wake: -1.1 dB, p = 0.0021; NREM: -2.1 dB, p = 0.025; REM: -1.1, p = 0.04). No changes in EEG spectra were observed 6 days after any drug treatment.

Conclusions: This study demonstrated acute and dose-dependent wake-promoting and REM sleep suppressing effects of psilocybin and ketamine in WKY rats, a rodent model of aspects of depression symptomatology. However, the acute effects of these drugs on frontoparietal EEG gamma band activity were markedly divergent. While high frequency power was increased by ketamine, a decrease was observed in response to psilocybin. Neither psilocybin nor ketamine produced long-lasting changes in this study. This preliminary analysis questions the existence of a single, simple relationship between drug-induced changes in sleep/wake behaviours and gamma band activity, parameters which have been previously considered to reflect aspects of the dissociative state and/or the potential for antidepressant efficacy. Future work should explore how these convergent markers relate to more detailed measures of neuronal activity and behaviour.

Keywords: Psilocybin, Psychedelic Medicine, Sleep Architecture, Quantitative EEG, Wistar Kyoto Rat

Disclosure: COMPASS Pathways Ltd, Eli Lilly & Co Ltd: Stock / Equity (Self)

M141. Abstinence-Dependent Dissociable Central Amygdala Microcircuits Control Drug Craving

Abstract not included.

M142. Role of Ventral Subiculum Neuronal Ensembles in Incubation of Oxycodone Craving After Electric Barrier-Induced Voluntary Abstinence

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Background: We recently developed a rat model of incubation of oxycodone craving after electric barrier-induced voluntary abstinence and showed time-dependent increases in drug seeking on abstinence day 15 and 30 compared to day 1. Here, we studied the role of ventral subiculum (vSub) neuronal ensembles in incubation of oxycodone seeking after electric barrier-induced abstinence, using the activity marker Fos, muscimol + baclofen (GABA agonists) inactivation, and the Daun02 chemogenetic inactivation procedure.

Methods: We trained either Sprague Dawley or Fos-lacZ transgenic rats to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d) for 14 days. We then introduced an electric barrier of increasing intensity (0.1 to 0.4 mA) near the drug-paired lever that caused cessation of oxycodone self-administration. We tested Sprague Dawley rats (n = 6-7/group) for relapse to oxycodone seeking (extinction tests) in the absence of shock and drug on abstinence day 15 and extracted the brains for Fos-immunohistochemistry or tested the rats (n = 10-12/group) after vSub injections of vehicle or GABA agonists (muscimol-baclofen). We tested the Fos-lacZ transgenic rats (n = 10-11/group) for relapse to oxycodone seeking on abstinence day 18 after selective inactivation of relapse test-activated Fos neurons in vSub on abstinence day 15 using the Daun02 chemogenetic inactivation procedure.

Results: Relapse after electric barrier-induced abstinence was associated with increased Fos expression in vSub, and both local inactivation of vSub and selective inactivation of vSub Fos-expressing neuronal ensembles decreased "incubated" oxycodone seeking.

Conclusions: Together, these data demonstrate a role of vSub neuronal ensembles in incubation of oxycodone craving after cessation of drug taking due to adverse consequences of drug seeking.

Supported by NIDA-IRP.

Keywords: Drug Relapse, Prescription Opioids, Oxycodone, Incubation of Drug Craving, Drug Self-Administration

Disclosure: Nothing to disclose.

M143. Investigating Mechanisms Unique to Memories Associated With Methamphetamine and Cocaine

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Background: Drug-associated memories are persistent, long-lasting, and perpetuate substance use disorders (SUD) by inducing motivation to seek drug. We previously reported that methamphetamine (METH)-associated memories can be disrupted by intra-basolateral amygdala (BLA) administration of Blebbistatin (Blebb), which inhibits the actin motor ATPase nonmuscle myosin II (NMII), leading to actin depolymerization. The effect is specific, as it does not interfere with other appetitive or aversive memories, including cocaine (COC). Further, it appears to be region-specific, as it does not have the same effect in dorsal hippocampus (dHPC) or nucleus accumbens (NAc). Therefore, we aimed to determine the differences between METH and COC to identify mechanisms contributing to the selective effect of NMII inhibition on BLA-specific METH-associated memories.

Methods: To examine the different mechanisms underlying COC- or METH-associated memories, we used the conditioned place preference (CPP) paradigm. First, to determine the influence of differences in drug half-life, mass spectrometry and programmable mini-pumps were used to mimic METH's clearance rates with COC. Second, RNA sequencing (RNA-seq) was used to determine broader transcriptional differences between COC- and METH-associated learning, and follow-up CPP experiments were used to determine the functional role of a specifically altered RNA candidate.

Results: Clearance rates of METH (2mg/kg) and COC (15mg/kg) were first determined following CPP conditioning, as METH exposure has previously been shown to slow subsequent clearance rates. Using mass spectrometry, METH's brain concentration was confirmed to be higher than COC's 15 minutes after injection on the final conditioning day, and its clearance rate was slower (below detection at ~8hr for METH and ~2hr for COC). Mini-pumps were then programmed to infuse COC at a rate that mimicked METH's clearance rate during CPP conditioning, followed by systemic Blebb administration prior to the first retention test. However, mimicking METH's half-life did not render the COC-associated memory susceptible to NMII inhibition, but CPP was absent at the second retention test, confirming our previous report that NMII inhibition disrupts reconsolidation. Next, RNA-seq identified potentially unique transcriptional changes in tissue samples collected from BLA, NAc, and dHPC after the final CPP training session for METH, COC, or saline. A relatively large

difference emerged between METH and COC-conditioned samples in BLA, as 638 differentially expressed genes (DEGs) had a p-value of <0.01 and a 1.5-fold change or greater, whereas fewer genes were identified when comparing METH or COC to saline (99 and 471, respectively), suggesting that METH and COC drive transcriptional changes in opposing directions. Moreover, there were far fewer DEGs in NAc or dHPC between METH and COC conditions (94 and 84, respectively). One gene selectively changed in BLA in METH-treated compared to COC-treated mice was crhr2 (Corticotrophin releasing hormone or factor (CRF) 2). CRF binds to CRF receptor 1 (CRFR1; gene: crhr1) and CRF receptor 2. Inhibiting CRFR1 disrupts COC- but not METH- associated memories or induced locomotion. Whereas, inhibiting CRFR2 has no effect on COC-associated memories or locomotion, but interferes with METH sensitization. Its role in METH-associated memories was unknown, therefore, we infused mice in BLA with vehicle or CRFR2 antagonist (Astressin-2B, AS2B) before each CPP conditioning session. AS2B-treated mice did not prefer the METH-paired chamber when tested 48 hours later, indicating CRFR2 is necessary for METH-associated learning. Interestingly, CRF2 inhibition after only the final training session was not sufficient to disrupt the memory, indicating that CRFR2 must be inhibited from the start of METH-associated memory acquisition to disrupt the memory. Experiments are ongoing to determine the relationship between CRFR2 and NMII.

Conclusions: Differences between different classes of commonly abused substances (e.g., stimulants and opioids) are often examined, but the differences within a class are under-studied. These experiments begin to assess those differences, which will allow for a better understanding of different substance use disorders and their interactions in the context of polydrug use. Moreover, identification of the mechanism(s) responsible for METH-associated memory's selective vulnerability to NMII inhibition may yield avenues to target other pathogenic memories, including other SUDs and traumatic memories.

Keywords: Memory and Learning, Myosin, Polydrug Use

Disclosure: Nothing to disclose.

M144. Heroin Extinction and Transient Cued Seeking Activates Cell-Type Specific Matrix Metalloproteinase Activation at Tetrapartite Synapse

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Background: Heroin abuse is a leading cause of drug overdose-related deaths in the United States, highlighting a need for further research elucidating effects of maladaptive neuroadaptations following prolonged heroin use. Activation of the tetrapartite synapse in nucleus accumbens core (NAcore), which comprises of pre- and postsynapse, astrocytic processes, and surrounding extracellular matrix (ECM), has been linked to increased relapse vulnerability. Specifically, degradation of the ECM by activated matrix metalloproteinases (MMPs) is involved in extracellular synaptic remodeling both constitutively and transiently. Following chronic heroin self-administration and extinction training, transient increases in MMP-9 activity in NAcore were elicited after 15 mins of cued heroin seeking compared to heroin-withdrawn and saline control rats. Although increases in MMP-2,9 fluorescence can be localized to the soma and dendritic processes of medium spiny neurons (MSNs) in accumbens, it is unknown which specific cell types of the tetrapartite synapse harbor changes in MMP-2/9 activity under heroin-withdrawn and cued reinstatement conditions.

Methods: We used a viral transfection strategy with a mCherry reporter to label NAcore D1- and D2-MSNs and astrocytes in transgenic rats expressing Cre recombinase selectively in D1- or D2-MSNs and in wildtype rats, respectively. Following heroin self-administration, we measured the localization of activated MMP-2,9 after FITC-gelatin microinjection under extinction-related and cue-induced heroin seeking conditions. Results were analyzed using Nested ANOVA following by Bonferonni post hoc tests for multiple comparisons for D1/D2 data, and Kruskal-Wallis with Dunn's post hoc comparisons for astrocyte data. Both sexes were included in these studies.

Results: For D1 MSNs, we observed increased transient MMP-9 gelatinolytic puncta localized proximal to dendritic surfaces in reinstated animals compared to both yoked saline controls and heroin-withdrawn animals. Moreover, after 120 min of cued reinstatement the increase in MMP-9 activity seen in the first 15 min of cued heroin seeking had decreased to levels below extinction baseline activity. Furthermore, co-registry between synaptic astroglial processes and MMP-2/9 activity was diminished following extinction, but transiently restored during cued heroin seeking. Conversely, D2 MSNs exhibited increased constitutive MMP-2 gelatinolytic activity following heroin-extinction, but was transiently reduced after 15 min of cued reinstatement. Within trial extinction of heroin cues after a 120 min reinstatement trial was associated with extinction-like elevations in MMP-2,9 activity. The transient reduction in gelatinase activity around D2 MSNs during heroin seeking was regulated by local inhibition by tissue inhibitors of metalloproteinases.

Conclusions: These findings reveal how NAcore extracellular matrix signaling and corresponding activity within the tetrapartite synapse underlie behavioral states related to opioid addiction. Our understanding of the integration of tetrapartite signaling is necessary to develop successful therapeutic manipulations of opioid-addicted synaptic pathologies.

Keywords: Matrix Metalloproteinase-9 (MMP-9), Nucleus Accumbens, Opioid Addiction, Tetrapartite Synapse

Disclosure: Nothing to disclose.

M145. Intermittent Access to Alcohol Drives Increased Colocalization of Glutamate and GABA Markers in Ventral Pallidum

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Background: Compulsive alcohol use is a major contributor to alcohol use disorder's intractable and persistent nature. Prior research has shown that neuroadaptations in corticostriatal projections to nucleus accumbens are critical for the development of compulsive-like alcohol use behaviors, including "aversion-resistant" drinking, in which animals continue to consume alcohol despite its adulteration with bitter quinine. Yet it remains unclear how alcohol-induced changes in nucleus accumbens can impact sensitivity to aversive outcomes during consumption and seeking of alcohol. The ventral pallidum is a major output of the nucleus accumbens, which is implicated in both appetitively- and aversively-motivated behaviors, largely via the actions of distinct cell types. Here we examine the effects of long-term intermittent access to alcohol on cell-type specific markers and activity in the ventral pallidum of rats tested for aversion-resistant alcohol consumption.

Methods: Male and female Wistar rats (n = 25; 11 males, 14 females) were tested for consumption of 15% ethanol with or without quinine adulteration (30 mg/L) following intermittent access to ethanol. The long-term access group (n = 12) received

intermittent access to 15% ethanol in the homecage, every other day, for 6 weeks, prior to completing alcohol consumption tests in operant boxes. The intermediate access group (n = 13) proceeded directly to operant box consumption tests. Both groups consumed alcohol in operant boxes three days a week for ~8 weeks, culminating in tests of aversion-resistant drinking. Brains were extracted immediately following a final drinking test, and then processed via in situ hybridization for mRNA markers of neural activity (Fos), as well as cellular identity (Slc17a6 [Vglut2], and Gad1).

Results: Here, we found that long-term alcohol access selectively increased alcohol consumption in the male but not female rats ($F(1,403) = 12.021$, $p < 0.003$), as intermediate access female rats drank more than their male counterparts and similarly to long-term access females. While long-term access rats were resistant to the adulteration of alcohol by quinine, they did not differ from intermediate access rats in this respect ($F(1,69) = 0.1577$, $p = 0.6925$), suggesting that our intermediate access protocol was enough to induce resistance to this concentration of quinine. We found no differences in cell-type specific recruitment of Fos following the final quinine-adulterated drinking test. Interestingly, we found that long-term access rats had more cells labelled for Gad1 mRNA ($t(23) = 2.8986$, $p < 0.01$) in comparison to the intermediate access group. This increase was primarily driven by an increase in the number of cells with co-localized vGlut2 and Gad1 mRNA ($t(23) = 4.3147$, $p < 0.001$) as the number of cells labelled for Gad1 but not vGlut2 did not differ between the groups ($t(23) = 1.6600$, n.s.).

Conclusions: Our results indicate that intermittent access to alcohol can alter the expression of cell-type specific mRNA markers in ventral pallidum. Specifically, long-term access increased the colocalization of markers for glutamate and GABA neurons. Given that ventral pallidum glutamate neurons are implicated in aversive processing, whereas ventral pallidum GABA neurons are implicated in reward processing, increased colocalization could alter the processing of both rewards and punishment during conflict. Increased GABA release by neurons that normally response to aversive events could be one mechanism by which alcohol exposure alters sensitivity to negative outcomes.

Keywords: Compulsive Models of Drug Use, Alcohol Drinking, Ventral Pallidum, Glutamate GABA, Reward and Aversion

Disclosure: Nothing to disclose.

M146. Impact of Ca2+ Imaging Processing Methods on Interpretation of Cellular Effects of Cocaine Self-Administration in the Rat

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Background: Development of genetically encoded calcium indicators has encouraged a bloom of research investigating activity of large cell populations at high spatiotemporal resolution. This, in turn, has encouraged the design and implementation of a torrent of new image processing methods. Among the diverse approaches to processing of Ca2+-imaging data is an often subjective decision of how to quantify baseline fluorescence or F0. We examine the effect of popular F0 determination methods on interpretation of neuronal and astrocyte activity in a single data set of rats trained to self-administer intravenous infusions of cocaine and compare them to an F0 independent, wavelet ridgewalking, approach to detecting significant Ca2+ transients. We specifically focus on spontaneous Ca2+ activity in individual cells and graph theory-based network measures arising from interactions between cells.

Methods: Two cohorts of 8 male Sprague-Dawley rats were injected with adeno-associated viruses driving expression of the genetically encoded calcium indicator, GCaMP6f, under control of either neuron-specific promoter, hSyn, or astrocyte-specific promoter, GFAP, in the nucleus accumbens (NAc) shell. Half of the rats from each cohort were trained to self-administer cocaine on a short-access (2 hours/day) fixed ratio 5 schedule for a period of two weeks, while the other half of each cohort served as yoked saline controls. After training, brains were extracted and coronal (300 μm-thick) slices containing NAc shell prepared for imaging of spontaneous Ca2+ events using epi-fluorescence conditions. Regions of interest were identified using an automatic activity-based detection algorithm, and the associated traces were subsequently processed by event identification algorithms to identify significant Ca2+ transients. Three F0 approaches (F0 smooth, F0 initial, and F0 minimal) each with four threshold parameters and a wavelet ridgewalking algorithm were chosen for analysis. Relative performance of these methods was compared between imaging data collected from cocaine-exposed/cocaine-naïve animals and a synthetic data set.

Results: We find that the choice of processing method has a profound impact on interpretation of imaging results. All dF/F0 thresholding methods tended to introduce spurious events and to fragment correctly identified events, leading to smaller calculated event durations and increased frequencies. Wavelet ridgewalking algorithm, in contrast, did not suffer from these shortcomings. Most dF/F0 methods, on their own, were unable to adequately account for bleaching of fluorescence, although F0 smooth method and the wavelet ridgewalking algorithm both did so. In general, choice of processing method led to different quantitative and sometimes opposing qualitative interpretation of the effects of cocaine self-administration both at the level of individual cells and at the level of cell networks. This was largely due to differences between transients identified as significant. For example, while several methods, including wavelet ridgewalking, converged on a significant increase in duration of neuronal calcium transients associated with cocaine self-administration, F0 smooth method identified a significant decrease in event duration while other approaches identified no significant differences. For both neurons and astrocytes, nearly every dF/F0 method was found to produce significantly different distributions of Ca2+ transient durations for both yoked saline and cocaine self-administration conditions. Wavelet ridgewalking algorithm proved superior to df/F0 methods in terms of correctly identifying significant Ca2+ transients as confirmed also with a simulated dataset. The improvement in performance was especially notably under relatively low signal to noise conditions common with physiological recordings and for analysis of astrocytic Ca2+ signals.

Conclusions: Choice of specific Ca2+ imaging processing approaches had a pronounced impact on interpretation of neuronal and astrocytic effects of cocaine self-administration. The wavelet ridgewalking algorithm broadly outperformed dF/F0-based methods for both neuron and astrocyte recordings. Given rising use of Ca2+ imaging across the neurosciences, researchers should be aware of limitations and tendencies associated with decisions to use particular processing methods. Both quantification and interpretation of effects of experimental manipulations, including drug self-administration, are strongly sensitive to such decisions.

Keywords: Cocaine Self-Administration, Calcium Imaging, Medium Spiny Neuron, Astrocytes, Nucleus Accumbens Shell

Disclosure: Nothing to disclose.

M147. Role of Organic Cation Transporter 3 in the Locomotor Sensitizing Effects and Rewarding Properties of Amphetamine in Male and Female Mice

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Background: The interaction between amphetamine and the dopamine (DA) transporter (DAT) is thought to mediate its abuse-related effects. However, strategies targeting DAT have yielded little benefit in treating addiction to amphetamine or other psychostimulants, raising the possibility that these stimulants have significant action elsewhere to modulate dopaminergic transmission. Consistent with this view, we found that the actions of amphetamine to evoke DA release and increase locomotor activity, are also dependent on organic cation transporter 3 (OCT3), a transporter capable of bidirectional monoamine flux (Mayer et al., 2018, PMID: 29773909). These findings raise the possibility that interactions with OCT3 contribute to the abuse-related effects of psychostimulants. The present studies, therefore, sought to investigate the role of OCT3 in the rewarding properties of amphetamine, and importantly, to examine potential OCT3-dependent sex differences, given the well-documented dichotomy in the response of males and females to psychostimulants, and the knowledge gap regarding sex differences in the role that OCT3 may play in the behavioral effects of these drugs. To this end, we used a combined genetic (constitutive OCT3 knockout (−/−) mice), and pharmacological (decynium-22 (D22), a blocker of OCT3) approach to examine the role of OCT3 in mediating sensitization to the locomotor stimulant effects of amphetamine, and conditioned place preference (CPP), in males and females. We hypothesized that the effect of amphetamine on sensitization and CPP would be attenuated in OCT3−/− mice compared with wild-type (OCT3+/+) mice and that D22 would attenuate the behavioral effects of amphetamine in OCT3+/+ mice, but not in OCT3−/− mice. Consistent with published literature, we expected females to be more sensitive to these effects of amphetamine than males. Finally, because estradiol is known to interact with OCT3, we anticipated revealing sex-dependent effects of D22.

Methods: Sensitization was simultaneously tested as part of the CPP paradigm in OCT3+/+ (17 male, 20 female) and OCT3−/− (15 male, 15 female) mice. The CPP procedure included three phases: habituation (one session), conditioning (eight sessions), and place preference test (one session). Each mouse was weighed and received two intraperitoneal injections (saline and saline, saline and amphetamine (1.0 mg/kg), or D22 (0.1 mg/kg) and amphetamine) approximately one hour apart. Immediately after the second injection, mice were placed in the center of the CPP apparatus for each 30 min session. Mice received saline only on habituation and preference test days. Time spent on the drug-paired floor and the saline-paired floor on preference test day was used to measure CPP.

Results: OCT3+/+ mice developed sensitization to the effects of amphetamine, which was reduced by D22 administration ($p = 0.01$). There was no significant sensitization to the effects of amphetamine in male OCT3−/− mice, regardless of pre-treatment with saline or D22. However, female OCT3−/− mice did develop sensitization to amphetamine ($p = 0.02$), which was attenuated by D22.

OCT3+/+ mice developed CPP for amphetamine ($p = 0.03$) and did so to similar extents in males and females. This preference was attenuated by D22 administration ($p = 0.04$). Though preliminary, our data further suggest that male OCT3−/− mice do not develop CPP for amphetamine and that D22 does not moderate this lack of CPP. In contrast, current data suggest that female OCT3−/− mice develop CPP for amphetamine ($p = 0.01$), which appears to be prevented by D22.

Conclusions: These preliminary data support a role for OCT3 in sensitization to the locomotor stimulant effects and rewarding properties of amphetamine in males. While data collection is ongoing, the sex-dependent trends observed so far in OCT3−/− mice suggest a more complex interplay between amphetamine-induced DAT activity and D22-sensitive transporters in females. In addition to OCT3, D22 inhibits the activity of OCT1, OCT2, and the plasma membrane monoamine transporter (PMAT). Of these, PMAT is a prime candidate for future studies, given its ability to transport DA and its strong expression in brain regions important for reward. If female OCT3−/− mice do, indeed, develop CPP for amphetamine, which is blocked by D22, this would suggest that OCT3 is not important for the emergence of this behavior in females, and therefore independent of estradiol interactions with OCT3.

Keywords: Organic Cation Transporters, Amphetamine, Sex-Differences, Addiction, Conditioned Place Preference

Disclosure: Nothing to disclose.

M148. Oxycodone-Induced Gene Adaptations in the Brain Reward Center in a Murine Model of Neuropathic Pain

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Background: 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 pain symptoms, and most available treatments show limited efficacy and tolerability and produce severe side effects. Approximately 20 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 robust gene expression adaptations in the brain reward center (Descalzi et al., Sci. Signaling, 2017). Here, we are using Next Generation RNA sequencing and upstream pathway analysis to gain insight on gene expression adaptations triggered by persistent exposure to oxycodone under chronic pain vs pain-free states.

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 physical symptoms (allodynia and hyperalgesia) of pain bi-weekly for nine weeks, followed by two weeks of daily s.cu oxycodone (30mg/kg) or saline injections. Following the two weeks of Oxycodone administration, animals underwent an extended period of drug withdrawal and where we monitored for physical and affective components of pain to understand the effects of oxycodone withdrawal in the context of chronic pain and also pain-free states. To this extend, we performed Novelty social recognition assay, marble burying, Light-dark box, and locomotor activity to assess emotional behaviors associated with drug withdrawal. 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. For the differential expressed gene (DEGs)

analysis of RNA seq data the p-value cut-off was p<0.05, and log2fold change cutoff was <0.5 and >-0.5.

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 as 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, and 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 to be affected in pain states when compared with non-pain states. ost robust gene changes in response to oxycodone under pain-free was i in the NAc were we saw 1759 genes upregulated and 871 downregulated, whereas SNI-oxy had greatest increase in gene expression in the mPFC; 351 upregulated and 508 downregulated . Overall genes affected by oxy in the the VTA where significantly less in both pain and non pain animals compared to the NAc and the PFC.

Conclusions: Our findings suggest that chronic pain states exacerbate the behavioral and transcriptomic signatures of oxycodone withdrawal. Overall, this work will provide new insights on the influence of long term pain in oxycodone-induced plasticity in the brain reward center. We are also evaluating if interventions in several genes or intracellular pathways may effectively alleviate oxycodone physical dependence in pain free or in chronic pain states.

Keywords: Opioid Dependence, Pharmacotherapy, Animal Model, Withdrawal, Neuropathic Pain, Next Generation Sequencing, Brain Reward Center, Gene Expression

Disclosure: Nothing to disclose.

M149. Identification of Epigenetically Regulated Novel Genes in the Amygdala During Alcohol Dependence

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Background: A primary risk factor for development of alcohol use disorder (AUD) is anxiety that develops during early withdrawal from chronic ethanol exposure. The cellular basis for the development of anxiety involves epigenetic regulation (histone acetylation) of gene expression in the amygdala during ethanol withdrawal that may alter synaptic plasticity mechanisms. We used RNA-sequencing (RNA-seq) and ChIP-sequencing (ChIP-seq) to identify epigenetically regulated gene networks in the amygdala during ethanol withdrawal after chronic ethanol exposure.

Methods: Adult Sprague-Dawley male rats were fed with either control (C) or ethanol (9% v/v) Lieber-DeCarli diet for 15 or 16 days. Chronic ethanol-fed rats were withdrawn for 0 (E) and 24 h (W). Amygdala tissue was collected for RNA-seq and ChIP-seq (H3K9/14ac) (Illumina®). Following standardized bioinformatic workflows, we identified a list of differentially expressed (DE) transcripts (RNA-seq; FDR<0.1 W vs C) and then merged our findings with ChIP-seq data to locate acetylation peaks around transcription start sites (TSS) (+/-2kb). Ingenuity Pathway Analysis® (IPA) was performed on the merged data. Validations of selected candidates were performed by ChIP (H3K9/14ac) and mRNA expression assays using qRT-PCR.

Results: Overall observations were that about 75% of DE genes were

annotated to a H3K9/14ac peak and about 25% of ChIP-seq peaks were in a gene promoter. From the merged data, we observed that 725 out of a total of 869 DE genes contained a TSS acetylation peak and these genes belonged to important biological networks such as cell cycle, RNA trafficking, RNA post-transcriptional modifications, cell signaling and nervous system development. Important gene candidates that showed down-regulation of both ChIP-seq fold changes and mRNA expression changes in withdrawal compared to the control group included Clcc1 (Chloride Channel CLIC Like 1), Grk2 (G Protein-Coupled Receptor Kinase 2), Pgrmc2 (Progesterone Receptor Membrane Component 2). Candidates that were up-regulated in both datasets included Abt1 (Activator of Basal Transcription 1), Plcl2 (Phospholipase C Like 2), and three members of the mitogen-activated protein kinase (MAPK) signaling pathway - Spry4 (Sprouty RTK Signaling Antagonist 4), Spry2 (Sprouty RTK Signaling Antagonist 2) and Dusp6 (Dual specificity phosphatase 6). We chose to validate Spry4, Spry2 and Dusp6 using ChIP and mRNA expression assays and observed increased histone acetylation at their promoters associated with an increase in mRNA levels in the amygdala during ethanol withdrawal.

Conclusions: The merger of two whole-genome data sets helped identify several important gene candidates that may play a role in ethanol-withdrawal related behaviors. We identified epigenetically regulated signaling molecules linked to MAPK signaling and changes in the expression of several other genes, which suggest that epigenetic and transcriptomic changes underlie ethanol-withdrawal related behavioral phenotypes. Future functional studies will validate the role of these targets in ethanol-withdrawal related anxiety-like behaviors in rats (Supported by NIH-NIAAA P50AA022538, UO1AA019971, U24AA024605 [NADIA] and by the VA merit grant and Senior Research Career Scientist award to SCP).

Keywords: Epigenetic Modification, Alcohol Dependence, Amygdala, Histone Acetylation, Alcohol Use Disorder

Disclosure: Nothing to disclose.

M150. Δ9-Tetrahydrocannabinol Self-Administration Induces Adaptations in the Nucleus Accumbens Core That Are Cell-Type Specific

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Background: Approximately 30% of chronic cannabis users develop cannabis use disorder (CUD), characterized by tolerance, anhedonia and withdrawal symptoms. Given the widespread use of cannabis, it is critical to identify neuroadaptations in the reward circuitry that might

contribute to CUD. The NAc core is an essential component of the mesocorticolimbic system and plays a prominent role in mood, motivation and reward behavior. GABAergic D1- and D2-medium spiny neurons (MSNs), which together comprise 90-95% of all neurons in the NAc, have opposing roles in depression-like behaviors and reward seeking behaviors. Distinct MSN-type specific adaptations after chronic cocaine and morphine result in a bias toward D1-MSN signaling which is thought to drive reward seeking behavior. Whether the adaptations induced by chronic THC use, such as loss of spines, functional tolerance of CB1R and metaplasticity, are also cell-type specific is unknown.

Methods: D1- and D2-Cre transgenic rats were transfected with Cre-dependent reporters and trained to self-administer a combination of two constituents of cannabis, THC and cannabidiol (CBD) or vehicle. After extinction training cell type specific spine morphology, basal glutamate transmission, CB1R function and synaptic plasticity and c-FOS expression were quantified.

Results: THC+CBD use induced a loss of large spines in D1- but not D2-MSN and a commensurate reduction in glutamate synaptic transmission. Also, CB1R function was impaired on D1-MSN glutamatergic synapses. The loss of the CB1R dependent auto-inhibition was paralleled by an augmentation in the capacity to potentiate glutamate transmission in D1-MSNs. THC+CBD use did not alter CB1R function or glutamate synaptic transmission on D2-MSN synapses.

Conclusions: Chronic THC+CBD exposure induces D1-MSN specific adaptations that shift the balance between D1-MSN and D2-MSN activity in an activity dependent manner that could explain negative and positive symptoms of CUD.

Keywords: Nucleus Accumbens, Cannabis Use Disorder, Medium Spiny Neuron

Disclosure: Nothing to disclose.

M151. Detecting Sign-Tracking in Human Substance Users

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Background: The goal tracker/sign tracker phenotype identified in animal research relates to the value an animal assigns to cues indicating reward may be available and is distinguished from the cognitive information a cue carries once learned. Animal models demonstrate an effect of this phenotype on acquisition of drug self-administration and indicate it may be a risk factor for drug dependence. Further, it has been proposed that assigning incentive value to drug cues may promote cue-induced relapse. However, it is not clear if adult humans display sign tracking. The current task attempted to identify sign tracking in healthy and substance use disordered humans by assessing willingness to work to see a picture of something that will not actually lead to obtaining it in the near future. Demonstrating sign tracking in adults may have implications for better understanding addictive and other neuropsychiatric disorders.

Methods: Thirty-one adults (10 (3F, mean age 38 years, 5 AA) smokers, 14(1 F, mean age 43 years, 8 AA) cocaine use disorder participants (CUD), 7 (2F, mean age 43 years, 5 AA) healthy controls (HC)) participated in a task that required them to view a series of pictures on a computer screen for 2 seconds each. If they wished to continue seeing the picture, they could press a button at least once per second. The picture stayed up for another 10 seconds or until pressing slowed to less than 1/second. If the picture disappeared before the 10 seconds, a fixation cross replaced it until the 10 seconds passed and the next picture appeared. 25 food, 25 drug (cocaine or cigarette, matched to the participant), 20 positive (landscapes, puppies), and 45 neutral pictures were presented in a pseudorandom order such that the same category did not repeat more than three times in a row. 6 of the 7 controls participated in the smoking version. In addition, five pictures of $5 were scattered throughout the task. If the participant pressed to keep the $5 up the full 10 seconds, they received $5 at the end of the task. This served primarily to maintain attention to the task. After completion, participants rated their liking of each picture they had seen.

Results: Across all groups, participants demonstrated a range of behavior on the task. 25 of the 31 pressed enough to receive all five $5 payments, while five collected four payments. Only one participant failed to collect any of the $5 payments. All groups demonstrated a range of button pressing behavior from pressing only for the $5 pictures to pressing more for pictures of preferred foods than for neutral pictures. All participants pressed far less frequently for non-money pictures than for the $5 pictures but 4 of 10 smokers, 4 of 14 CUD and 3 of 7 HC pressed to see pictures they could not consume. Differential pressing for pleasant, food or drug pictures over neutral pictures (a measure of pressing for the incentive value of the picture rather than general boredom) was measured by subtracting presses for neutral pictures from presses for another category and dividing by the sum plus 1 (to avoid dividing by 0 when no pressing took place). Both smokers and cocaine users demonstrated significantly less sign tracking as measured by responses to food and pleasant pictures than did controls ($p = .006$-$.05$ for the various comparisons). Both smokers and cocaine users showed a negative relationship between relative viewing time of food pictures and cigarettes per day ($r = -.5$) or cocaine use per week ($r = -.5$). Smokers pressing for cigarette cues correlated with pressing for food cues at $r = 0.69$ while CUD pressing for cocaine cues appeared unrelated to pressing for food with at $r = -.21$.

Conclusions: Humans do display a range of sign tracking behavior in a task measuring willingness to work to see a picture. Interestingly and contrary to predictions from rat data, in this pilot sample, healthy controls displayed higher levels of sign tracking behavior than substance users. Further, sign tracking behavior was negatively related to drug consumption in both smokers and cocaine users. Sign tracking does appear to exist in humans, but these preliminary data suggest that sign tracking is associated with less drug consumption, perhaps due to users being more interested in the goal of drug use rather than assigning incentive value to the cues.

Keywords: Sign-Tracking, Cocaine Addiction, Nicotine Addiction

Disclosure: Nothing to disclose.

M152. Do Behavioral Pharmacology Findings Predict Clinical Trials Outcomes? A Proof-Of-Concept in Medication Development for Alcohol Use Disorder

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Background: Behavioral pharmacology paradigms have been used for early efficacy testing of novel compounds for alcohol use disorder (AUD). However, the degree to which early efficacy in the human laboratory predicts clinical efficacy remains unclear. To address this gap in the literature we employed a novel meta-analytic approach.

Methods: We searched the literature for medications tested for AUD using both behavioral pharmacology (i.e. alcohol administration) and randomized clinical trials (RCTs). For behavioral pharmacology, we computed medication effects on alcohol-induced

stimulation, sedation, and craving during the alcohol administration (k=51 studies, 24 medications).

Results: For RCTs, we computed medication effects on any drinking and heavy drinking (k=73 studies, 17 medications). We used medication as the unit of analysis and applied the Williamson-York bivariate weighted least squares estimation to preserve the errors in both the independent and dependent variables. Results revealed a significant and positive relationship between medication effects on alcohol-induced stimulation (β=1.18, p<0.001) and craving (β=3.39, p<0.001) in the laboratory, and drinking outcomes in RCTs, such that medications that reduced stimulation and craving during the alcohol administration were associated with better clinical outcomes. For sedation the relationship was negative (β=-0.330; p<0.001), such that medications that increase sedation during alcohol administration were associated with reduced drinking.

Conclusions: This proof-of-concept study demonstrates that behavioral pharmacology endpoints of alcohol-induced stimulation, sedation, and craving track medication effects from the human laboratory to clinical trials outcomes. These novel methods and results can be applied to a host of clinical questions and can streamline the process of screening novel compounds for AUD.

Keywords: Clinical Trial, Behavioral Pharmacology, Alcohol

Disclosure: Nothing to disclose.

M153. Neural Correlates of Social Reward Processing Differ Between Young Adult Smokers and Non-Smokers

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Background: Social relationships are critical to young adult development. Growing evidence suggests that social deficits such as low subjective social status, insecure attachment, and social isolation are important risk factors for substance use, including tobacco smoking. Importantly, individual differences in social connectedness and relationship quality may be driven, in part, by differences in socio-emotional processing instantiated in neural circuitry that is directly impacted by nicotine administration and withdrawal. In particular, social rewards (e.g., being liked) engage key reward-related circuitry implicated in the pathophysiology of smoking. However, the neural basis of social reward processing among smokers has yet to be explored. In the current study, we examined differences in reward-related brain activation between young adult (ages 18-29) overnight-abstinent smokers (n = 21) and nonsmokers (n = 22) in response to an fMRI social "likeability" task previously shown to elicit robust activation in regions involved in reward and self-referential processing (e.g., medial prefrontal cortex; mPFC). Based on prior work demonstrating blunted striatal and mPFC activation to monetary reward, we hypothesized that smokers, relative to nonsmokers, would exhibit a similar pattern of hypo-activation in these regions in response to social reward. We further hypothesized that hypo-activation in these regions would correspond to behavioral factors such as loneliness, low subjective social status, and anhedonia.

Methods: Participants completed an initial training session, in which they were presented with photos of fictitious peers and asked to rate how much they thought they might like each individual if they were to meet them. Participants were informed that their own photo would also be rated by others. During the fMRI scan, participants were presented with a subset of the photos they had previously rated, and were provided with "feedback" in each of three categories: 1) being rated highly by those whom they previously rated highly (mutual liking condition), 2) being rated highly by those they had previously rated lower (received liking condition), or 3) being told that the individual pictured had not had a chance to rate them (neutral condition). Group differences between smokers and non-smokers in response to mutual > received liking and all positive > neutral feedback were evaluated in SPM12 using independent samples t-tests, with cluster significance determined by AFNI's 3dclustsim (voxel threshold of p<.001 and cluster significance of p<.05). Primary analyses were conducted within an a priori mask of the ventral striatum (VS) and mPFC; follow-up analyses explored additional activation across the whole brain.

Results: Across all subjects, mutual > received liking elicited activation in the bilateral temporoparietal junction, mPFC, and right anterior insula. All positive > neutral feedback elicited activation in the bilateral VS, mPFC, occipital cortex, and bilateral anterior insula. A main effect of smoking status was observed, with smokers exhibiting greater activation to mutual versus received liking in the bilateral VS (81 and 32 voxels for right and left VS, respectively) and mPFC (444 voxels). Additional whole-brain analyses revealed a similar pattern of greater activation among smokers compared with nonsmokers in the left hippocampus, left inferior frontal gyrus, and bilateral superior parietal cortex and fusiform face areas. No group differences were observed for all positive > neutral feedback. Regarding behavioral measures, smokers reported higher levels of anhedonia (t=2.1, p<.05) and lower subjective social status (t=2.4, p<.05) than non-smokers. Group differences in loneliness did not reach significance (t=2.0, p = .06). Anhedonia was positively correlated with mutual > received liking contrast data extracted from a priori regions of interest including the right (r=.34, p<.05) and left (r=.37, p<.05) VS and mPFC (r=.32, p<.05). However, anhedonia did not explain the association between smoking and brain activation.

Conclusions: In contrast to our hypotheses, smokers exhibited increased activation throughout the VS and mPFC in response to positive social feedback, and greater activation in these regions was positively correlated with anhedonia. These results contrast with prior studies of monetary reward indicating deficits in reward processing among smokers, but are consistent with a prior study demonstrating an association between greater mPFC activation and social anhedonia in young adults. Heightened VS and mPFC activation among smokers in the present study may indicate greater salience of positive social feedback, potentially against a backdrop of diminished positive social experiences, or a heightened ruminative process during self-reflection. These findings provide preliminary evidence of altered neural processing of social stimuli among smokers, which may have important implications for understanding pathways of risk for smoking progression and/or consequences of repeated nicotine exposure. Future work should examine the impact of acute nicotine administration and withdrawal on neural processing of social reward, and determine if observed differences in the present study are a risk factor or consequence of repeated nicotine use.

Keywords: Social Brain, Smoking, Reward, Nicotine

Disclosure: Nothing to disclose.

M154. Neuroimaging the Effects of Drug-Related Cue-Reactivity on Inhibitory Control in Cocaine Use Disorder

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Background: Drug addiction is characterized by impaired Response Inhibition and Salience Attribution (iRISA), where exposure to drugs is associated with amplified salience and

valence of drug-related cues and reinforcers at the expense of other salient reinforcers and with concomitant impairments in inhibitory control. Though these functions are associated with mesencephalic and corticostriatal regions, their interaction in cocaine use disorder (CUD) remains unclear.

Methods: We developed a novel stop-signal fMRI task, administered to individuals with CUD and demographically matched healthy control subjects [CUD: n = 26, Age: 44.1±8.2, Male/Female: 22/4, Race (Black/White/Other/Unreported): 17/3/4/2; Control: n = 26, Age: 42.7 ±7.0, Male/Female: 21/5, Race(Black/White/Other/Unreported): 16/4/6/0, all ps>.05], to investigate the putative iRISA interaction. Participants were shown a word (salient—drug/food/threat-related, or neutral) in white text and performed Go responses via button presses as soon as text color turned blue or green. Occasionally, text color turned red following a stop-signal delay (SSD), signaling to withhold the prepotent response (Stop trials). Using SSD and Go latency distributions, we derived the stop-signal reaction time (SSRT; a classical inhibitory control measure). We hypothesized drug words to uniquely elicit higher inhibitory control demands (longer SSRT) in CUD vs. controls. We further expected aberrant prefrontal and mesencephalic activations during the inhibition of drug vs. non-drug cues (via the cardinal contrast of successful vs. failed inhibition) in CUD compared to controls. We also collected measures relating to CUD severity (e.g., frequency of use) to reveal potential individual differences in neural recruitment during inhibition in our CUD sample.

Results: Despite drug cue-related SSRT similarities across groups [CUD mean drug SSRT: 409.6 (sd=68.4) ms, Control mean drug SSRT: 404.8 (sd=91.2), t(50)=.21, p = .83], fMRI analyses revealed diminished dorsomedial prefrontal cortex (dmPFC) activity in CUD during inhibitory control across all cue types (peak Z=5.04, cluster-corrected, p<.05), and diminished dorsolateral PFC (dlPFC) activity during the inhibition of drug vs. food words in CUD (peak Z=3.89, cluster-corrected, p<.05) . A region-of-interest analysis targeting the midbrain showed increased mesencephalic activity during the inhibitory control of drug cues compared to neutral cues in CUD relative to controls (p<.05). Additionally, greater frequency of cocaine use (mean use in past 30 days: 6.1±8.3, range: 0-25) positively correlated with dmPFC (R-squared=.31, p<.05), dlPFC (R-squared=0.48, p<.001), and orbitofrontal cortex (R-squared=0.34, p<.01) activity during the inhibitory control of drug vs. food cues.

Conclusions: We report an alteration of prefrontal and dopaminergic signaling during inhibitory processes, particularly in a drug cue-reactive context, in CUD. Specifically, we showcase the dampened involvement of regions associated with cognitive control (e.g., dlPFC) and amplified involvement of a region associated with incentive salience (e.g., midbrain) during inhibitory control for the CUD group under drug cue reactivity. The positive correlation between cocaine use frequency and PFC function, which could support a self-medication hypothesis, needs to be explored further. Overall, bolstering the iRISA model, these results highlight that our novel task parses the neurobehavioral bases of inhibitory control and salience attribution, elucidating how drug-related cue-reactivity (vs. other reinforcers) impacts the neural signature of inhibitory control in CUD.

Keywords: Functional MRI (fMRI), Cocaine Addiction, SSRT, Drug Cues

Disclosure: Nothing to disclose.

M155. The Medial Septum Enhances Strategy Switching When Cognitive Demand is High

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Background: Cognitive flexibility is a broad construct that encompasses a wide range of cognitive processes. In general, it is the act of adjusting a behavior or strategy (cognitive plan to achieve a goal) to account for an unexpected reduction in positive outcomes. Strategies that result in stable reward acquisition, and are repeated over time, eventually become outcome-insensitive, motor-stereotyped habits. Because a decrease in outcome sensitivity will necessarily lead to a decrease in trial-by-trial flexibility, switching strategies becomes increasingly more difficult and requires more cognitive effort as repetitions increase. However, these processes are not often discussed in tandem and the circuitry and mechanism that precipitates the increased cognitive effort required to switch a well-learned, habitual strategy is not known. Furthermore, the inability to flexibly switch strategies is a key symptom in disorders that feature maladaptive habits and compulsions, such as addiction, schizophrenia, and OCD. Therefore, identifying the specific part of cognitive flexibility circuitry that increases cognitive effort when cognitive demand is high, such as when the original strategy is well-learned, could reveal novel drug targets to treat psychiatric illnesses.

Methods: Previous studies, including our own, have hinted at a role for the medial septum (MS), a sub region of the basal forebrain, in cognitive flexibility. However, the extent to which the MS is involved in the broad construct of cognitive flexibility is not known. To answer this question, we first activated the MS of male and female Sprague-Dawley rats with designer receptors exclusively activated by designer drugs (DREADDs) and measured their performance on an operant-based strategy switching task, following regular (1 day) and extended (10 days) training.

Results: The extended training group that received the Gq-containing DREADD in their MS and clozapine-n-oxide (DR/CNO), i.e. MS activation, performed better than controls. They performed the strategy switch in significantly fewer trials and made significantly fewer mistakes. However, rats in the regular training group saw no benefit of MS activation compared to controls. Male and female rats performed similarly at both training time points.

Conclusions: This data presents the possibility that MS activation could be key in increasing cognitive effort switch strategies when demand is high.

Keywords: Medial Septum, Dopamine, Strategy Switching, Cognitive Flexibility, Basal Forebrain

Disclosure: Nothing to disclose.

M156. Longitudinal Assessments of Incubation of Cue-Induced Drug Craving in Cocaine-Addicted Individuals

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Background: Cue-induced craving is a major contributor to relapse in treatment-seeking addicted individuals. Animal studies have shown that cue-induced drug-seeking increases (or incubates) during the initial phase of abstinence, presumably reflecting a period of heightened relapse vulnerability. In contrast, most human studies that use self-reports showed a steady decline in craving with increasing abstinence duration. In a previous cross-sectional study, we employed an EEG-derived late positive potential (LPP) as an objective biomarker for cue-induced craving, providing evidence for incubation of cue-induced craving in human cocaine addiction. Here, we used a within-subjects longitudinal design spanning a baseline and 4 follow-ups in individuals with cocaine use disorders (iCUD) in an effort to replicate the previous results and provide translation from the animal findings to human addiction.

Methods: Twenty-one mostly treatment-seeking iCUD completed five assessments, at a baseline (mean abstinence = 21.4 days) and

then at 4 follow-ups, each three months apart. At each visit, participants completed the Cocaine Craving Questionnaire to report their unprovoked subjective craving. In addition, EEG data were recorded to assess cue-reactivity as participants passively viewed 30 cocaine and 30 neutral pictures. For each picture, participants rated the intensity of cocaine 'wanting' (i.e., subjective cue-induced drug craving). The LPP elicited by cocaine-related relative to neutral pictures was extracted.

Results: Subjective cue-induced cocaine wanting ratings decreased significantly [$F(1,20)=7.92$, $p = .011$] with the subjective unprovoked craving reports showing a similar linear trend [$F(1,20)=3.37$, $p = .081$] such that both were highest at baseline, linearly decreasing with every subsequent follow-up. Importantly, the LPP amplitudes showed a significant quadratic effect [$F(1,14)=4.54$, $p = .051$], such that LPP amplitudes elicited by drug-related cues (relative to neutral cues) showed initial increase from baseline to the first follow-up (3 months), staying elevated until the second follow-up (6 months) before gradually decreasing in the subsequent follow-ups (9 and 12 months).

Conclusions: To our knowledge the current study is the first to use both subjective and objective indices to show longitudinal within-subject evidence of incubation and subsequent decline in cue-induced craving in iCUD. These results underscore the need for incorporating objective measures of cue-induced craving in addition to subjective measures of craving, which are commonly used in clinical settings. Analyses are underway to explore whether measures of cue-reactivity and incubation predict longitudinal clinical outcomes in iCUD.

Keywords: Incubation of Drug Craving, Cue Reactivity, Cocaine Addiction

Disclosure: Nothing to disclose.

M157. Effects of Working Memory Training on Cocaine- and Cannabinoid-Seeking in Abstinent Male Rats

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Background: Evidence from clinical and preclinical studies suggests that cognitive training may promote resistance to the development of problem drug use or dependence. Training in tasks that improve working memory, response inhibition, and goal-directed learning may also serve as a treatment option to promote continued abstinence in individuals with substance use disorders, or prior to initiation of substance use. These tasks require the involvement of the prefrontal cortex, and it is hypothesized that developing methods to strengthen top-down cortical control of behavior will increase successful treatment of substance abuse. Using rodent models of cannabinoid and cocaine self-administration, we investigated the possibility of a protective effect of cognitive training. We hypothesized that training on a cognitively taxing working memory task prior to drug exposure, but not training on a task requiring low cognitive engagement or no training at all, would reduce self-administration and drug-seeking across multiple classes of drug.

Methods: Adult male Sprague Dawley rats ($n = 197$) were first trained on a delayed-match-to-sample working memory task. During this task, rats learn to nose poke into one of 5 illuminated sample ports. After responding in a specific port during the sample phase, a 0.5 s delay period elapsed before initiation of a choice phase where the sample port and 2 directly adjacent ports were illuminated. The rat must then choose the originally sampled port to receive a sucrose pellet reward. Rats trained on the simple task remained at this stage of training, while rats trained on the more cognitively taxing version of the task were required to engage their working memory during delays ranging from 0 – 24 s before the choice phase. A separate group of animals remained in the home cage during this training period and were handled, weighed, and fed sucrose pellets daily. Tissue was taken from one cohort of animals ($n = 33$) and analyzed for brain derived neurotrophic factor (BDNF) protein expression after completion of training or an equal amount of time spent in the home cage. Separate cohorts of rats were trained to self-administer the synthetic cannabinoid WIN 55,212-2 (WIN, 12.5 μg/kg/infusion, $n = 90$) or cocaine (1.0 mg/kg/infusion, $n = 74$) over 14 days. These rats were then tested in abstinence for cued drug-seeking or working memory performance over 35 days. Protein expression and behavioral outcomes were compared across groups using t-tests or repeated measures ANOVA where appropriate.

Results: We found that training on both the simple and challenging working memory task resulted in BDNF expression in the prelimbic area of the prefrontal cortex compared to untrained controls. Training on the simple or challenging version of the working memory task did not significantly alter lever pressing or drug intake during 14 days of WIN or cocaine self-administration. When tested during abstinence, performance on the challenging working memory task was reduced in both WIN- and cocaine-exposed rats compared to pre-drug baseline performance. Additionally, deficiency in pre-drug baseline performance at the most challenging delays was correlated with increased WIN-self-administration, but not cocaine self-administration. The amount of drug taken was correlated with the degree of cued reinstatement for animals that self-administered WIN, but not those that self-administered cocaine. Interestingly, there was no difference in cued reinstatement of WIN- or cocaine-seeking between working memory-trained animals and untrained controls, and pre-drug working memory performance was not correlated with cued reinstatement for either drug.

Conclusions: While cognitive training on tasks that engage executive functioning is proposed to help blunt drug-taking or promote abstinence, we found limited evidence for the effectiveness of working memory training to blunt substance-associated outcomes for either a cannabinoid or cocaine. Prefrontal BDNF expression was induced by simple and challenging working memory training relative to untrained controls, suggesting engagement of this structure and potential synaptic plasticity. Performance on the working memory task was only associated with eventual drug-taking for the synthetic cannabinoid WIN but not cocaine, suggesting a potential discrepancy in the effectiveness of cognitive training due to the rewarding effects of different drugs. This study is limited by the use of only male animals and future studies should investigate the possibility of sex-specific effects of cognitive training on substance abuse-related outcomes.

Keywords: Cannabinoid, Cocaine Self-Administration and Reinstatement, Working Memory, Cognitive Training

Disclosure: Nothing to disclose.

M158. Cannabis Users Demonstrate Enhanced Neural Reactivity to Reward: An Event-Related Potential and Time-Frequency EEG Study

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Background: Disruptions in neural measures of reward responsiveness are implicated in risk for and the development of Substance Use Disorders (SUDs) in general, but it is not clear if this is also true for Cannabis Use Disorder (CUD). To date, no studies have examined neural reward responsiveness in cannabis users using EEG.

Methods: Cannabis users (CU; n = 67) and non-users (NU; n = 60) were drawn from larger studies of individuals with and without internalizing and externalizing psychopathology. Groups were matched on current and lifetime psychopathology. Participants completed a validated monetary reward task during electroencephalogram (EEG). One-way between subject analysis of covariance (ANCOVA) models examined group differences in four EEG indicators of reward responsiveness - the reward positivity (RewP) and feedback negativity (FN) event-related potential residuals and two time-frequency measures (reward-related delta and loss-related theta).

Results: CU demonstrated an enhanced RewP to the attainment of monetary reward compared to NU ($p = .004$, $\eta 2 = .07$), even after controlling for relevant covariates. Secondary analyses found that occasional CU, but not current CUD or remitted CUD, showed enhanced RewP compared to NU ($p = .04$). There were no significant differences in FN, reward-related delta, or loss-related theta time-frequency measures between groups (p-values > .05).

Conclusions: To our knowledge, this is the first study to show preliminary evidence that CU have an enhanced RewP to reward and the extent of disruption may be related to CUD status. Our findings suggest that greater neural reward responsiveness may only be seen among CU with occasional use, not necessarily among CU with current or remitted CUD.

Keywords: Cannabis, Event-Related Potentials, Marijuana, Reward, Cannabis Use Disorder

Disclosure: Nothing to disclose.

M159. Sleep Disturbances are Associated With Cortical and Subcortical Atrophy in Alcohol Use Disorder

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Background: Sleep disturbances are prominent in patients with alcohol use disorder (AUD) and predict relapse. So far, the mechanisms underlying sleep disruptions in AUD are poorly understood. Because sleep-related regions vastly overlap with regions where patients with AUD showed pronounced grey matter (GM) reduction, we hypothesized that GM structure could contribute to sleep disturbances associated with chronic alcohol use.

Methods: We combined sleep EEG recording and high-resolution structural brain imaging to examine the GM-sleep associations in 36 AUD vs. 26 age and gender-matched healthy controls (HC). The whole-brain vertex/voxel-wise analyses were performed for cortical thickness (CT) and GM density (GMD). A generalized linear regression model was applied for the independent effects of group, sleep (REM, N2, N3) and their interactions on GM structure. Furthermore, we performed mediation analyses to test whether GM reductions mediate the effect of chronic alcohol use on N3 and REM sleep in AUD.

Results: The patterns of GM-sleep associations differed for N3 vs REM sleep and for AUD vs HC. For CT, CT-sleep associations were significant in AUD but not in HC and were lateralized such that lower CT in right hemisphere was associated with shorter N3, whereas in left hemisphere was associated with shorter REM sleep. For the GMD, we observed a more extensive positive GMD-N3 association in AUD (right orbitofrontal cortex, cerebellum, dorsal cingulate and occipital cortex) than in HC (right orbitofrontal cortex), and the GMD-REM association was positive in AUD (midline, motor and paralimbic regions) whereas negative in HC (the left supramarginal gyrus). GM structure mediated the effect of chronic alcohol use on the duration of N3 and the age by alcohol effect on REM sleep. A family-wise error corrected $p<0.05$ was used to report significant effects.

Conclusions: Our findings provide evidence that sleep disturbances in AUD was associated with GM reductions. Targeting sleep-related regions might improve sleep in AUD and enhance sleep-induced benefits in cognition and emotional regulation for recovery.

Keywords: Alcohol Use Disorder, Slow-Wave Sleep, REM Sleep, Cortical Thickness, Grey Matter Morphometry

Disclosure: Nothing to disclose.

M160. Prelimbic Activity During a Distress Tolerance Task Predicts Cocaine-Seeking Behavior in Male, but Not Female Rats

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Background: Distress tolerance (DT) is defined as the ability to persist in challenging goal-directed behavior in the face of stress, and individuals with low DT exhibit heightened drug-seeking behavior. However, no preclinical studies have examined the neurobiology underlying this phenomenon.

Methods: To assess this, we used in vivo electrophysiology in Long Evans rats (n = 15 female, n = 20 male) during a DT task to record neural activity in the prelimbic cortex (PrL) and nucleus accumbens (NAc) core, two brain regions implicated in drug-seeking. For the DT task, rats were initially trained to press a lever within a brief window of time in order to receive a sucrose pellet. On the DT test day, this brief window of time was progressively shortened so that it became impossible for animals to obtain a correct response, and we assessed how long animals persistently responded in the task as our measure of DT. Following the test day, rats underwent two weeks of self-administration for either water/saline or cocaine (1 mg/kg/inf) for 6 hr/day. Animals then began a 1-month period of experimenter-imposed abstinence to induce heightened drug-seeking behavior (Grimm et al., 2001). On day 28 of abstinence we reassessed DT and neural activity, and on day 30 we assessed cocaine-seeking behavior. Data were analysed with 2 x 2 x 2 ANOVAs (Drug Group x Sex x Abstinence) and Pearson correlations.

Results: Males had significantly higher DT than females ($p = 0.009$). Additionally, male (but not female) rats with low DT after 28 days of abstinence had significantly heightened drug-seeking behavior (male: $p = 0.048$; female: $p = 0.624$). Furthermore, we found that a subset of PrL neurons tracked behavioral persistence during the DT task. Critically, task-induced activity in these neurons (but not those in the NAc core) negatively correlated with subsequent drug-seeking behavior in males ($p = 0.027$).

Conclusions: hese findings suggest that males that can maintain heightened PrL activity during stressful/challenging contexts are less likely to persistently seek out drug later. Conversely, DT may not play as large of a role in drug-seeking in females. In total, these data demonstrate an important role for the PrL in DT and link this neural activity and behavior to drug seeking.

Keywords: Alcohol and Substance Use Disorders, Prelimbic Cortex, Distress Intolerance

Disclosure: Nothing to disclose.

M161. Developmental Methamphetamine Exposure Causes Sex Dependent and Persistent Changes in Behaviour Across Adolescence and Adulthood in Rats

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Background: Methamphetamine (METH) abuse is not only a serious health problem for the user, it also significantly impacts on the health and well-being of babies exposed to METH while in utero, during the prenatal period. In a recent study it was reported that 1 in 4 pregnant women seeking treatment for drug use, were METH dependent. Research from the Infant Development, Environment and Lifestyle (IDEAL) study have highlighted the impact of prenatal METH exposure on the development of USA and New Zealand children from 0-36 months. At 4 months of age differences in brain white matter trajectories have been reported, with the effects greater in females than in males. Further studies in children aged 7.5 years, have demonstrated impairments to cognitive function following prenatal METH exposure, 3 times greater than in controls. Here we used a refined model of developmental METH exposure (gestation to postnatal day 21) in rats that fully equated to human prenatal METH exposure to identify the effect on behaviour at adolescence and adulthood. Specifically, we measured the impact of developmental METH exposure on anxiety, social behaviour, depression and reward-related learning. Due to sex-difference effects of prenatal METH exposure in humans, the behavioural outcomes on both sexes were examined in rats.

Methods: Female Sprague-Dawley rats (n = 16) were mated and administered either saline or METH (5mg/kg, s.c.) from gestation day 0 to birth, with pups continuing drug administration until postnatal day (PND 21, equivalent to third trimester in humans), henceforth 'developmental exposure'. Behavioural testing was conducted in one cohort at adolescence (PND 27, n = 62), with the other cohort in adulthood (PND 70, n = 80), with approximately equal numbers of males and females in each cohort. Behavioural testing was conducted over an 18-day period and consisted of: elevated plus maze (anxiety), social interaction, Pavlovian Conditioned Approach (PCA) task (reward learning) and forced swim test (depression). For statistical analyses ANOVA and Bonferroni corrections were applied as appropriate. All experimental procedures were approved by the Macquarie University Animal Ethics Committee.

Results: Following developmental METH exposure, adolescent rats showed a significantly greater anxiety response (open arm time: $F(1,57) = 10.77$, $p = .002$, $\eta p^2 = .174$) and reduced social investigation ($F(1,53) = 37.75$, $p < .001$, $\eta p2 = .430$) regardless of sex. Social play was significantly reduced by prenatal METH exposure, only in males (P<0.001). Adolescent female rats exposed to prenatal METH showed greater 'sign-tracking' behaviour (lever focused over food goal) in the PCA task than controls (($F(2.77, 77.60) = 3.72$, $p = .001$, $\eta p^2 = .11$), however there was no difference in PCA behaviour for the adolescent males. Adolescent rats showed increased immobility in the FST after developmental METH exposure ($F(1,58) = 31.96$, $p < .001$, $\eta p^2 = .367$), when compared to controls, regardless of sex.

In adulthood, both male and female rats showed higher levels of anxiety following developmental exposure to METH when compared to controls (open arm time: $F(1,61) = 6.26$, $p = .013$, $\eta p^2 = .103$), with males showing greater anxiety than females overall ($F(1,61) = 4.48$, $p = .039$, $\eta p^2 = .072$). Both sexes showed decreased prosocial behaviours (head to head sniff: $F(1,51) = 13.71$, $p < .001$, $\eta p^2 = .222$) and increased anti-social behaviours (biting: $F(1,51) = 4.50$, $p = .039$, $\eta p^2 = .086$) following developmental METH exposure compared to controls. There were no treatment or sex effects in the PCA task. Only females in adulthood showed increased immobility in the FST after developmental METH exposure when compared to controls (p<0.001).

Conclusions: Overall, developmental METH exposure produced significant behavioural alterations, however the nature of these effects were contingent on age and sex. Given the paucity of research on the long-term outcomes of developmental METH exposure, these findings provide important insights into age and sex-specific effects that may facilitate the development of early intervention strategies that aim to improve the status of infants exposed to METH while in utero.

Keywords: Methamphetamine, Prenatal Drug Exposure, Depression and Anxiety, Reward Learning, Social Interaction

Disclosure: Nothing to disclose.

M162. Sex-Dependent Consequences of Early Life Adversity on Reward Circuit Development Promote Opioid Addiction Vulnerability

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Background: The epidemic of opioid use, addiction, and overdose is an ongoing public health problem in the U.S. Whereas opioid over-prescription and genetic predispositions play a in this epidemic, these factors alone cannot explain the exponential rise in opioid abuse. Individuals who have experienced early life adversity (ELA) such as poverty or abuse are overrepresented among opioid abusers, and addicted women report experiencing such adversity at a disproportionate rate, suggesting that they may be uniquely vulnerable to this risk factor. The mechanisms by which ELA confers increased vulnerability to opioid addiction are still poorly understood, and in humans, it is impossible to dissociate ELA from other co-existing vulnerabilities. Therefore, we employ a naturalistic ELA model in rats to examine the sex-specific impacts of ELA on reward and stress circuit development, and concomitant opioid drug seeking behaviors.

Methods: ELA was modeled in male and female Sprague Dawley rats by limiting bedding and nesting from postnatal day 2-9, whereas controls were housed in standard cages. As adults, rats were tested on intravenous heroin self-administration, extinction, and reinstatement, as well as in a measure of microeconomic demand elasticity for opioids. We assessed ELA-induced changes in reward- and stress-circuit nodes which might convey susceptibility to the addictive effects of opioids using two approaches(a) the activation of brain regions (nodes) of the circuit by heroin in naïve and in opioid-experienced animals. (b) gene expression changes in the same nodes/regions for a panel of reward- and stress-related molecules All experiments included 7+ rats per sex and rearing condition, and data were analyzed using t-test or ANOVA with Bonferroni post-hocs.

Results: ELA robustly increases opioid addiction-like behavior in female, but not male rats. Compared to controls, ELA females persisted in seeking heroin longer during extinction (e.g., number of days until extinction criterion t12=2.509; P=0.0274; n = 7/group), showed greater cue-induced (ELA vs. CTL active lever presses: t24 = 4.676; P=0.0002; n = 7/group) and heroin-primed reinstatement (ELA vs. CTL active lever presses: t24 = 4.676; P=0.0002; n = 7/group), and showed less elastic opioid demand, similar to a phenotype seen in humans addicted to opioids (t28=2.630; P=0.0137; n = 15/group). In contrast, ELA males did not exhibit opioid addiction-like behaviors. Preliminary findings suggest that ELA-induced opioid vulnerability in females may

involve altered heroin-induced activity in nucleus accumbens, especially in a dorsomedial accumbens shell opioid "hedonic hotspot." Initial results also suggest sex-dependent changes in pleasure- and stress- related molecule expression in several of the nodes of the reward and stress circuits.

Conclusions: ELA produces a sex-specific pro-opioid addiction phenotype in female rats, which may be caused by disrupted neurodevelopment of reward and stress circuits, leading to aberrant neural responses to opioid drugs.

Keywords: Opioid Addiction, Early life Adversity, Reward Circuitry, Nucleus Accumbens, CRH

Disclosure: Nothing to disclose.

M163. Common Grey Matter Reductions in Alcohol Use Disorder and Obsessive-Compulsive Disorder: A Meta-Analytic Approach

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Background: A hallmark of alcohol use disorder (AUD) is compulsive alcohol use, characterized by a tendency to continue seeking alcohol despite negative or aversive consequences. One way to investigate the underlying neural mechanisms of compulsive behavior is comparing AUD with other disorders involving compulsive actions, such as obsessive-compulsive disorder (OCD). The goal of this study was to examine the overlap in neural structures between AUD and OCD using a meta-analysis approach.

Methods: We performed separate metanalyses of journal articles using voxel-based morphometry (VBM) to investigate grey matter (GM) volume alterations in AUD and OCD. A systematic search strategy was used with the databases PubMed, Web of Science, Science Direct, Scopus and EMBASE. Inclusion criteria were: (1) for AUD: a DSM diagnosis; for OCD: diagnosis based on DSM or ICD-10; (2) use of whole-brain VBM to analyze GM differences; (3) comparison to healthy controls; (4) Talairach or Montreal Neurological Institute coordinates; (5) significance corrected for multiple comparisons or uncorrected with spatial extent thresholds; (6) peer-reviewed studies; (7) results reported in English. Exclusion criteria included: (1) review or metanalysis articles; (2) studies that re-analyzed previously published data; (3) A minimum of 10 patients. Articles were independently reviewed by two blinded raters and reviewed for conflicts. The SDM-PSI software package was used to analyze the data. Jackknife sensitivity analyses were performed to assess reliability of the results. To investigate if there were regions of common grey matter decreases or increases between the AUD and OCD metanalyses, the final mean analysis statistical images from each metanalysis were turned into binary images and multiplied to identify regions in common to both OCD and AUD.

Results: A total of 22 articles for AUD (794 AUD, 885 controls) and 35 for OCD (995 OCD, 1,177 controls) were included in the metanalyses. The AUD metanalysis revealed several large clusters of brain regions with grey matter differences compared to controls. These included: (a) a bilateral middle cingulate cluster, spanning the entire cingulate cortex from anterior to posterior, as well as the medial superior frontal gyrus and supplementary motor area; (b) a left postcentral gyrus cluster including the insula, superior and middle temporal gyri, precentral gyrus and putamen; and (c) a right insular cluster, which also included the putamen, amygdala and the superior and middle temporal gyri. Jackknife analysis showed consistent results, with all the clusters remaining in 18 of the 22 studies. Metanalysis of OCD versus controls revealed a relatively small number of clusters with differences in grey matter volume. The OCD group had increased grey matter volume in two small clusters including the left thalamus and bilateral cortico-spinal projections in the brain stem. In addition, the OCD group had decreased grey matter in a cluster spanning the anterior cingulate and medial superior frontal gyrus. They also had decreased grey matter in two smaller clusters in the insula. Jackknife analysis gave consistent results with all clusters surviving in 19-24 of the 27 studies. When comparing the overlap between the AUD and OCD metanalyses, there were three common clusters that showed decreased grey matter in cases compared to controls. These included a cluster spanning the anterior cingulate and medial superior frontal gyrus, and two small clusters in the posterior insula and middle insula.

Conclusions: Results indicate significant overlap in alterations in the anterior cingulate and insula between AUD and OCD. These brain regions are associated with executive function and cognitive control as well as in control of internal emotional and visceral states, and suggest that decreased volumes in these regions may underlie the compulsive behaviors associated with both OCD and AUD.

Keywords: Alcohol Use Disorder, Obsessive Compulsive Disorder, Voxel-Based Morphometry (VBM), Anterior Cingulate Cortex (ACC), Insula

Disclosure: Nothing to disclose.

M164. A "No Brainer"? Using Brain-Based, Relapse-Relevant, Endophenotypes in Medication Development: Testing a Dopamine D3-Preferring Partial Agonist (Cariprazine) Against Cue -Triggered Limbic Activation in Cocaine Patients

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Background: Despite elegant preclinical neuroscience on the brain circuits important for addiction and for relapse vulnerability, the clinical addictions field still awaits an FDA-approved medication for several drug classes, including cocaine use disorders. Though candidate cocaine medications often have stated brain targets, most of the clinical medication trials have not been informed by brain measures – either by confirmation of receptor occupancy (by PET) at the study dose and/or by confirmation of action on the intended brain systems (by functional MRI). Getting brain information for a brain-directed intervention may seem like a "no brainer", but it is still rare. Brain information may be critical for understanding why a medication 'failed' – e.g., did the medication even reach the intended brain target, at the tested dose? Equally important -- the brain information may be helpful in understanding why the medication 'succeeded' for some patients but not others, a heterogeneity familiar in most clinical trials. Brain information can provide rational guidance for the 'next step' in developing a medication, and in selecting the responsive clinical population.

Which brain endophenotypes are ready for targeting with a candidate medication? Drug cue-triggered (learned) activation of the brain's limbic motivational circuitry has been well-characterized: this endophenotype is linked to dopamine (DA) release (animals and humans), to reinstatement of drug use in animal models, and to drug-taking in humans. Dopamine D3 receptors are anatomically-localized in nodes of this reward / motivational circuitry (e.g., ventral striatum), and antagonism of D3 receptors has shown substantial promise in pre-clinical models of the learned response to drug reward cues (e.g., reinstatement and conditioned place preference). Though D3-specific agents are still in (long-pursued) development, the recently approved atypical

anti-psychotic cariprazine is strongly D3-preferring, with D3 affinity that is higher than the D3 affinity of DA itself. As compared to other atypicals, cariprazine is thus unique for its in vivo occupancy of D3 receptors. We are testing whether this D3-preferring partial agonist can blunt cue-triggered limbic activation in cocaine patients – offering a window onto its potential mechanism for reducing cocaine use in an ongoing clinical trial.

Methods: We studied a small case series of treatment-seeking cocaine patients (males with multi-year history of chronic cocaine use by the smoked route of administration) who gave witnessed, informed consent for fMRI imaging during study of a medication with potential therapeutic benefit. As part of their study participation, each individual received inpatient stabilization and induction onto cariprazine (3mg daily, 86-96% occupancy at D3 receptors by [11-C]-(+)-PHNO PET) vs. placebo across 10-12 days, followed by a functional magnetic resonance imaging (fMRI) session with several tasks. For the current analyses, we examined the brain response to brief cues administered within a "fast" event-related BOLD (Blood Oxygen-Level-Dependent) fMRI paradigm. The cues (24 unique cues per category, repeated once) were cocaine-related and comparison (sexual, aversive or neutral) visual stimuli of 500 msec duration. Average interstimulus interval was 1500 msec (TR=2 sec). Data were smoothed, normalized, realigned and batch-analyzed within the SPM 12 pipeline. Pre-planned contrasts compared the brain response to cocaine vs. neutral cues (first task half, to minimize the impact of cue repetition) for the small case series of cariprazine patients ($n = 4$) and for an historical comparator group of demographically-similar, unmedicated cocaine patients ($n = 14$). The resulting statistical parametric maps were thresholded at $2<t<5$, for display and examination.

Results: The unmedicated cocaine comparator patients showed the expected drug cue-triggered activation of the brain's motivational circuitry (as well as bilateral regions of the visual association cortex). In contrast, for the case series of patients receiving 3 mg of daily cariprazine, the brain activation maps were "quiet" – lacking the widespread activation pattern evident in the unmedicated group; activation was instead confined to a small region of the posterior insula and the VMPFC (p set at 0.05 for examining the limbic mask; k=20 contiguous voxels).

Conclusions: These findings offer a preliminary demonstration that a high-affinity D3 partial agonist, cariprazine, may impact a relapse-relevant brain endophenotype, cue-triggered limbic activation, in patients with cocaine use disorder. Though the size of the case series is small, and makes use of an historical comparator group, they do support the hypothesis that this D3 agent can impact a druggable brain target – and that fMRI circuit-level information may be a potentially informative measure in the context of ongoing clinical efficacy trials – with the eventual goal of linking brain response to clinical response. Further, as preclinical studies with D3 partial-agonist and antagonists have shown promise in blunting cue-related drug motivation across multiple drug classes, the current study offers additional encouragement for testing cariprazine (and other D3-specific agents, as they become available) in other addiction populations, including those with the use of multiple drugs, e.g., opioids and stimulants. Our laboratory is in the regulatory stage of this work.

Keywords: Functional MRI (fMRI), D3 Receptor Partial Agonist, Cue Reactivity, Cariprazine, Cocaine

Disclosure: Nothing to disclose.

M165. Preliminary Assessment of [11C]PBR28 PET Imaging Sensitivity to Alcohol in People

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Background: Alcohol elicits complex effects on the brain's immune system, yet limited in vivo tools exist to study these phenomena in people. Positron emission tomography (PET) imaging of the 18-kDa translocator protein (TSPO) provides a biomarker that is sensitive to dynamic changes in the immune system. For example, the immune stimulus endotoxin increases [11C]PBR28 VT by 40-50% in human brain(Sandiego et al., 2015). The goal of this work was to determine whether [11C]PBR28 PET imaging is sensitive to acute immune effects elicited by an oral alcohol challenge.

Methods: Participants were six people (4M, 2F; Age 21-31) who reported recent drinking experience consistent with a binge alcohol event. A baseline dynamic [11C]PBR28 PET scan was acquired in the morning, followed by a standardized lunch. Subjects then drank alcohol designed to achieve blood alcohol concentrations (BAC) of 80 mg/dL over 90 min. Following an additional 60 min rest period, a second post-alcohol [11C]PBR28 PET scan was acquired. Arterial blood samples were acquired at 30 min intervals to measure BAC. Dynamic PET data were acquired with a Siemens Biograph mCT PET/CT scanner for at least 90 min following injection of 427±142 MBq [11C]PBR28. Arterial blood samples were collected to measure the metabolite-corrected input function. Multilinear analysis was used to estimate [11C]PBR28 distribution volumes (VT) in 9 brain regions.

Results: In five subjects with available BAC measures, peak levels of 89±23 mg/dL were achieved 90-120 min after initiating the alcohol session. BAC levels were, on average, 65±14 mg/dL during PET imaging sessions. Averaged across all subjects and brain regions, the alcohol challenge increased [11C]PBR28 VT by 21% (range, 3%-38%). There was no initial evidence for regional patterns in alcohol's effects on [11C]PBR28 VT.

Conclusions: These preliminary data suggest that [11C]PBR28 VT is sensitive to an oral alcohol challenge in people. Data collection is ongoing to confirm the findings in a larger cohort of moderate drinkers, and begin to assess sensitivity in people with alcohol use disorder.

Keywords: TSPO and [11C]PBR-28 PET, Alcohol Drinking, Immune Biomarkers

Disclosure: Nothing to disclose.

M166. Ovarian Hormones and Contraceptive Estrogen Alter Nicotine Demand Intensity and Estrogen and Dopamine Receptor Expression Within the Reward Pathway

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Background: Many women use some form of oral contraceptives while smoking, and ovarian hormones across the menstrual cycle can affect craving and smoking relapse propensity. Ethinyl estradiol (EE), a synthetic, orally bio-available estrogen, is the most common estrogen in oral contraceptives, and is currently prescribed to women chronically.

Methods: The current study examined the impact of 17-β-estradiol (E2), a major form of the sex hormone estrogen, as well as EE on nicotine self-administration, demand, reinstatement, and protein expression within the nucleus accumbens core (NAcore) and ventral tegmental area (VTA) following ovariectomy (OVX) or sham surgery. Linear mixed effect modeling was used for analysis, with Bonferroni post-hoc correction.

Results: OVX vehicle-treated females self-administered significantly less nicotine, had significantly lower intensity of demand,

and reinstated significantly less as compared to sham vehicle-treated females. OVX E2 and EE treatment groups showed a rebound of nicotine intake later in training and Q0 levels of consumption were partially rescued in both groups. Further, E2 but not EE reversed the abolishment of reinstated nicotine seeking induced by OVX. Estrogen receptor (ER)-β protein expression was downregulated in OVX vehicle compared to sham vehicle-treated animals in both the NAcore and VTA, and this was only reversed by hormone supplementation in the NAcore. No differences in ER-α expression were found between groups. Dopamine D2 receptor expression was altered in the VTA. Specifically, D2 receptor expression was increased following OVX within the VTA compared to sham, which was reversed by EE treatment.

Conclusions: Taken together, these results demonstrate that ovarian hormones and EE play a critical role in mediating the neurobehavioral effects of nicotine, and future studies are needed to increase our understanding of how synthetic hormones contained within oral contraceptives interact with smoking.

Keywords: 17-β-estradiol, Nicotine Demand, Ethinyl Estradiol, ER-β, Reinstatement

Disclosure: Nothing to disclose.

M167. Antagonism of the Mineralocorticoid Receptor as a Potential Pharmacotherapy for Alcohol Use Disorder: Convergent Evidence From Rodent and Human Studies

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Background: The role of the hypothalamic-pituitary-adrenal axis in the neurobiology of alcohol drinking behaviors has been well established. To date, most of the research in this regard has been focused on the glucocorticoid receptor and its primary ligand (i.e., cortisol in humans, corticosterone in rats). Recent evidence suggests that the mineralocorticoid receptor, which binds to both cortisol and aldosterone, is also involved in alcohol seeking and consummatory behaviors. Accordingly, we hypothesize that antagonism of the mineralocorticoid receptor may represent a potential pharmacotherapy for alcohol use disorder (AUD). As a proof-of-concept, the effects of treatment with the mineralocorticoid receptor antagonist spironolactone on alcohol-related outcomes was examined in both rodents and humans.

Methods: The first study tested the effects of spironolactone in a preclinical model of alcohol dependence. Wistar rats were made dependent on alcohol via alcohol-vapor exposure (n = 7), whereas nondependent rats were exposed to air (n = 9). Operant alcohol self-administration (0.1 mL, 10% w/v ethanol) was measured in bi-weekly, 30-min sessions (fixed-ratio 1 schedule of reinforcement). Procedures were performed during acute withdrawal in alcohol-dependent rats. Spironolactone (50 mg/kg dissolved in 4% Tween 80 in saline) was administered intraperitoneally 1 h before the test session. The second study was a pharmacoepidemiological investigation which utilized data from the Veteran Birth Cohort to examine the association between spironolactone use (prescribed for any indication) and alcohol consumption in humans aged 40 to 70 years. Spironolactone-exposed individuals (n = 10,726) were each propensity-score matched to up to five unexposed individuals. Multivariable linear regression models estimated changes in Alcohol Use Disorders Identification Test - Consumption (AUDIT-C) scores before and after spironolactone exposure.

Results: In the first study, following alcohol vapor exposure, alcohol-dependent rats escalated their alcohol self-administration, whereas nondependent rats exhibited stable levels of alcohol self-administration. Intraperitoneal injection of spironolactone (50 mg/kg) significantly reduced alcohol self-administration in both dependent and nondependent rats (treatment effect: $p = 0.0003$). In the second study, among substance use disorder treatment-seeking patients, AUDIT-C scores decreased 0.34 points (95% CI: 0.16, 0.52) more in individuals exposed to spironolactone, compared to those not exposed ($p = 0.0003$). This observation was stronger in 1) individuals who had a baseline AUDIT-C score of ≥ 8 [0.55 (0.25, 0.86), $p = 0.0003$], which is a standard cut-off indicative of hazardous or harmful alcohol use, and 2) individuals who were exposed to ≥ 50 mg/day of spironolactone [0.89 (0.56, 1.22), $p < 0.0001$].

Conclusions: Together, these data from both rodent and human studies support the involvement of the mineralocorticoid receptor in the neurobiology of alcohol consumption and provide preliminary evidence suggesting that antagonism of the mineralocorticoid receptor may represent a novel pharmacotherapeutic approach for AUD.

Keywords: Alcohol, Addiction, Mineralocorticoid Receptor, Spironolactone, Translational Pharmacology

Disclosure: Nothing to disclose.

M168. Discovery of Novel Biomarkers of Substance Use Disorders by Leveraging Untargeted Metabolomics and Microbiome Multi-omic Analysis

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Background: In the last few decades it has become clear that the microbes that live in and on humans contribute to both healthy and disease states. The microbiome is involved in educating the immune system, digesting nutrients, secreting signaling molecules, protecting from pathogens, and a myriad of other functions that are still under investigation. Recent evidence has demonstrated that the gut-brain axis, the bi-directional communication between the gut microbes and our brain, are involved in modulating states of intoxication and withdrawal from illicit substances such as opioids and stimulants.

Methods: To further understand the complex communication between the gut microbiome and functional metabolites that are released into the blood stream, we are using large cohorts of Heterogenous-outbred (HS) rats which self-administer cocaine or oxycodone, as well as Wistar rats which self-administer alcohol to establish the changes in the microbiome and the metabolome which occur after long-term drug use. We conducted multi-omic phenotyping of (n = 66 cocaine, n = 95 oxycodone, and n = 50 alcohol) in order to discover novel metabolites associated with individuals that were either resistant or vulnerable to becoming dependent on each substance. We profiled the gut microbiome (16s sequencing) at baseline and post-escalation timepoints. We also applied untargeted metabolomic profiling in plasma at matched timepoints. Following the data collection and curation, we then implemented random-forest and other computational approaches to analyze and establish biomarkers for vulnerable and resistant animals for each drug group.

Results: Our analysis identifies novel biomarkers of both metabolites and microbes that can be used to predict drug use behavior in these preclinical models. Of these metabolites and microbes, Verrucomicrobia, and Actinobacteria have emerged as a predictor of vulnerable individuals in oxycodone animals. Metabolites in the bile-acid family were also altered in vulnerable individuals when compared to resistant individuals in oxycodone self-administering animals. Analysis of the cocaine and alcohol groups is still ongoing. Furthermore, a differential analysis will be

performed to determine common and divergent measures across drug classes.

Conclusions: Multi-omic analysis across multiple drug types and sample inputs identified phyla and metabolite changes in vulnerable versus resistant animals. The potential mechanisms involved, and duration of change is still yet to be determined. However, it provides a foundation to use an unbiased multi-omic approach to evaluate potential targets that impact drug intake.

Keywords: Microbiome, Metabolomics, Multi-omics

Disclosure: Nothing to disclose.

M169. Induction of Toll-Like Receptor 7 and Interferon Signaling in Alcohol Use Disorder: A Role in Stress Reactivity During Withdrawal

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Background: Background: Neuroimmune activation is a key feature of neuropathology in alcohol use disorder (AUD). Interactions between neurons and glia likely mediate this pathology; however, the role of neuroimmune mechanisms in the genesis of behavioral pathology associated with alcohol abuse are unknown. Further, specific druggable signaling targets that could promote behavioral dysfunction associated with alcohol abuse need to be identified. We have reported alcohol abuse causes induction of Toll-like Receptors 7 (TLR7) in brain with release of its endogenous ligand miRNA let-7b (Coleman et al JNI 2017). One of the downstream consequences of TLR7 is induction of interferon (IFN) signaling. IFNs cause induction of numerous interferon-stimulated genes (ISGs) capable of modulating neuronal circuitry. Exogenous interferon (IFN) administration is known to cause progressive onset of negative affect in humans and mouse models. Negative affect and stress hyperactivity during withdrawal are thought to drive ongoing drinking and relapse in AUD. Therefore, we investigated the role of IFN signaling in alcohol use disorder and its contribution to negative affect and stress-related behaviors associated with alcohol withdrawal.

Methods: Methods: Human Postmortem Brain Assessment – Cortical and hippocampal tissue from human alcohol brains (N = 10/group) were obtained from the New South Wales Brain Tissue Resource Centre (NSW-BTRC). Levels of TLR7 and IFN mRNA were measured by RT-PCR. Induction of TLR7 immune transcription factors IRF7 and NFkB was assessed by RT-PCR and immunohistochemistry (IHC). Chronic Intermittent Ethanol (CIE) Exposure in vivo – WT or IFN receptor (IFNR) KO mice received CIE (5g/kg, i.g. 5-days on, 2-days off) for 5 weeks. Anxiety-like (elevated plus maze and light/dark preference) were assessed at 48 and 96 hours into withdrawal respectively. One week into withdrawal locomotor activity (open field) was assessed and mice underwent acquisition of conditioned-fear memory using a Pavlovian foot-shock method. Three weeks into withdrawal mice underwent the forced swim test. Retention of conditioned fear memory was assessed 4 weeks into withdrawal followed by acoustic startle and sensory-motor gating (prepulse inhibition) at 7 weeks into withdrawal. Mice were sacrificed a week later and induction of IFNs and ISGs measured in whole brain by RT-PCR. In vitro studies – The effect of ethanol on IFNs was measured in neuronal (SH-SY5Y), microglial (BV2) and astrocyte (U373MG) cells. Conditioned media transfers were done to assess for neuronal induction of IFNs by mediators secreted by glia. RNA-Seq was performed to assess for ethanol-induced changes in SH-SY5Y neurons.

Results: We found a novel glial to neuronal signaling pathway, involving TLR7 and its endogenous agonist, miRNA let-7, contributes to interferon (IFN) induction, and may contribute to negative affect. In postmortem human alcoholic cortex and hippocampus, TLR7 mRNA and protein were increased (2-fold), with downstream signaling molecules IRF7, NFkB and IFNs induced in human cortex (*$p<0.05$). In mice, CIE ethanol caused behavioral dysfunction during withdrawal, increasing anxiety-like behavior with increased dark preference (*$p<0.05$), and conditioned fear memory evidenced by persistently increased context and cue-induced freezing (~2-fold increases in each). These behavioral disruptions were associated with persistent increases in IFNα and IFNβ gene expression in whole brain 8 weeks into withdrawal and correlated with indices of context-induced freezing ($R=0.85$ and 0.74 respectively, *$p<0.05$) IFNR KO mice, however, were protected from persistent context-induced freezing during withdrawal from CIE. In vitro, RNA-Seq analysis found that ethanol (100mM, 24h) caused significant changes in 45 genes ($p<0.05$, $q=0.01$) of which 21 were ISGs (44%). Ethanol-conditioned media from U373MG astrocytes induced IFNα and IFNβ gene expression (2-fold) in naïve SH-SY5Y, which was blocked by let-7 antagomirs and TLR7 siRNA treatment of recipient neurons prior to media transfer.

Conclusions: Conclusion: IFN in brain plays a role in AUD and mediates chronic stress responses during withdrawal from chronic ethanol treatment in vivo. Findings support a glial to neuronal signaling mechanism mediated by secretion of the TLR7 ligand let-7b from glia that induces IFN signaling in neurons. This work was supported by NIAAA and the Bowles Center for Alcohol Studies.

Keywords: Neuroimmune Mechanisms, Alcohol Use Disorder, Stress Reactivity, Conditioned Fear Memory, Anxiety

Disclosure: Nothing to disclose.

M170. Validation of a Nicotine Vapor Self-Administration Model in Rats With Relevance to Electronic Cigarette Use

Abstract not included.

M171. Dual Approaches to in Vitro and in Vivo Detection of Opioid Peptides

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Background: Endogenous opioid peptides are critical for analgesia, reward processing, and negative affect, however, research on their function has been challenging due to an inability to reliably detect dynamic release. To begin to address this we have developed two complementary methods to allow both in vitro and in vivo, rapid and sensitive detection of opioid peptides. We have developed an electrochemical detection approach using microimmunoelectrodes (MIEs), to allow for increased spatiotemporal resolution. This approach enables detection of opioid peptide changes over a number of seconds both in brain slices and in vivo. We have further increased the sensitivity and reproducibility of our microdialysis/nano-liquid chromatography-mass spectrometry (nLC-MS) approach to allow for the quantification of opioid peptide release during behavior.

Methods: MIEs: We custom-make carbon fiber-based electrodes, which are coated with antibody selective to the opioid peptide of interest. An electrical potential is applied to the electrode and an electrical current is measured from the oxidation of electroactive species. Opioid peptides contain an electroactive tyrosine residue, which we oxidize using square-wave

voltammetry to measure their presence. MIEs detect the oxidation of tyrosine at ~0.65V. An antibody is used to coat the MIE thereby allowing specific detection of the tyrosine residue on the opioid peptide of interest. To confirm specificity, oxidative current is also measured from tyrosine and other opioid peptides.

nLC/MS method development: We use a Q-Exactive LC-MS to optimize the utility of charged standards for dynorphin, Leu-Enkephalin and Met-Enkephalin. We were able to stabilize Met-Enkephalin from further oxidation during analysis by modifying it to its sulfone form. We developed a solid phase extraction process to allow improved reproducibility and sensitivity without compromising the limits of detection. We are now beginning to pilot in vivo studies measuring changes in opioid peptides.

Results: We show that MIEs are sensitive to increasing concentrations of dynorphin in the fmol range and are optimal at detecting low concentrations of dynorphin. Furthermore, we show that current scales linearly with concentration. We have negligible non-selective signals and we are able to distinguish detection of each of our opioid peptides (dynorphin, Leu-Enkephalin and Met-Enkephalin).

Using out nLC/MS approach our limits of detection are now in the subfmol range for dynorphin 1-8, Leu-Enk and Met-Enk. We have now chemically stabilized Met-Enkephalin to prevent further oxidation during detection. This has allowed for the simultaneous detection of all 3 peptides. We are now able to detect met-enkephalin levels at baseline in wildtype mice, prior to any manipulation/behavioral assessment, which will be critical in measuring functional decreases, a major limitation thus far.

Conclusions: Here we show the development and optimization of two methods to detect opioid peptides in vitro and in vivo at a subfmol range with spatiotemporal resolution. We plan to expand this to not only include other opioid peptides but also neuropeptides in general. This will give much needed insight into role and/or changes in endogenous neuropeptides that occur in neuropsychiatric diseases such as addiction.

Keywords: Opioid Peptides, Electrochemistry, Liquid Chromatography/Mass Spectrometry

Disclosure: Nothing to disclose.

M172. A Multi-Site Preclinical Model of Individual Variation in Vulnerability Versus Resiliency to Opioid Use Disorder

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Background: There has been a significant rise in opioid use disorder (OUD) in the United States over the past decade, making the understanding of the behavioral components that contribute to OUD necessary to explore. There is considerable individual variation in vulnerability to drug-taking and seeking behavior in humans, and we sought to create a rodent model that captures such individual variation. The goal of this project is to assess the behavioral correlates across multiple time points that contribute to opioid resiliency versus dependency in an effort to establish a translationally relevant model of OUD.

Methods: Adult male and female heterogeneous stock rats were used in this study in order to better capture genetic and behavioral diversity within a rodent model. Rats were first assessed for stress and anxiety-related behaviors via the elevated-plus maze and an open field task, followed by a tail-flick test to determine analgesic threshold. Next, rats underwent 3 weeks of intermittent long-access (LgA, 12-hr sessions) heroin self-administration training, immediately followed by a progressive ratio test to determine the motivation to work for the dug. Heroin-taking behavior was then reestablished prior to a within-session extinction training and heroin-induced reinstatement test (0.25 mg/kg). Rats underwent 6 days of extinction training prior to a test for cue-induced reinstatement. The behavioral assays assessing stress, anxiety and analgesic threshold were then repeated. To control for effects of laboratory environment on behavioral results, rats are run in tandem at two distinct locations: our facility at MUSC and the University of Camerino, Italy (UCAM).

Results: A total of 304 rats (MUSC, $n = 170$; UCAM, $n = 134$) have completed all training and testing procedures. After correcting for differences between the two sites, a Bayesian stochastic block model was applied to separate rats into clusters based on behavior over the course of training. Results showed three behaviorally distinct clusters emerged. Cluster 1 (MUSC, $n = 21$; UCAM, $n = 32$) contained rats that showed higher levels of heroin consumption and escalation of intake, greater motivation to work for drug delivery, and high levels of heroin-primed reinstatement of drug-seeking behavior compared to clusters 2 and 3 ($p<0.01$ for all). Compared to cluster 3 (MUSC, $n = 56$; UCAM, $n = 35$), clusters 1 and 2 (MUSC, $n = 83$; UCAM, $n = 67$) also showed greater cue-induced reinstatement of drug-seeking behavior ($p<0.01$ for both). In general, behavior in cluster two was between clusters 2 and 3.

Conclusions: Our model showed approximately 20% of rats demonstrate a vulnerability to OUD (cluster 1), with 30% of the population showing a distinct resiliency (cluster 3). The other half of the population exhibited traits associated with both resiliency and vulnerability (cluster 2). This stratification of individual variation in addiction-related behaviors is akin to what is observed in the human population, lending translational validity to our model. Current analyses are assessing the functional connectivity associated with resiliency versus vulnerability to OUD.

Keywords: Individual Variation, Opioid Abuse, Cluster Analysis, Resiliency, Vulnerability

Disclosure: Nothing to disclose.

M173. The Relationship Between Impulsivity, Craving, and Nighttime Drinking in a Subjects Being Treated for AUD

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Background: Impulsivity is a complex multifaceted construct that has been implicated as a transdiagnostic behavioral feature influencing AUD and other psychiatric conditions. The evidence suggests that the link between impulsivity and AUD is reciprocal, with impulsivity contributing to alcohol use, and heavy alcohol use increasing impulsive behavior. We aimed to study the role of daily (momentary) impulsivity and its relationship to daily craving and nighttime drinking, as assessed by measures on automated questionnaires administered by daily phone calls in subjects being treated for AUD in clinical trials.

Methods: The subjects with AUD $N = 162$ were recruited from the community as part of an ongoing 16-week medication trial. All subjects complete daily phone calls using an interactive voice response (IVR) data collection system that asks about craving, impulsive behavior, and drinking behavior. Because the study is ongoing, it remains blinded, so we focused this analysis on the first four weeks of treatment in which the medication is still being titrated up on the dose, and is unlikely to active yet (it takes seven weeks to get to the target dose). Multilevel modeling was used to study the effects of craving and impulsivity on nighttime drinking.

We modeled the effect of nighttime drinking on following day stress, desire, and impulsive (spur of the moment) behavior.

Results: The results show nighttime drinking did not significantly affect subsequent day stress (p = .125) once accounting for previous day stress. However, nighttime drinks did affect subsequent day desire/craving (b = .04, S.E. = 0.08, p<.001) after controlling for previous day desire and did affect subsequent impulsive behavior (b= 0.010, S.E.= 0.004, p = .005) after controlling for previous day impulsive behavior.

Conclusions: This preliminary exploratory analysis suggests that greater nighttime drinking leads to more craving and state impulsive behavior the following day. We plan to continue to explore the relationship and develop the model further with regard to other important factors such as mood, and alcohol expectancies. We anticipate the main blinded clinical trial will be finished in the next 8 months and we will also explore the effect of medication in this model over a much longer period of time (16 weeks).

Keywords: Impulsivity, Alcohol, Alcohol Use Disorder - Treatment, Perceived Stress, Craving

Disclosure: Nothing to disclose.

M174. Acute Objective and Subjective Effects of Cannabis Among Flower Versus Edible Users

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Background: In accordance with growing public acceptance of cannabis in the United States, the potency of legal market cannabis products has increased substantially in recent years. However, published studies examining the effects of cannabis have largely utilized forms of cannabis that are not representative of the products currently available on the legal market, and thus research on the health risks associated with high potency products is critically needed. The present analysis uses data from a novel observational research methodology to: 1) characterize associations between blood cannabinoid levels and amount of THC consumed among participants taking cannabis edibles, and 2) examine differences in the objective and subjective effects of cannabis among individuals who smoked high-potency flower versus took an edible.

Methods: N = 83 participants (55 smoked cannabis flower, 28 used edible cannabis; mean age=31.82 years [SD=12.2], 46% female, average self-reported cannabis use days in the past month at baseline=20.24 days [SD=10.5]) were recruited to participate in the study. Following a baseline appointment, subjects were asked to obtain either one gram of cannabis flower or a cannabis edible of their choice from a local study-partnered dispensary. At the experimental appointment, which took place in a mobile laboratory, participants completed a blood draw to assess plasma cannabinoid levels, measures of subjective drug effects (self-reported vigor, tension, and acute cannabis effects) and cognition (performance on the International Shopping List task [ISLT]) at three time points: pre-use, acute post-use, and one-hour post-use (for flower users) or two-hours post use (for edible users; note longer timespan before final timepoint for edible users was selected to account for the longer metabolism of edible cannabis). Some subjects were missing complete blood and cognitive data; the edible group n = 18 for blood and n = 16 for cognitive analyses, and the smoking group n = 50 for blood and n = 33 for cognitive analyses. RM-ANOVAs testing within-between interactions across 2 groups and 3 timepoints, expecting a medium effect size and assuming a correlation among repeated measures of at least .5 requires a sample size of 44 for α=.05. Thus, power is sufficient for the proposed analyses. Both linear and quadratic effects were tested in all models. The quadratic effect is presumed to be higher order, thus when both linear and quadratic effects were observed, only the quadratic effect was reported.

Results: In the edible group, correlations between blood-THC and self-reported THC consumed were strong at acute post-use [r(18) = .56, p = .016] and two-hours later [r(18) = .647, p = .005]. RM-ANOVAs revealed a significant interaction between group and quadratic time on blood-THC F(1,198) = 23.719, p < .001, generalized eta2=.107. Both groups showed increased blood-THC from pre-use to acute post-use and decreased blood-THC from acute post-use to one/two-hours post-use, but flower users had a higher peak at acute post-use. There were significant effects of group (F(1,243)=9.750, p =.002, eta2=.039) and quadratic time (F(1,243)=6.069, p =.014, eta2=.024) on self-reported vigor. Both groups showed increased vigor acutely post-use and a decrease at the final timepoint. The flower group reported higher vigor over the course of the experiment, but no group by time interaction emerged. Both groups showed quadratic (F(1,243)=6.222, p =.013, eta2=.025) effects of time on tension, such that tension increased at the acute post-use timepoint and decreased at the final timepoint. Acute drug effects showed the same pattern and significant quadratic effect (F(1,243)=39.958, p <.001, eta2=.141). Correct ISLT responses showed linear time effects (F(1,141)=4.352, p = .039, eta2=.030) such that correct responses were decreased at acute post-use timepoint and remained lower than baseline at the final timepoint. Quadratic (F(1,141)=5.854, p = .017, eta2=.040) effects of time were observed for ISLT errors. No group differences were observed for ISLT performance, tension, or acute drug effects. All patterns and significant effect remained for subjective and blood measures when sex was included as a covariate in all models. Including sex in the cognitive models produces significant group F(1,140)=5.943, p = .016, eta2=.041) and sex effects F(1,140)=11.522, p <.001, eta2=.076 for ISLT correct responses, and significant group F(1,140)=7.413, p =.007, eta2=.05 and sex effects on ISLT errors F(1,140)=8.513, p = .004, eta2=.057. Males made more errors than females and produced fewer correct responses over the course of the experiment.

Conclusions: Findings indicate positive correlations between blood-THC and amount of THC ingested and suggest that flower users experience higher blood-THC after using cannabis compared to edible users. Flower user reported higher positive mood effects (vigor) over the course of the experiment than the edible users, but across other subjective measures, significant group differences did not emerge. Participants who used either flower or edibles demonstrated decreased cognitive performance immediately after using cannabis, and an improvement in performance one hour later. These results have clinical and public health implications and represent a contribution to the sparse literature on high potency forms of cannabis and cannabis edibles.

Keywords: Cannabis, THC, High Potency THC

Disclosure: Nothing to disclose.

M175. Dose-Dependent Analgesic, Subjective, and Cardiovascular Effects of Oral Cannabidiol in Healthy Volunteers

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Background: Cannabidiol (CBD) elicits an antinociceptive response in animal models of neuropathic pain. Preclinical studies also point to CBD's positive cardiovascular effects. While these

effects are hypothesized to translate to humans, limited data are available supporting CBD's analgesic and cardiovascular effects when administrated alone. This outpatient double-blind, placebo-controlled, within-subject study sought to determine the analgesic and cardiovascular effects of three acute doses of oral cannabidiol compared to placebo. Pain response was assessed using the Cold Pressor Test (CPT), an experimental pain test that has predictive validity for therapeutics used to treat chronic pain. Ratings of subjective drug effects known to be associated with abuse liability and intoxication and mood states proported to be affected by CBD administration were also measured

Methods: Healthy male and female volunteers without pain (N = 14) were administered oral cannabidiol (0, 200, 400, and 800 mg); analgesic, cardiovascular, and subjective effect endpoints were assessed before and at several time points after drug administration (0.5 – 6.0 hours). For the CPT, participants immersed their hand in cold water (4°C) and times to report pain (pain threshold) and withdraw the hand from the water (pain tolerance) were recorded; subjective ratings of the 'Painfulness' and 'Bothersomeness' of the cold water stimulus were also measured. Pairwise comparisons assessed difference in 1) average, peak, and trough analgesic and cardiovascular effects between CBD (200, 400, and 800 mg) and placebo and 2) average subjective drug effect ratings between CBD (200, 400, and 800 mg) and placebo.

Results: CBD (200, 400, and 800 mg) decreased subjective ratings of 'Bothersomeness' of the cold water stimulus compared to placebo ($p < 0.05$); other measures related to pain were either not affected (pain threshold) or increased (subjective ratings of 'Painfulness' and pain tolerance) with CBD administration relative to placebo ($p < 0.05$). All CBD doses significantly reduced resting diastolic blood pressure ($p < 0.05$), an effect also observed for resting systolic blood pressure for the two lower doses ($p < 0.05$), with minimal effect on heart rate. CBD also reduced systolic blood pressure relative to placebo after the CPT (200, 400, and 800 mg CBD, $p < 0.01$) with minimal effect on heart rate. Some doses of CBD produced small but significant decreases in ratings of 'Good Drug Effect' and 'Alert' ($p < 0.05$); no other changes in subjective drug effects or mood states were observed.

Conclusions: Under double-blind, placebo-controlled conditions, acute oral CBD administration did not elicit a reliable analgesic effect in the CPT in healthy participants. This is in contrast to the analgesic effects of acute delta-9-tetrahydrocannabinol (THC) administration observed in earlier studies using the CPT. Subjective mood effects often attributed to CBD (decreased arousal and anxiety) and intoxication were also not observed in the current study. However, reliable decreases in blood pressure observed across doses replicates earlier findings with a single CBD dose and provides further evidence for the potentially favorable effects of CBD on cardiovascular endpoints. While analgesia was not detected with acute oral CBD administration, investigating CBD's analgesic effects using chronic administration paradigms, additional pain tests, and in pain populations may further inform its clinical utility to relieve pain.

Keywords: Cannabidiol, Cannabis, Abuse Liability, Pain

Disclosure: FSD Pharma: Advisory Board (Self); Insys Therapeutics: Grant (Self)

M176. Cocaine and Amphetamine Regulated Transcript (CART) Signalling in the Central Nucleus of the Amygdala Modulates Stress-Induced Alcohol Seeking

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Background: The central nucleus of the amygdala (CeA) is a key hub of the neural circuitry regulating alcohol and stress interactions. However, the exact neuronal populations that govern this interaction are not well defined. One subpopulation within the CeA that may regulate alcohol seeking includes neurons that produce the neuropeptide cocaine and amphetamine regulated transcript (CART). CART was named due to upregulation following cocaine and amphetamine administration and since has been implicated in depression-like, anxiety-like, learning, memory and reward- related behaviours. CART is expressed throughout the brain, including dense expression in the CeA, therefore we examined the distribution and role of CeA CART in alcohol seeking.

Methods: The CeA is a heterogenous structure, therefore, we first used immunohistochemistry and in situ hybridisation to examine the distribution and molecular phenotype of CeA CART neurons. To examine If CeA CART neurons were activated by yohimbine (1 mg/kg) or yohimbine-induced alcohol seeking we next using CART and Fos immunohistochemistry. To determine a functional role for CeA CART signalling we microinjected a neutralising CART antibody within the CeA and examined stress (yohimbine)-induced alcohol and sucrose seeking. We further examined whether exogenous CART 55-102 peptide within the CeA precipitates alcohol seeking in the absence of stress (yohimbine). Finally, we examined the role of CeA CART signalling in motivation using a progressive ratio schedule and anxiety-like behaviour in the light-dark box following yohimbine administration in withdrawal.

Results: We found that CART-containing neurons are predominantly expressed in the capsular/lateral division of the CeA and are a subpopulation of protein kinase Cδ (PKCδ) cells, distinct from corticotrophin releasing factor (CRF)-expressing cells. Both stress and stress-induced alcohol seeking activated CART cells (Two way ANOVA, Bonferroni's post hoc adjustment, vehicle vs. yohimbine/reinstatement, p's < 0.001), while neutralisation of endogenous CeA CART signalling attenuated stress-induced alcohol (RM two-way ANOVA, Bonferroni's adjustment, vehicle vs. CART Ab $p < 0.0001$), but not sucrose seeking (vehicle vs. CART Ab $p > 0.9999$). However, administration of exogenous CART 55-102 peptide within the CeA did not precipitate relapse in the absence of yohimbine (RM two-way ANOVA, $p = 0.286$). Further, blocking CART signalling within the CeA did not alter the motivation to obtain and consume alcohol (Paired t-test, vehicle vs. CART Ab, $p = 0.233$) but did attenuate stressor-induced anxiety-like behaviour during abstinence from alcohol (One-way ANOVA, Bonferroni's adjustment, vehicle vs. CART Ab, $p < 0.05$).

Conclusions: In summary, here we first identify CART cells as a novel discrete subpopulation of PKCδ cells within the CeA. Examination of CeA CART signalling showed they are robustly activated by, and functionally regulate, stress-induced alcohol seeking. Our data show that this action is not mediated through a reduction in the motivation for alcohol, but a reduction in stress-induced anxiety-like behaviour during abstinence from alcohol. Together our data suggest yohimbine (stress) causes release of endogenous CART within the CeA that contributes towards the reinstatement of alcohol seeking; however, exogenous CART administration within the CeA does not precipitate alcohol seeking in the absence of stress.

Keywords: Alcohol and Substance Use Disorders, Stress and Anxiety Behavior, Central Nucleus of the Amygdala, Alcohol Relapse Treatment, Neuropeptides

Disclosure: Nothing to disclose.

M177. Effect of Social Housing on Context-Induced Drug-Seeking Behavior in Rats With a History of Adolescent Drug Exposure

Abstract not included.

M178. Multiple α7 Nicotinic Acetylcholine Negative Allosteric Modulators Can Prophylactically Prevent Adolescent Binge Alcohol Exposure Enhancement of Adult Alcohol Consumption

Abstract not included.

M179. Early Adolescent Subchronic Low Dose Nicotine Exposure Increases Subsequent Pychostimulant and Opioid Self-Administration in Sprague Dawley Rats

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Background: Initiation of nicotine products typically occurs in adolescence. Recent escalation of e-cigarette use among teens highlights the necessity to understand adolescent nicotine exposure effects on substance use. Adolescence is a critical period in development (12-18 years in humans, postnatal days (PN) 28-42 in rodents) where the maturation of brain neurocircuitry is vulnerable to nicotine. Rodent studies have shown that nicotine exposure in early adolescence consistently increases subsequent drug intake and reward. However, very few studies have assessed adolescent nicotine exposure effects on opioids such as fentanyl. We hypothesize that early adolescent, but not adult, nicotine exposure will enhance fentanyl intake.

Methods: Prior to testing our hypothesis, we first replicated previous studies using an established low-dose, 4-day nicotine paradigm. Male rats (n = 9-13/group) were pretreated with nicotine (2x, 30 μg/kg/0.1 mL, intravenous) or saline for 4 consecutive days during early adolescence (PN 28-31) or adulthood (PN 86-89). One day following nicotine exposure (PN 32 or PN 90, respectively), animals underwent operant self-administration for the psychostimulant, cocaine (500 μg/kg/infusion (inf)). We then used the 4-day nicotine pretreatment paradigm in male and female rats (n = 25-34/group) followed by fentanyl operant self-administration (2.5 μg/kg/inf).

Results: Cocaine self-administration mean response data over time was analyzed by a repeated measure four-way ANOVA for pretreatment x age x reinforced/non-reinforced responses x time, with a repeated measure on reinforced/non-reinforced responses and time. Our data illustrate that adolescent nicotine exposure potentiates cocaine intake (pretreatment x age x reinforced/non-reinforced response x time, $F_{7,266}=4.51$, $p = 0.0001$). Post hoc analysis illustrates that adolescent nicotine, but not saline treated animals exhibit enhanced cocaine intake at all time points ($p<0.05$, Bonferroni corrected 1-tailed t-test) and preference for reinforced over non-reinforced responding after 105 minutes ($p<0.05$, Bonferroni corrected 1-tailed t-test). When evaluating total mean response during cocaine self-administration by a repeated measure three-way ANOVA for pretreatment x age x reinforced/non-reinforced responses, with a repeated measure on reinforced/non-reinforced responses, our results illustrate that adolescent, but not adult, nicotine exposure enhances cocaine self-administration (reinforced/non-reinforced responding x age x pretreatment, $F_{1,39}=9.35$, $p = 0.004$). Post hoc analysis illustrates that nicotine versus saline pretreated adolescents have higher cocaine reinforcement ($p<0.05$, Bonferroni corrected 1-tailed t-test). Further, nicotine pretreated adolescents exhibit discrimination for reinforced versus non-reinforced responding, highlighting a preference for cocaine intake ($p<0.05$, Bonferroni corrected 1-tailed t-test).

Fentanyl self-administration mean response data over time was analyzed with a repeated measure three-way ANOVA for pretreatment x age x reinforced/non-reinforced responses, with a repeated measure on reinforced/non-reinforced responses. Our results show that adolescent nicotine exposure potentiates fentanyl intake (age x reinforced/non-reinforced x time, $F_{7,770}=9.36$, $p = 0.0001$; pretreatment x reinforced/non-reinforced x time, $F_{7,770}=3.20$, $p = 0.002$). Post hoc analysis illustrates that adolescent nicotine versus saline treated animals exhibit enhanced fentanyl intake after 105 min ($p<0.05$, Bonferroni corrected 1-tailed t-test) and preference for reinforced versus non-reinforced responding after 30 minutes ($p<0.05$, Bonferroni corrected 1-tailed t-test). Fentanyl self-administration total response data was analyzed by a four-way ANOVA for age x sex x pretreatment x reinforced/non-reinforced responses, with a repeated measure on reinforced/non-reinforced responses in the second hour of fentanyl self-administration. Our results illustrate that adolescent, but not adult, nicotine exposure enhances fentanyl self-administration (reinforced/non-reinforced responding x age x pretreatment, $F_{1,111}=4.02$, $p = 0.047$). Post-hoc analysis illustrates that nicotine versus saline pretreated adolescents have higher fentanyl reinforcement ($p<0.01$, Bonferroni corrected 1-tailed t-test).

Conclusions: We successfully show that adolescent, but not adult, nicotine exposure enhances cocaine reinforcement in male rats. Similarly, we illustrate adolescent nicotine exposure enhances fentanyl self-administration, independent of sex. Overall, our findings highlight that adolescence is a unique period in development that is susceptible to nicotine-induced enhancement for psychostimulant and opioid self-administration in rats.

Keywords: Vaping, Drug Addiction, Fentanyl

Disclosure: Nothing to disclose.

M180. The Role of Dorsal Raphe Projections to the Ventral Tegmental Area in Reward: Modulation by CB1 Receptors

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Background: Serotonin and glutamate co-releasing neurons originating in the dorsal raphe (DRN) synapse onto ventral tegmental area (VTA) dopamine neurons. These connections excite neurons, enhance terminal dopamine release in the nucleus accumbens (NAc) and promote conditioned place preference. The expression of CB1 receptors in this pathway may act to curtail this excitation and dampen reward processes.

Methods: To investigate the contribution of CB1 receptors to the rewarding effect of this pathway, we deleted CB1 receptors in DRN to VTA neurons in both male and female CB1 floxed mice (n = 10). To achieve this, we used a dual viral approach. A retrograde flp virus (pAAV-EF1a-mCherry-IRES-Flpo) was injected into the VTA, and a flp dependent cre (pAAV-EF1a-fDIO-Cre) into the DRN. This strategy produced selective deletion of CB1 receptors only in VTA to DRN projecting neurons. In addition, the genetically encoded dopamine sensor, GRAB-DA4.4 was expressed in the NAc and an optical fiber implanted to record real time dopamine release during reward related behaviors with fiber photometry. Mice performed operant responding - lever pressing - for sucrose pellets on fixed ratio 1, fixed ratio 5 and progressive ratio schedules.

Results: We found that CB1 deletion did not affect lever press responding for food on fixed ratio schedules (1 or 5 lever presses) $p>.05$. However, there was an unexpected and significant decrease in responding on progressive ratio (PR) measures ($p<.01$), reflecting a reduction in motivated pursuit of reward in the deleted condition. In addition, dopamine release was substantially reduced

in knock out animals in both FR5 and PR experiments in response to both reward receipt and reward cue events.

Conclusions: These results suggest there may be complex, indirect mechanisms involved in CB1 modulation of DRN to VTA neurons which require further investigation. Future experiments will characterize the extent of deletion in these animals, evaluate the success of this viral approach and investigate the effects of cannabinoid drugs in this paradigm.

Keywords: Endocannabinoid System, Dopamine, Dorsal Raphe, Serotonin, Motivation

Disclosure: Nothing to disclose.

M181. Vaping Nicotine and COVID-19: Effects of Nicotine on Lung ACE2 Expression

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Background: The COVID-19 pandemic has presented a great challenge worldwide in the last 4 months. Global urgency currently surrounds the need to learn more about disease pathology and individual differences in infection and outcome. Interestingly, patient reports have suggested a possible correlation between nicotine smoking and severe respiratory symptoms following infection. Further, it has been demonstrated that angiotensin-converting enzyme 2 (ACE2) in the lungs may serve as a mechanism for viral entry. In these studies, we sought to investigate whether e-cigarette vapor exposure alters the expression of ACE2 and related proteins in the lungs and blood of male and female mice.

Methods: In the first group, male and female wildtype C57BL6/J mice (n = 6 per group for each sex) were exposed to e-cigarette nicotine or vehicle vapor in custom chambers (LJARI, La Jolla, CA) across five sessions. Vehicle consisted of propylene glycol (PG) and vegetable glycerin (VG) at a 1:1 ratio, and the nicotine solution concentration was 7.5 mg/ml free base. Each day, exposure consisted of two second puffs every five minutes across a one hr session. Constant air flow in the chambers was maintained at one liter/min. On the final treatment day, blood, lungs and brain tissue were collected two hours after the first nicotine or vehicle puff. RT-qPCR analysis was conducted to determine the mRNA levels of ACE2 and its associated protein transmembrane serine protease 2 (Tmprss2). Given that nicotine directly acts on nicotinic acetylcholine receptors, we also examined for differences in two receptor subunits – alpha5 and alpha7 – that are expressed in the lungs and have been associated with cancer and inflammation, respectively. To assess whether ACE2 protein is released into the blood in response to nicotine treatment, blood plasma samples were analyzed with an ELISA assay. Finally, to determine if the differences were specifically due to nicotine, a second group of male and female mice (n = 5 per group for each sex) were subcutaneously injected with saline or nicotine daily for five consecutive days. On the final day, mice were sacrificed two hours after the injection, and lung tissue was collected and processed for ACE2 expression. All procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The University of California, Irvine.

Results: We found a significant increase in ACE2 mRNA in lung tissue of male mice exposed to nicotine-containing vape, as compared to the vehicle control (Unpaired t test, $t(11)=2.572$, $p = 0.0260$). Surprisingly, this difference was sex-specific, as the female groups did not differ in ACE2 mRNA expression in lung tissue ($t(11)=0.6122$, $p = 0.5529$). Therefore, we next analyzed the auxiliary protein Tmprss2, but no differences were found between the experimental groups (Male: $t(12)=0.2787$, $p = 0.7852$; Female: $t(11)=0.2339$, $p = 0.8193$). Similarly, protein quantification of ACE2 in the blood did not detect any change following vapor exposure (Male: $t(11)=0.4002$, $p = 0.6967$; Female: $t(11)=1.888$, $p = 0.0857$). When we analyzed the expression of the nicotinic acetylcholine subunits, no differences were found between the experimental groups for alpha5 (Male: $t(12)=1.272$, $p = 0.2276$; Female: $t(11)=1.259$, $p = 0.2341$) or for alpha7 (Male: $t(12)=0.0076$, $p = 0.9940$; Female: $t(11)=0.1523$, $p = 0.8817$). Therefore, in the second study, we analyzed whether the increase in ACE2 could be attributed to nicotine alone via systemic exposure. However, no significant differences were observed between groups following peripheral nicotine treatment (male: $t(8)=0.1173$, $p = 0.9095$; female: $t(7)=0.4304$, $p = 0.6799$), indicating that inhalation of nicotine with the vehicle mitigated the increase in ACE2 expression.

Conclusions: The data derived from these preliminary investigations highlights a putative direct link between e-cigarette vapor and increased ACE2 expression in the lungs of males. Future studies will be necessary to determine whether these nicotine vapor-mediated effects subsequently lead to altered pathology and lung function following viral COVID-19 infection, which may thus inform on individual differences found in patient populations.

Supported by the National Institute on Drug Abuse (NIH DA039658 to CDF) and Tobacco-Related Disease Research Program (TRDRP T30FT0967 to VL).

Keywords: Nicotine, COVID-19, Electronic Cigarette (e-cigarette)

Disclosure: Nothing to disclose.

M182. Examining Partial Versus Full mGlu5 Negative Allosteric Modulators on Cognition and Brain Function Using EEG in Rats

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Background: Functional antagonism of the metabotropic glutamate receptor subtype 5 (mGlu5) remains a promising potential treatment for many neuropsychiatric disorders including anxiety, depression and multiple symptoms associated with substance use disorders (SUD). However, historical data suggest that mGlu5 antagonists may also engender adverse effects including sedation, cognitive impairments and psychotomimetic-like effects. Although drug discovery programs have achieved a higher degree of subtype selectivity via development of negative allosteric modulators (NAMs) targeting mGlu5, concern surrounding adverse effect potential remains. One strategy for potentially decreasing adverse effect liability is through development of partial mGlu5 NAMs. Partial mGlu5 NAMs block less than 100% of the effects assessed in vitro when compared to a full mGlu5 NAM, at concentrations that fully occupy the allosteric binding site on mGlu5. Partial mGlu5 NAMs, represented by M-5MPEP, still produce many of the potential therapeutic effects in preclinical models (including anxiolytic- and antidepressant-like and decreasing cocaine-related behaviors). Further understanding of the adverse effects associated with full and partial functional antagonism of mGlu5 and possible underlying mechanisms are necessary. Herein, we describe studies comparing behavioral and functional effects of the partial mGlu5 NAM M-5MPEP with the full mGlu5 NAM, VU0424238. First, effects of both compounds were examined in rats trained to perform a paired-associates learning (PAL) task via a touch-sensitive computer screen, a measure of visuo-spatial memory shown to be sensitive to glutamatergic manipulations. Secondly, using electroencephalography (EEG) studies in freely moving rats, we evaluated effects of these full

and partial mGlu5 NAMs on brain function using quantitative EEG (qEEG). Lastly, given that mGlu5 is structurally and functionally coupled to the NMDA receptor and NMDA receptor hypofunction induces cognitive disruptions, we examined mGlu5 NAMs in combination with the NMDA receptor antagonist MK-801 on cognition and brain function to investigate one purported mechanism underlying adverse effects.

Methods: Male Sprague-Dawley rats (n = 8) were trained to break an infrared beam in front of a computer screen (e.g. touchscreen) to receive a liquid reward. Rats learned to track and respond on one of three stimuli, each of which was only deemed a "correct" response when presented in a specific location on the touchscreen. Following stable performance (>75% accuracy for 3 consecutive days) effects of 18-56.6 mg/kg M-5MPEP, 3-30 mg/kg VU0424238 or vehicle (10% Tween 80) and 0.1-0.3 mg/kg (s.c.) MK-801 were tested alone. Lastly, effects of 30 or 56.6 mg/kg M-5MPEP and 1 or 30 mg/kg VU0424238 were evaluated in combination with a subthreshold dose of MK-801 (0.1 mg/kg s.c.).

For electroencephalography studies, male Sprague-Dawley rats (n = 8) were implanted with surface electrodes in contact with the dura above the frontal and contralateral occipital cortex. EEGs were recorded from each rat's home cage for 8 hours beginning with light onset. Effects of M-5MPEP, VU0424238, MK-801 (same doses as above) were evaluated on brain function using quantitative EEG (qEEG) when administered two hours into the light cycle. Based on behavioral data, effects of M-5MPEP and VU0424238 were evaluated in combination with 0.1 mg/kg MK-801. All experiments were approved by the Wake Forest University Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. For all studies, ANOVAs were used for statistical analysis to examine differences from respective vehicle-treated control groups and, when appropriate, followed by post-hoc Dunnett's t-tests.

Results: Neither M-5MPEP nor VU0424238 decreased percent accuracy on the PAL task, at doses spanning 50-90% ex vivo receptor occupancy. MK-801 does-dependently decreased percent accuracy. In combination, only 30 mg/kg VU0424238 decreased percent accuracy in combination with the subthreshold dose of 0.1 mg/kg MK-801. Consistent with previously published studies, MK-801 induced elevations in gamma band oscillatory activity (30-100Hz). 30 mg/kg VU0424238 potentiated MK-801-induced elevations in high frequency gamma power a potential correlate for cognitive disrupting effects. M-5MPEP did not potentiate MK-801-induced elevations in gamma power. Importantly, M-5MPEP and VU0424238 did not induce significant effects on gamma power or alone.

Conclusions: These results suggest that highly selective mGlu5 NAMs alone, at dose exceeding >80% receptor occupancy, do not disrupt visuospatial learning. Cognition and EEG studies further support the hypothesis that disruptive effects of mGlu5 antagonism may occur through interaction with NMDA receptor inhibition. However, doses of VU0424238 that exacerbated MK-801 effects may be outside the therapeutic range based on prior preclinical studies. Together, data suggest that partial negative allosteric modulation of the mGlu5 receptor may be less likely to induce adverse effects than full mGlu5 NAMs. These data add to literature reiterating the importance of considering dose ranges producing possible therapeutic versus adverse effects of full mGlu5 NAMs as opposed to a generalization that functional inhibition of mGlu5 induces adverse effects.

Keywords: Cognition, Quantitative Electroencephalography (qEEG), mGlu5-NAM

Disclosure: Nothing to disclose.

M183. Does Exposure to Nicotine and Cannabinoids During Adolescence Make Nicotine Relapse More Likely in Adulthood?

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Background: Recent studies suggest that adolescent exposure to substances of abuse, including nicotine or cannabis, may alter neuromaturation and neurocognitive function during adulthood. Nicotine, the main psychoactive component in cigarettes and e-cigarettes, acts on neuronal nicotinic acetylcholine receptors in the brain. The main psychoactive component in cannabis, THC, acts on cannabinoid receptors. Here, we examine the effects of adolescent exposure to nicotine, a cannabinoid receptor agonist (WIN55-212,2), or co-exposure to both substances on nicotine relapse-related behaviors in adult male and female mice.

Methods: During adolescence (postnatal days 38-49), mice were injected with vehicle (n = 10 males, 10 females), nicotine (n = 8 males, 10 females), WIN55-212,2 (n = 9 males, 10 females), or both substances (n = 10 males, 11 females) across 12 consecutive days. During adulthood, mice were trained in a food self-administration paradigm in operant boxes. Subsequently, catheters were intravenously implanted in the right jugular vein, and following a recovery period, the mice were given access to self-administer nicotine. Following stable responding with intravenous nicotine self- administration, mice were tested in the incubation of craving protocol to assess nicotine relapse-related behavior. This was measured via lever pressing behavior in the absence of nicotine, on either day 1 or day 24 post-nicotine. Data was analyzed using repeated-measures one-way ANOVA with Prism 7 software (GraphPad, La Jolla, CA, USA). Significant effects were followed by Tukey's post-hoc multiple comparisons test. The criterion for significance was set at $\alpha = 0.05$.

Results: Our findings reveal differential effects in relapse-related behavior within each sex, dependent on adolescent drug exposure. As expected, control male and female mice displayed a significant increase ($p<0.05$) in nicotine seeking behavior after the incubation period. Males exposed to WIN55-212,2 alone ($p<0.01$), or co-exposed to nicotine/WIN55-212,2 ($p<0.05$), and females exposed to WIN55-212,2 ($p<0.05$) alone also exhibited an incubation effect with increased lever pressing behavior in adulthood. Conversely, males exposed to nicotine and females exposed to nicotine alone, or nicotine/WIN55-212,2, did not exhibit a significant change in nicotine seeking following the incubation period.

Conclusions: Together, these data provide evidence that adolescent exposure to nicotine and/or cannabinoids alters later nicotine relapse-related behaviors in a sex-dependent manner during adulthood.

This research was supported by the Tobacco and Related Disease Research Program (TRDRP) award 26IP-0043 to CDF and the National Science Foundation Graduate Research Fellowship (NSF GRFP) award DGE-1839285 to AJE. Unique Data: All of these findings are new and unpublished.

Keywords: Nicotine, Cannabinoids, Adolescence

Disclosure: Nothing to disclose.

M184. Adolescent Social Isolation Drives Increased Heroin Vulnerability and Dysregulates the Dopamine System

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Background: The United States is in the midst of a major opioid epidemic, with >30 million people reporting opioid use and nearly 1 million Americans reporting heroin use at least one time in 2016. To combat the opioid crisis effectively, we need a deeper understanding

of the factors that drive vulnerability to develop opioid use disorder (OUD). Chronic psychosocial stress has been linked to increased risk for a host of negative outcomes, including depression, anxiety, and substance use disorders (SUDs). In addition, exposure to these stressors during adolescence drives even higher risk for future psychiatric disorders that may drive or worsen SUDs in adulthood. Our group and others have shown that chronic adolescent social isolation (aSI) stress in rodents leads to persistently increased drug responsiveness and negative affective behaviors when compared to adolescent group-housed (aGH) control. The robust impact of aSI on drug and alcohol self-administration (SA), coupled with strong evidence that aSI-induced alterations in the dopamine system potently regulate vulnerability to drug-seeking, lead us to predict these effects would be applicable opioids as well. Here, we will assess the impact of aSI on heroin self-administration.

Methods: Male Long-Evans rats (N = 96) were housed in groups (4/cage) or isolation (1/cage) from postnatal day (PND) 28-70 following a 7-day acclimation period. Following the housing paradigm, all rats were assayed for anxiety-like behavior and response to a novel environment, implanted with jugular catheters, and individually housed in operant chambers that serve as both the home cage and SA chamber. Following recovery, rats were given access to a lever and trained to self-administer heroin (FR1, 0.025 mg/kg/infusion). After acquiring heroin SA, rats were tested for dose-responsivity (FR1), motivation for heroin seeking using progressive ratio (PR), and escalation of intake using a long access paradigm (unlimited infusions, 6hr/session, FR1). Next, the combination of cue- and stress-induced reinstatement responding using the pharmacological stressor, yohimbine (1.25 mg/kg, IP), were evaluated following the extinction of responding. A group of aSI and aGH were given sham surgeries to serve as heroin-naïve controls. To measure dopamine alterations after chronic heroin exposure (or LgA), we utilized ex vivo fast-scan cyclic voltammetry (FSCV) to measure dopamine release and uptake kinetics and terminal receptor functioning in brain slices containing the nucleus accumbens (NAc). Lastly, negative affect elicited by heroin withdrawal was assessed by recording ultrasonic vocalizations (USVs) prior to the last LgA session.

Results: In support of previous studies, we found that aSI rats have increased anxiety-like behavior and locomotor response to a novel environment. Our SA results revealed that aSI rats have increased rates of heroin SA acquisition, escalation of heroin responding on LgA, responding during extinction sessions, and cue- and stress-induced reinstatement responding. In contrast, we found that both aSI and aGH have similar responding to various doses of heroin on both FR1 and PR schedules of reinforcement. Using FSCV, we found that heroin-naïve aSI rats have increased electrically stimulated dopamine release and uptake rats, indicating increased DA system functioning. Following LgA, stimulated dopamine release was reduced in both aSI and aGH rats, however; dopamine uptake rates were only reduced in heroin-exposed aSI rats, compared to their respective heroin naïve counterparts, suggesting a greater downregulation of the dopamine system in heroin-exposed aSI rats. In addition, we found that heroin aSI rats had increased activity at D2/D3 autoreceptors, which may play a role in the profound decrease in NAc dopamine terminal function in aSI rats after heroin. Further, analysis of USVs revealed increased 22 kHz calls in heroin aSI rats compared to heroin naïve aSI and heroin-exposed aGH rats. Consistent with our results demonstrating downregulated dopamine functioning in the NAc, this data suggests that heroin-exposed aSI rats experienced greater negative affect during withdrawal from heroin, which may, in part, drive the increased heroin seeking exhibited during SA.

Conclusions: Our results demonstrate that exposure to chronic psychosocial stress during adolescence results in robust behavioral and neurobiological adaptions that lead to increased vulnerability to opioid seeking. In addition, the intersection of adolescent stress and heroin vulnerability may be linked to altered dopaminergic functioning in reward-related brain regions.

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

Disclosure: Nothing to disclose.

M185. Granulocyte-Colony Stimulating Factor Mediates Cocaine Reward and Reinstatement Through Glutamatergic Mechanisms

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Background: Pathological substance use disorders, including psychostimulant use disorder, represent a major public health concern. Addiction to cocaine and other psychostimulants remains a major cause of this morbidity. The pathophysiological mechanisms that lead to persistent and dysregulated drug use remain incompletely understood, and there are currently no FDA-approved pharmacotherapies for treatment of psychostimulant use disorders. There is growing evidence that dysregulation of the immune system plays a role in the pathophysiology of multiple psychiatric disorders including major depressive disorder, Alzheimer's disease, and schizophrenia. While cocaine is known to have immunomodulatory effects, the link between these immune interactions and pathological use behaviors has only recently been investigated. Recent work from our laboratory implicates granulocyte-colony stimulating factor (G-CSF) as a neuroimmune modulator of cocaine reward. Levels of G-CSF are increased in blood and brain following cocaine administration and levels of G-CSF correlate with cocaine intake. Injections of G-CSF enhance low-dose cocaine self-administration in a threshold task, and inhibition of G-CSF in the nucleus accumbens (NAc) prevents formation of cocaine conditioned place preference. Since publication of these earlier studies, we have performed extensive work examining the, cellular, molecular, and behavioral mechanisms that underlie G-CSF signaling. Here we show that treatment with G-CSF can lead to reduced reinstatement of cocaine self-administration, use cutting-edge proteomics to identify how G-CSF treatment affects cells in the prefrontal cortex and nucleus accumbens, and use viral circuit isolation experiments to identify interactions of G-CSF with specific glutamatergic afferents that project to the nucleus accumbens.

Methods: To assess the effects of G-CSF on reinstatement of cocaine seeking, male Sprague-Dawley rats were first trained to self-administer cocaine on a fixed-ratio 1 schedule until stably responding. Animals then received daily injections of G-CSF (n = 9) or vehicle (n = 8) during incubation and reinstatement. The rate of cue and cocaine-induced reinstatement responding was then measured. Samples from the nucleus accumbens and prefrontal cortex of these same animals were then sent for global proteomics analysis. We utilized cutting-edge data independent acquisition mass spectrometry analysis to increase coverage of the proteome. Significantly altered proteins were then analysis using the STRING software package to identify highly interacting proteins, and gene ontology analyses were utilized to identify functional pathway changes. Due to preliminary results suggesting that glutamatergic inputs to the nucleus accumbens may be the strongest drivers of behavioral and molecular effects, we then performed experiments to interrogate these inputs. Utilizing retrograde Cre-recombinase viral infection in the NAc, and Cre-dependent expression of designer receptors activated exclusively by designer drugs (DREADDs) in multiple projecting nuclei (prefrontal cortex, basolateral amygdala, ventral hippocampus) we activated/inactivated these pathways to observe how they modulate the expression of G-CSF and G-CSF receptor in the NAc with and without cocaine. 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: We find that treatment with G-CSF during abstinence leads to a marked reduction in cue-induced cocaine seeking. Proteomic analysis identified 125 proteins differentially regulated between G-CSF and vehicle treated animals with the most robust changes found in the prefrontal cortex. Pathway analysis indicated that many of these proteins localize to glutamatergic synapses (top gene ontology terms included glutamatergic synapse, cell membrane, postsynapse, and regulation of chemical synaptic transmission). DREADD experiments revealed activation of the prefrontal cortex to nucleus accumbens recapitulates the effects of cocaine on G-CSF and G-CSF receptor expression and leads to discrete molecular changes in the accumbens. These same effects were not noted in the other brain regions examined.

Conclusions: Our results demonstrate that the pleiotropic cytokine G-CSF reduces cue-induced cocaine reinstatement, and that this behavioral effect is likely due to effects of G-CSF on the prefrontal cortex to nucleus accumbens circuit. These studies further the importance of G-CSF as a key signaling molecule in addiction-like behaviors and provide a solid basis for the mechanism of the effects of G-CSF in the brain.

Keywords: Cocaine, Neuroimmune Mechanisms, Glutamate

Disclosure: Nothing to disclose.

M186. Mesolimbic Circuit Dynamics Underlying Individual Alcohol Drinking

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Background: Harmful alcohol use remains a serious public health issue, resulting in 3 million deaths globally and contributing to more than 200 disease and injury conditions per year. Within the United States alone, the prevalence of Alcohol-Use Disorder (AUD) has increased significantly from 8.5% to 12.7% over the last 10 years and whose complex etiology has limited the number of effective therapeutics currently available. An interesting phenomenon is the variability of alcohol consumption occurring within the human population; some individuals drink casually while others drink in an uncontrolled or compulsive manner, leading to future diagnoses of AUD. Furthermore, it is known that a hallmark of the progression of AUD is the dysfunction of dopamine (DA) neurons projecting from the ventral tegmental area to the nucleus accumbens (VTA-NAc), a neural circuit critical to encoding the salience of both drug and naturalistic stimuli. Previously, we found that low alcohol drinking mice exhibited hyperdopaminergic activity in this circuit after exposure to a 12-day two-bottle choice, voluntary alcohol access paradigm, whereas high alcohol drinking mice exhibited neuronal firing activity similar to alcohol-naïve controls. In this study, we investigated VTA-NAc DA circuit dynamics before and after the establishment of individual alcohol drinking phenotype, in an effort to determine if there was a neural signature that predicted low or high alcohol drinking and how this neural signature was reciprocally affected by alcohol drinking behavior in itself.

Methods: Methods: To capture this phenomenon of individual alcohol drinking variability, we utilized isogenic C57BL/6J male mice, an inbred mouse strain typically used to study alcohol drinking behaviors. To assess how VTA-NAc DA circuit-specific function may underly these distinct alcohol drinking phenotypes, we utilized a cell- and circuit-specific viral approach (GCaMP6s) within a transgenic TH-BAC-Cre mouse line. Using in vivo fiber photometry calcium imaging recordings in freely behaving animals, we next performed longitudinal recordings before and after alcohol drinking within the same animals over time. We used a series of naturalistic behavioral assessments of reward sensitivity and motivation as a proxy for overall circuit activity to ensure the natural emergence of alcohol drinking phenotype would not be disrupted. These naturalistic behavioral assessments, including social interaction, novel object investigation, and sex-related reward (female urine), allowed us to determine the neural population response of the VTA-NAc DA circuit before and after the establishment of alcohol drinking phenotype, to see how this phenotype attenuated or augmented the VTA-NAc DA circuit response broadly and to illuminate the transition to low or high alcohol drinking. All data sets were tested for normality and homoscedasticity before assumptions were made about the distributions and the appropriate statistical tests were utilized.

Results: Our preliminary fiber photometry recordings from VTA-NAc DA neurons show that while individuals exhibit a canonical response to sex-related reward and social interaction at baseline in the form of GCaMP6s fluorescence, the magnitude, timing, and sustained activity of this neural population response differs between individuals and is predictive of future alcohol drinking phenotype. Interestingly, our behavioral results show that individuals that spend a greater percent of time with natural reward at baseline become low alcohol drinkers in the future ($n = 34$, Pearson correlation, $r = -0.44$, $p = .0092$**). Further, animals that exhibit a greater latency to engage in investigation of this reward, become high alcohol drinkers ($n = 34$, Pearson correlation, $r = 0.3676$ $p = .0298$*). After the establishment of alcohol drinking phenotype, our fiber photometry recordings show a potentiation of VTA-NAc DA activity in response to reward in high alcohol drinking mice, whereas this effect is seen to be decreased in low alcohol drinking mice. In corroboration, our behavioral results show that high alcohol drinking is associated with an increase in exploration and social interaction, whereas low alcohol drinking is associated with a decrease.

Conclusions: By assessing the VTA-NAc DA neuronal profile of activity during naturalistic mammalian behaviors prior to and after voluntary alcohol drinking, this project will provide novel insight into physiological and real-time predictors of future, individual alcohol drinking phenotypes that are relevant to the general population and reveal how alcohol actively and reciprocally attenuates or exacerbates VTA-NAc DA circuit function leading to subsequent adaptive or maladaptive behaviors.

Keywords: Fiber Photometry, Alcohol, Dopamine, Ventral Tegmental Area (VTA), Nucleus Accumbens

Disclosure: Nothing to disclose.

M187. Optogenetic Inhibition of Cue-Elicited Dopamine Activity Attenuates Sign-Tracking Behavior to a Pavlovian Food Cue

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Background: Environmental cues can guide behavior in an adaptive manner, bringing one into close proximity to valuable resources. However, for some individuals, such cues attain inordinate control and can lead to maladaptive behavior. In rodents, individual differences in cue-motivated behaviors can be captured using a Pavlovian conditioned approach (PavCa) paradigm, wherein presentation of a discrete cue (conditioned stimulus, CS) is followed by delivery of a food reward (unconditioned stimulus, US). Following PavCa training, two distinct phenotypes emerge – goal-trackers (GT) and sign-trackers (ST).

While both GTs and STs attribute predictive value to the reward cue, STs also attribute incentive value to the cue. The attribution of incentive motivational value, or incentive salience, transforms the cue into an attractive and desirable stimulus. For STs, both food- and drug-associated cues gain excessive incentive value and elicit maladaptive behaviors. The ST/GT model, therefore, can be utilized to elucidate the neurobiological mechanisms that encode adaptive or maladaptive cue-driven behaviors. STs and GTs rely on distinct neurobiological mechanisms. Notably, sign-tracking, but not goal-tracking, behavior is dopamine (DA)-dependent, and cue-elicited DA in the nucleus accumbens (NAc) is thought to encode the incentive value of reward cues. Here we exploited the temporal resolution of optogenetics to determine if selective inhibition of cue-elicited DA would attenuate the propensity to sign-track. In addition, we assessed the distribution of the transgenic Long-Evans rat population that would allow us to do so.

Methods: To assess the phenotype distribution, 115 male Long-Evans rats were trained in a PavCa paradigm for 6 sessions, in which they received 25 CS-US (lever-food) pairings each session. For the optogenetics experiment, we utilized 13 male tyrosine hydroxylase (TH)-Cre Long Evans rats, which express cre-recombinase in DA neurons. Stereotaxic surgeries were performed to infuse and express virus selectively in DA neurons within the ventral tegmental area (VTA), and to implant optogenetic probes above the VTA. Animals either received a control virus (pAAV5-Ef1a-DIO-EYFP) or an optogenetic viral construct containing halorhodopsin (pAAV5-Ef1a-DIO eNpHR 3.0-EYFP), a light-sensitive inhibitory channel. Following 21 days of virus incubation, animals were tested in the PavCa paradigm, in which they received 25 CS-US (lever-food) pairings for 6 sessions. During the first 3 sessions, laser light (10 mW; 593 nm) was concurrent with CS presentation. For the final 3 PavCa sessions, CS presentation was not accompanied by laser light. Animals were euthanized within 5 days of experiment completion and tissue was sliced to confirm virus expression and optogenetic probe placement.

Results: First, we assessed the tendency of male Long-Evans rats to sign-track (i.e. without optogenetic manipulation), and found that, out of a population of ~115 rats, ~72% are sign-trackers. Further, out of the ~45 TH-Cre Long-Evans rats, ~85% are sign-trackers. We then assessed whether laser-induced inhibition paired with CS presentation would prevent the development of sign-tracking behavior. Control animals ($n = 6$) displayed no impact of laser light on lever-directed behaviors. A linear mixed-effects model analysis compared session 3 (final laser light-CS pairing) to session 6 for halorhodopsin (eNpHR)-expressing animals ($n = 7$) - revealing an increase in lever-directed behaviors. Number of lever presses ($p = 0.021$) and probability to approach the lever ($p = 0.002$) significantly increased from session 3 to session 6 in eNpHR-expressing animals. Further, eNpHR-expressing animals also had a significant increase in response bias [response bias = ((lever contacts – magazine entries)/(lever contacts + magazine entries))] ($p = 0.006$) from session 3 to session 6.

Conclusions: These results suggest that male Long-Evans rats tend to be skewed toward sign-tracking behavior. Moreover, rats with optogenetic inhibition of cue-elicited DA exhibited a bias towards goal-tracking, rather than sign-tracking behavior. Thus, the pairing of laser-light with CS presentation during the first 75 trials of CS-US (lever-food) presentations decreased the tendency to sign-track. When laser inhibition was terminated, these same rats then began to develop a sign-tracking response. These findings demonstrate that cue-elicited DA release is critical for incentive learning processes.

Keywords: Individual Differences, Incentive Salience, Optogenetics, Th-Cre

Disclosure: Nothing to disclose.

M188. Endocannabinoids Control the Neural Substrates of Interval Timing in the Nucleus Accumbens

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

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

Results: We found that interval timing was encoded by bidirectional ramping activity of NAc ensembles and progressive increases in gamma frequency power of the local field potential. Increasing levels of the endocannabinoid 2-AG via the MAGL inhibitor JZL184 (18 mg/kg, ip) resulted in an acceleration of time estimation and attenuation of interval encoding in a CB1 receptor-dependent manner. However, endocannabinoid-mediated disruptions in interval timing were reversed by optically-driven NAc network oscillations at gamma frequencies.

Conclusions: These results reveal a significant role for endocannabinoids in the accumbal network dynamics that guide timing behavior and may have important implications for the use of pharmacotherapies targeting the endocannabinoid system and for the recreational use of plant-based and synthetic cannabinoids.

Keywords: Endocannabinoids, Nucleus Accumbens, Timing, Reinforcement, Gamma Oscillations

Disclosure: Nothing to disclose.

M189. Investigating Behavioral and Physiological Sex Differences in a Model of Opioid Withdrawal

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Background: Stemming from the over prescription of opioids, women are showing precipitous increases in opioid use and overdose, compelling researchers to investigate sex differences in opioid use disorder. Previously we found that both male and female c57BL6/J mice exhibit profound precipitated withdrawal (WD) from moderate doses of morphine. Investigating the bed nucleus of the stria terminalis (BNST), a region regulating both somatic and affective components of opioid WD. We found that an opposite effect on spontaneous inhibitory postsynaptic currents (sIPSCs) in male and female mice in acute WD (Luster, Cogan et al., 2020). This paradigm results in the development of a protracted WD syndrome lasting for over 6 weeks following initial treatment. Interestingly, the behavioral plasticity expressed in protracted WD is distinct in male and female mice (Bravo et al., 2020). Here we further explore the behavioral and physiological manifestation of opioid WD in male and female mice.

Methods: Opioid WD in adult (at least 10 weeks old) male and female c57BL6/J mice was conducted as in Luster, Cogan et al., 2020.

Electrophysiological was conducted as in McElligott et al., 2010, recorded ClampEx, and analyzed using ClampFit and Synaptisoft Minianalysis. Miniature excitatory postsynaptic currents (mEPSCs) had 500 nM tetrodotoxin in the bath.

Sleep analysis used the PiezoSleep System (Signal Solutions). Mice were placed into the chambers for 6 days to establish baseline sleep rhythms and subsequently exposed to the WD paradigm. They were then monitored for 8 days following. The 6th day, animals were deprived of sleep for the first 4 hours into their light cycle.

Statistics: Statistics were analyzed using GraphPad Prism (versions 6-8). 2-way ANOVAs were used to test between groups with Bonferoni post-hoc tests performed when there was a significant interaction ($p<0.05$).

Results: Examining excitatory synapses in the BNST, we found that the paradigm increases the frequency of mEPSCs in both male and female mice ($p<0.05$). To examine which circuits may underly this adaptation, we injected mice with fluorescent retrobeads in the ventral tegmental area, and then recorded from fluorescent cell bodies in the BNST. We found that there was a significant decrease in the paired pulse ratio onto these BNST->VTA neurons ($p<0.05$).

To further investigate behavioral adaptation following morphine exposure and withdrawal, we next examined how our paradigm of opioid WD modulated sleep behavior. We found that our paradigm significantly altered the sleep patterns of male and female mice during the three days of treatment ($p<0.0001$ both sexes). Interestingly, it also dysregulated sleep during the subsequent days following the treatment (day 1 males: $p<0001$, females $p<0.05$; day 2 males $p<0.001$), however by day 3 post treatment, there were no significant differences in either male or female withdrawn mice as compared to their controls. We then examined how the mice would respond to a 4-hour sleep deprivation experiment one week into protracted WD. While the male mice did not differ from their controls, the female WD mice demonstrated significantly enhanced active period sleep ($p<0.05$).

Conclusions: These data suggest that there is significant physiological and behavioral adaptation occurring in mice following opioid WD in sleep relevant circuitry. Interestingly we observed that female mice were more sensitive to sleep deprivation, which may suggest changes in their homeostatic sleep drive. We have several plans to follow up on these studies to mechanistically investigate how this circuitry may be altered in opioid WD.

Keywords: Opioid Abuse, Sleep, Withdrawal, Sex Differences

Disclosure: Nothing to disclose.

M190. Dissecting the Role of Accumbal D1 and D2 Medium Spiny Neurons in Information Encoding

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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 1 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, designer receptor exclusively activated by designer drugs (DREADDs), 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 activating and inhibiting D1 and D2 MSNs via designer receptors exclusively activated by designer drugs (DREADDs) affected learning and performance on these tasks.

Results: First, we showed that mice responded for optical self-stimulation of both cell types, suggesting D2-MSN activation is not inherently aversive. While optogenetic approaches give some information about how cellular activation can modulate behavior, they eliminate the temporal specificity of neural activity patterns that encode information in behaving animals. To understand how real-time activity in these populations is linked to behavioral execution, we expressed the genetically encoded calcium indicator (GCaMP6f) within D1 and D2 MSNs coupled with in vivo fiber photometry and miniature microscopes to record from these cell populations in awake and behaving animals during multiple learning and memory tasks. Utilizing complex reinforcement schedules as well as Pavlovian learning paradigms that allow dissociation of stimulus value, outcome, cue learning, and action, we demonstrated that D1 MSNs respond to the presence and intensity of unconditioned stimuli – regardless of value. Conversely, D2 MSNs responded to the prediction of these outcomes during specific cues rather than responding divergently to positive and negative stimuli.

Conclusions: Overall, these results provide foundational evidence for the discrete aspects of information that are encoded within the NAc D1 and D2 MSN populations, which ultimately goes beyond simply encoding rewarding versus aversive stimuli. 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 Neurons, Information Encoding, Reward Learning, Aversive Learning

Disclosure: Nothing to disclose.

M191. Resting State Functional Connectivity and mGlu5 Receptor Availability at Multiple Abstinence Time Points in Patients With Alcohol Use Disorder

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Background: People recovering from alcohol use disorder (AUD) show persistent alterations in resting brain activity during abstinence [1,2]. Importantly, functional connectivity changes after months of abstinence can be predictive of later relapse [2]. It is not yet known how these resting state alterations develop in the earliest stages of recovery. Given high rates of relapse within the first month of treatment [3], early changes in brain activity may be particularly important in recovery.

Glutamate is a key neurotransmitter influenced by chronic alcohol use, with the metabotropic glutamate 5 (mGlu5) receptor

emerging as a target of high interest to reduce drinking. MGlu5 receptors are an important regulator of synaptic plasticity, and so dysregulation in this molecular marker could be associated with alterations in brain network activity. The objectives of this study were (1) to assess changes in resting state functional connectivity in AUD patients longitudinally in early abstinence and (2) to explore patterns of change in mGlu5 receptor availability associated with these alterations.

Methods: Seventeen subjects who met DSM-5 criteria for AUD (42.3 ± 8.65 years; 13 men and 4 women) participated in the study. Subjects completed an inpatient recovery protocol with medically supervised abstinence. Functional magnetic resonance imaging (fMRI) scans to assess resting state functional connectivity and positron emission tomography (PET) scans using [18F]FPEB to measure mGlu5 receptor availability were acquired at two separate time points, approximately two and four weeks after inpatient admittance (T1 and T2, respectively). Imaging outcomes were compared to 23 healthy control volunteers (HCs) (41.0 ± 12.9 years; 17 men and 6 women) scanned with each modality at a single time point.

For fMRI, resting state networks were identified by entering all scans into an independent component analysis (ICA). To assess spontaneous activity within these networks, fractional amplitude of low frequency fluctuations (fALFF) was computed for each component. In exploratory analyses to identify group differences outside of canonical resting state networks, the intrinsic connectivity distribution (ICD) [4] was used to assess average whole-brain connectivity of anatomically defined cortical and subcortical regions of interest (ROIs). For PET, tracer volume of distribution (Vt) was computed as a ratio of equilibrium concentration in each ROI to that in venous blood. Two-way analysis of variance with group and component/ROI as factors and post hoc t tests were used to assess differences in fALFF, ICD, and [18F]FPEB Vt values between HC and AUD subjects at each time point.

Results: Fourteen resting state network components were identified in ICA. Relative to HCs, fALFF was lower in the AUD group in the right dorsal attention network (DAN) at T1 ($p = 0.012$) and T2 ($p = 0.0019$) and in anterior and posterior default mode network (DMN) at T2 ($ps < 0.006$). Lower activity in sensorimotor networks was also observed in AUD at T1 ($ps < 0.04$). In the ICD analysis, global connectivity within the left orbitofrontal cortex was higher in the AUD group relative to HCs at T1 ($p = 0.011$) and T2 ($p = 0.0007$).

MGlu5 receptor availability was higher in AUD compared to HCs at T1 in dorsolateral prefrontal cortex ($p = 0.0016$), orbitofrontal cortex ($p = 0.014$), and occipital cortex ($p = 0.0024$), with similar trends of higher Vt but no significant differences from HCs at T2.

Conclusions: In AUD, neural activity in functional networks at resting state was lower than in HCs, with the largest differences in the DMN and the right DAN following four weeks of abstinence. This suggests that network activity is disrupted during extended alcohol abstinence. DMN activity is linked to internal states and self-referential thoughts, while the DAN is important for processing external stimuli. Disruption in both these systems may reflect impaired ability to modulate focus on appropriate internal and external cues. Increased global connectivity of the orbitofrontal cortex, a region involved in reward processing and motivated behavior, might also reflect reorganization of these processes in early recovery.

Higher mGlu5 receptor availability in cortical regions was also found in AUD patients compared to HCs in early abstinence, identifying one possible link to molecular signaling pathways that facilitate synaptic plasticity. Efforts are ongoing to use multimodal analysis techniques including joint ICA and PET-weighted ICD to explore relationships between cortical mGlu5 receptor availability during alcohol abstinence and concurrent changes in resting network activity.

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Keywords: Alcohol and Substance Use Disorders, Resting State fMRI, mGlu5, PET, Glutamate

Disclosure: Nothing to disclose.

M192. Regulation of Alcohol-Associated Phenotypes by Lateral Habenula and Bed Nucleus of the Stria Terminalis Serotonin 5HT2c Receptor-Containing Neurons

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Background: Negative affect associated with alcohol withdrawal is a critical driver of relapse to alcohol drinking. Both the lateral habenula (LHb) and the bed nucleus of the stria terminalis (BNST) have been shown to drive negative emotional states and are dysregulated by alcohol exposure. Here, we investigated the contribution of serotonin 5HT2c receptor neurons in the BNST to LHb in alcohol drinking and alcohol-associated negative affect.

Methods: We exposed male and female mice (n~8/group) to the binge-like alcohol exposure paradigm Drinking in the Dark (DiD) and assessed anxiety, arousal, and social behaviors at 7 and 28 days withdrawal using the open field test, acoustic startle test, 3-chamber sociability test, and free social interaction test. We traced serotonin inputs to the BNST and LHb from the DRN using retrograde tracers, and 5HT2c-receptor containing neuron outputs from the BNST and the LHb using anterograde AAVs (n~4/group). We performed in-vivo fiber photometry recordings of 5HT2c LHb and BNST neurons during alcohol consumption, social testing, anxiety testing, and arousal testing (n~7/group). Finally, we chemogenetically manipulated LHb and BNST 5HT2c receptor containing neurons or functionally deleted 5HT2c from the LHB and BNST and determined the effects on alcohol drinking, anxiety, arousal, and social behavior (n~8/group).

Results: Prolonged withdrawal from DiD induced sex-specific anxiety, arousal, and social phenotypes. Tracing experiments revealed that LHb and BNST 5HT2c neurons receive inputs from the same population of caudal dorsal raphe (DR) neurons while sending overlapping projections to the DR and the ventral tegmental area (VTA), among other regions. Fiber photometry showed that DiD alters LHb 5HT2c neural responses to alcohol ($p = 0.04$), but not water consumption ($p = 0.75$). Deletion of 5HT2c in the BNST in males reduced the initial acquisition of alcohol drinking behaviors ($p = 0.02$), while chemogenetic activation of 5HT2c BNST neurons in females increased acoustic startle responses ($p = 0.008$).

Conclusions: These findings demonstrate both distinct and overlapping roles of LHb and BNST 5HT2c neurons in male and female mice in alcohol-associated phenotypes.

Keywords: Alcohol and Substance Use Disorders, Serotonin 5-HT2C Receptor, Lateral Habenula, BNST

Disclosure: Patent pending for use of Orexin receptor 2 antagonists to treat aggression: (Self)

M193. Neuronal L-Type Calcium Channels in Cerebellar Neurodevelopment and Function

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Background: L-type voltage-gated calcium channels (LVGCCs) have important roles in neurogenesis, regulation of neuronal activity, and excitation-transcription coupling. From a developmental perspective, LVGCCs have also been implicated in activity-dependent neurite outgrowth, which comprises two processes: initiation of new neurites and neurite elongation. Here we used primary cerebellar granule neurons to differentiate between potential LVGCC effects on these two specific components of activity-dependent neurite outgrowth. We also hypothesized that if LVGCCs are important for activity-dependent neurite outgrowth in cerebellar granule neurons, they may also have important roles in cerebellar function. Therefore, we tested cerebellar function in mice lacking specific LVGCCs using the rotarod and Erasmus Ladder tasks.

Methods: Primary mouse cerebellar granule neurons (CGNs) were cultured from 129SvEv pups at P4-P6. Potassium chloride (50mM) was used to stimulate neuronal cultures and isradipine (either 10nM or 20nM) was added to culture medium to inhibit all LVGCCs nonspecifically for the time periods indicated in figures. For conditional Cav1.2 deletion in most neurons, we crossed Cav1.2 conditional knockout mice (Cav1.2-cKO) to Syn-Cre mice. For conditional deletion in cerebellar granule neurons, we crossed Cav1.2-cKO mice to Atoh1-Cre mice. The Cav1.2-cKO line was maintained on a 129SvEv background; all others were maintained on a C57BL/6NTac background. For behavioral assays with conditional Cav1.2 deletion lines, wild-type ($n = 12$) and knockout ($n = 8$-12) littermates were maintained on a mixed 129SvEv x C57BL/6NTac F2 genetic background. For constitutive Cav1.3 deletion, we used WT ($n = 31$) and KO ($n = 27$) littermates. Behavioral tasks included open field, rotarod, and Erasmus Ladder. Data were analyzed with sexes combined and with sexes separated to assess for sex as a biological variable. Studies were analyzed by one-way ANOVA, two-way ANOVA, or generalized linear mixed model, where appropriate.

Results: Cultured cerebellar granule neurons exhibited an increase in neurite initiation (as measured by number of neurites) but elongation when stimulated with potassium chloride, consistent with previous reports of activity-dependent neurite outgrowth in this cell type. LVGCC inhibition with isradipine blunted the KCl-induced primary and secondary neurite initiation ($p<0.01$ compared to KCl treatment alone; no difference between unstimulated CGNs and CGNs treated with both KCl plus isradipine). However, we observed no change in the length of either primary or secondary neurites with isradipine treatment with or without KCl stimulation. In our behavioral experiments, we observed no deficits in open field, rotarod, or Erasmus Ladder when Cav1.2 was deleted in most neurons (driven by Syn-Cre expression) or in cerebellar granule neurons (driven by Atoh1-Cre expression). In contrast, loss of Cav1.3 was associated with impaired motor learning in the rotarod task ($p<0.05$) without evidence of ataxia on Erasmus Ladder.

Conclusions: Our data support a specific role for LVGCCs in activity-dependent cerebellar granule cell neurite initiation but not neurite elongation. Future studies will examine the specific contribution of individual Cav1 channels to activity-dependent dendritogenesis in the cerebellum. Our behavioral studies show that while loss of Cav1.2 does not impact motor learning functions of the cerebellum as measured by rotarod and Erasmus Ladder, loss of Cav1.3 does cause motor learning impairments on the rotarod. These results provide new evidence that LVGCCs are important for activity-dependent neurite initiation in cerebellar neurons, and that Cav1.3 specifically is involved in neural circuits subserving motor learning.

Keywords: Voltage-Gated Calcium Channel, Dendritic Morphogenesis, Cerebellum

Disclosure: Nothing to disclose.

M194. Novel Mouse Lines to Study the Role of Human FKBP5 Polymorphisms in Differential Glucocorticoid Responsiveness and Stress Resilience

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Background: Glucocorticoids are the essential communicators of the stress response. The sensitivity to glucocorticoids, and regulators of such sensitivity, may be addressed at the cellular level. The gene FKBP5 encodes the chaperone protein FKBP51 that functionally inhibits glucocorticoid signaling and thus contributes to the regulation of stress. In the context of childhood trauma, differential expression of FKBP5 has been found in psychiatric patients compared to controls. These variations in expression levels of FKBP5 were reported to be associated with differences in stress responsiveness in human carriers of the single nucleotide polymorphism (SNP) rs1360780. Similar SNPs do not occur in rodents naturally. For a better understanding of the pathophysiological processes underlying the association between FKBP5 genotypes, early life adversity (ELA), stress coping and psychiatric disorders, mouse lines carrying rs1360780 SNPs have been developed.

Methods: The mouse lines carried either the risk (A/T) allele or the resilient (C/G) allele of rs1360780. Transgenic mice were subjected to maternal separation paradigm comprising 3 hours of separation from mother and peers between an age of 2-21 days at different times per day. Effects of this model of early life adversity compared to normal development were assessed in adulthood. Behavioral readouts including open field, dark-light, T-maze and 3-social-chamber test. HPA-axis performance was investigated using peak and nadir basal corticosterone levels, acute responsiveness to restraint stress and a dexamethasone suppression test. Blood counts, cytokine levels and next generation sequencing of adrenals, spleen, hypothalamus as well as dorsal and ventral hippocampus were carried out postmortem. Furthermore, primary murine cells from CNS (astrocytes, microglia, and neurons) were analyzed for their basal expression levels of FKBP5 and their responsiveness to glucocorticoids.

Results: Experiencing ELA was associated with a forward shift in diurnal HPA-axis rhythmicity that was still detectable in adulthood and resulted in differential activity compared to controls. This forward shift in diurnal rhythm in the maternal separation group was paralleled by higher locomotion in the open field test in the late afternoon were ELA-exposed mice. At night (18:30 – 05:30), females with ELA spent more time active (3.48h ± 48.2min) and traveled more distance (3.68km± 1.05km) than controls (active: 3.02h ± 51.4min, $d = .56$; distance: 3.07km ± .95km, $d = .6$, $F(1) = 6.5$, $p = .01$). Resilience C/G-allele carrying females showed the strongest effect ($t(65) = 3.7$, $p = .0055$, $d = 1.8$). At sunrise, ELA-exposed males consequently decreased their activity (-4.5 ± 9s/min) more than controls (-.4 ± 7.4s/min, $d = .5$, $W = 665.5$, $p = .02$). In the dark-light test, mice exposed to early life adversity spent more time in the light (24.4 ± 11 s/min) than controls (18.9 ± 5.9 s/min, $d = .6$, $F(1,70) = 6.67$, $p = .01$), with risk (A/T) allele

carrying females showing the biggest effect of ELA ($t(20) = 3.3$, $p = .02$, $d = 1.25$). With respect to sociability and working memory as assessed in the social chamber test or T-maze, no effect of early life adversity or mouse line was detected. Responsiveness of the HPA-axis to challenge like restraint stress or dexamethasone injection was functional in all groups. More lymphocytes were detected in risk (A/T)-allele carrying females after maternal separation (2.9 ± 0.3 1000/μl) compared to resilience allele carriers (1.8 ± 0.41000/μl, $d = 2.9$, $F(2) = 6.3$, $p = .009$). Furthermore, the size distribution of erythrocytes in controls was much narrower (13.6 ± 0.8%) than in ELA-exposed mice (14.1 ± 0.7%, $d = 0.7$, $W = 177$, $p = .008$). Differential expression of FKBP5 was found in primary neurons, microglia and astrocytes, with astrocytes expressing the least (5.10 ± 1.35 Cycles to Threshold (CT)) and neurons expressing highest levels of FKBP5 (3.43 ± 0.6 CT, $F(2,101) = 33.80$, $p < .0001$). The basal expression levels of FKPB5 were negatively correlated with cellular glucocorticoid responsiveness (Nfkbia: $\beta = -.46$, $p = .006$; Tsc22d3: $\beta = -.42$, $p = .01$). Astrocytes revealed the strongest transcriptional response with the risk-allele (A/T) being associated with greater induction of FKBP5 than the resilience allele ($F(8, 260) = 14.1$, $p < .0001$). Transcriptomic analysis of multiple tissues indicates differential transcriptional response between the two SNP carrying lines.

Conclusions: Novel FKBP5 -humanized mice display differential glucocorticoid responsiveness and adaptation to early life adversity due to a single intronic single nucleotide polymorphism. While not comprehensive, these data indicate the two humanized mouse lines show moderate behavioral and physiological differences, and interact with early life adversity – as is the case in humans. Overall, the novel FKPB5-humanized mouse lines will allow for further study of the role that FKBP5 SNPs have in risk and resilience to stress pathology. These mice are currently available for use via Taconic Biosciences.

Keywords: Early Life Stress, Stress Resilience and Susceptibility, Transgenic Models

Disclosure: Boehringer Ingelheim: Employee (Self)

M195. Translational EEG and Behavioral Measures of Cognitive Flexibility in a Cross-Species (Human/Rat) Probabilistic Reversal Learning Task

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Background: Behaving in a flexible manner and appropriately updating action-outcome associations in an uncertain and dynamic environment is impaired in several psychiatric and neurological disorders, including depression, schizophrenia, and Parkinson's disease. Our objective was to develop neurophysiological and behavioral measures of cognitive flexibility that could be assessed similarly in humans and rodents to support preclinical drug discovery efforts. We assessed cognitive flexibility in humans and rats using a functionally equivalent cross-species probabilistic reversal learning (PRL) task with simultaneous measurement of neurophysiological activity using electroencephalogram (EEG). To improve translation across species, we designed the tasks in parallel with similar parameters and used identical data processing methods and reinforcement learning models to analyze behavioral and neurophysiological data.

Methods: Adult male and female Wistar rats ($n = 15$) were implanted with intracranial (i.e., local field potential; LFP) and extracranial (i.e., EEG) recording electrodes and tested in an operant PRL task to respond for sucrose pellets. Three EEG electrodes were implanted over frontal and parietal areas and five LFP electrodes were implanted in prefrontal (i.e., lateral orbitofrontal and anterior cingulate (ACC) cortices), striatal (i.e., nucleus accumbens shell and caudate nucleus), and primary auditory cortical areas. Humans – EEG data from 96 equidistant scalp electrodes were recorded from male and female participants ($n = 45$) during performance of a computer-based PRL task where participants responded for a monetary reward. PRL Task Design – During PRL testing, subjects were required to choose between two stimuli – a target stimulus that was reinforced on 80% of responses and a non-target stimulus that was reinforced on 20% of responses. Positive (reward) or negative (no reward) feedback was signaled by high and low frequency tones (counterbalanced between subjects) prior to outcome. The target or non-target assignment of the stimuli reversed if subjects selected the target stimulus on eight consecutives trials, irrespective of feedback.

Results: Healthy humans and rats both performed several reversals during a single test session [humans: 13.5±0.75 (mean ±SEM); rats: 7.3±0.73], reflecting cognitive flexibility. This was accompanied by a significantly higher probability to repeat reinforced target responses (i.e., win-stay), reflecting sensitivity to positive outcomes, relative to a lower probability to abandon target responding following unreinforced trials (i.e., lose-shift), reflecting sensitivity to negative outcomes, across species [humans: $t(44)=13.84$, $p<0.001$; rats: $t(14)=8.4$, $p<0.001$]. After successfully selecting the target stimulus, a feedback-related negativity (FRN) emerged in frontal (e.g., FCz in humans; ACC in rats), but not parietal, areas when negative feedback was presented relative to positive feedback trials [humans: Feedback main effect $F(1,45)=14.83$, $p<0.001$; rats: Feedback main effect $F(1,9)=19.29$, $p<0.01$]. Reinforcement learning analyses revealed more positive Q values associated with target relative to non-target responses in both humans and rats [humans: $t(44)=26.74$, $p<0.001$; rats: $t(14)=7.41$, $p<0.001$]. Additionally, prediction error values were similarly positive for target responses and negative for non-target responses in both species [humans: $t(44)=12.2$, $p<0.001$; rats: $t(14)=6.23$, $p<0.001$].

Conclusions: Our results demonstrate a frontal FRN effect in both humans and rats performing a conceptually and procedurally analogous PRL task used to assess cognitive flexibility. The FRN reflects an error signal when a target response was not followed by a positive outcome (i.e., a negative prediction error). In rats, this FRN was most robust in the ACC, consistent with previous findings suggesting that the FRN originates in this frontocingulate area. Additionally, these results demonstrate feasibility in recording task-based neurophysiology in a similar manner across species and implementing identical data processing and analytical methods to both human and rodent behavioral and neurophysiological data. This approach may be used to evaluate the effects of putative therapeutics on both neurophysiological (e.g., FRN) and behavioral markers of cognitive flexibility and provides a bridge to support the translation of early stage preclinical discoveries into novel clinical treatments for cognitive impairment and other symptoms of psychiatric disorders.

Keywords: Cognitive Flexibility, Animal Models, EEG/ERP Electrophysiology, Probabilistic Reversal Learning, Reinforcement Learning Modelling

Disclosure: Nothing to disclose.

M196. Concordant Neurophysiological Signatures of Cognitive Control in Humans and Rats

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Background: Cognitive control is an adaptive process that involves error detection and response correction. Dysregulation of cognitive function, including deficits in reward sensitivity and cognitive control, is a common feature of virtually all neuropsychiatric disorders. The development of improved therapeutics may be enhanced by a focus on treating these cognitive deficits. The Eriksen Flanker task is commonly used to assess cognitive control in humans, but a rodent version of the task has never been developed. As part of an NIMH initiative to create reliable and valid cross-species assays of cognitive function, we developed a touchscreen-based Flanker task for rats. We then tested humans and rats in parallel version of the task using identical stimuli and endpoints and collected continuous neurophysiological data in both species. Here, we examine cross-species similarity in behavioral and neurophysiological signatures of cognitive control and whether these signals could be modulated by modafinil, which can enhance some features of cognition in humans.

Methods: Using fading and correction procedures combined with touch-sensitive response technology, we trained male and female Long-Evans rats (n = 11) to discriminate between detailed photographic stimuli (green leaf/violet flower). Discrimination was deemed successful when the criterion of 70% response accuracy during the session was reached on two consecutive days. Following training, rats underwent stereotaxic surgery for implantation of surface and depth electrodes for neurophysiology data collection. Using a within-subjects design, five 300-trial Flanker Task test sessions subsequently were conducted during which rats received vehicle (100% DMSO) or modafinil (16, 32, and 64 mg/kg IP, 30-minute pretreatment time); continuous EEG and LFP data were collected throughout test sessions. Healthy human subjects (n = 26) were tested in parallel with either modafinil (100, 200 mg oral, 2-hour pretreatment time) or placebo. We analyzed behavioral performance and neurophysiological signatures of cognitive control, including target- and response-locked event related potentials (ERPs) and spectral power to assess cross-species similarity in task-evoked responses.

Results: In both species, the task elicited the expected Flanker interference effect of reduced accuracy on incongruent compared to congruent trials (Human $F(1,24) = 110.64$, $p < .001$, $\eta 2p = 0.82$; Rat $F(1,9) = 255.00$, $p < 0.001$, $n2p = .966$). We analyzed target-locked signals and found increased theta power on incongruent trials at frontal electrodes in both species (Human $t(25) = 4.93$, $p < 0.001$, Cohen's $d = 0.966$; Rat $t(8) = 3.07$, $p = 0.015$, Cohen's $d = 1.02$). While we did not observe congruency differences in target-locked ERPs in rats, we did find the expected N200 component in humans at frontal electrodes ($t(25) = 5.78$, $p < 0.001$, Cohen's $d = 1.13$). For response-locked signals, we found a significant negative deflection following errors compared to correct responses (Human $t(25) = 9.65$, $p < 0.001$, Cohen's $d = 1.89$; Rat $t(10) = 5.77$, $p < 0.001$, Cohen's $d = 1.74$), known as the error-related negativity (ERN) in humans. Additionally, we found significant response-locked changes in spectral power, however the characteristics of the effect differed across species. Errors caused increases in midfrontal theta power in humans ($t(25) = 6.15$, $p < 0.001$, Cohen's $d = 1.21$) whereas they caused suppression of delta power in rats ($t(10) = 3.45$, $p = 0.006$, Cohen's $d = 1.04$). Modafinil did not affect behavioral performance or physiological responses in either species.

Conclusions: Here we developed a translationally-aligned flanker task and tested humans and rats in parallel. The task evoked both behavioral and neurophysiological responses that were similar across species, raising the possibility that these signals may serve as common biomarkers of cognitive control. Further pharmacological studies will seek to determine whether this task can be effective in screening candidate therapeutics to hasten drug development for psychiatric disorders.

Keywords: EEG Biomarkers, Flanker Task, Touchscreen

Disclosure: Nothing to disclose.

M197. Decomposing Resilience and its Relationship With Mental Health during COVID-19 Pandemic Outbreak

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Background: Resilience is key in maintaining mental health during stressful times. Quantifying resilience factors and their relative contribution to outcomes may advance interventions. COVID-19 pandemic is a major global stressor requiring individuals to recruit their resilience resources to endure. We aimed to identify resilience factors and assess their contribution to mental health during COVID-19.

Methods: Participants (N = 5,717, mean age 39, 70% females) were recruited through an online platform (covid19resilience.org), April 6 - May 5, 2020. Resilience was assessed using a 21-item battery tapping 5 factors: self-reliance, emotion regulation, positive (confidence) and negative (hostility) aspects of close relationships and perception of neighborhood environment. Current mental health screening included anxiety, depression and sleep. Factor analyses were conducted on resilience items. Resilience factors' effects on mental health were tested using structural equation modeling that included multiple distal risk factors and proximal COVID-19 related exposures, controlling for multiple covariates.

Results: Resilience factors correlated moderately among themselves (strongest $r<0.50$), suggesting that resilience is a multifaceted construct. Bifactor modeling revealed a general resilience factor with robust "buffering" effects on mental health outcomes during the pandemic (effect size~0.60), greater than the risk-increasing effect of previous depression/anxiety (effect size~0.35). Among resilience factors, emotion regulation showed the greatest buffering effect, moderating association between past and current depression.

Conclusions: Resilience factors can be quantified using a brief online tool that can inform on mental health trajectories. Emotion regulation may be a modifiable target for enhancing resilience. Interventional studies are warranted to translate findings for improving mental health following the pandemic.

Keywords: Resilience, COVID-19, Emotional Regulation, Stress Coping

Disclosure: Taliaz Health: Advisory Board, Stock/Equity (Self),

M198. Dextroamphetamine-Enhanced 5C-CPT Performance and Neural Correlates of Cognitive Control in Healthy Subjects: A Proof of Concept Study

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Background: People with schizophrenia (SZ) exhibit marked deficits across cognitive constructs. These cognitive deficits contribute to significant socio-occupational impairment and disability burden in SZ. Decades of research to identify pro-cognitive drugs for SZ have mostly yielded negative results. These failures are compounded by a lack of biomarkers that can i)

identify behaviors relevant to cognitive constructs, ii) be easily translated across species and iii) be altered by a pro-cognitive drug. Here we focused on identifying neurophysiological biomarkers of cognitive control processes that can be measured across species and can be altered by a pro-cognitive drug. We chose dextroamphetamine (Adderall), an FDA-approved treatment for attention deficit hyperactivity disorder (ADHD), as the pro-cognitive drug. Adderall enhances cognitive control processes of attention, vigilance, learning, working memory and impulsivity in healthy participants (HP), and people with SZ. We determined the impact of dextroamphetamine on neurophysiological measures relevant to cognitive control using a reverse-translated 5 choice continuous performance test (5C-CPT) in HP

Methods: Twenty-three carefully screened healthy men and women between the age of 18-35 years completed the study. Subjects received either placebo or one of two active doses of dextroamphetamine (10 or 20 mg) in a counterbalanced, randomized, double blind within-subject study, conducted across 3 test days separated by one week. After 120 minutes of ingesting the study medication, HP completed the 5C-CPT with simultaneous electroencephalogram (EEG) recordings. The 5C-CPT has Go and NoGo trials within separate hard (visually masked) and easy (unmasked) conditions. Event related potential (ERP) components were quantified for NoGo conditions (N1, P2, N2 and P3a) at FCz electrode and for Go condition (P3b) at Pz electrode. Time frequency measures (cue and response locked theta power), were computed using wavelet decomposition. Mixed liner modeling was utilized to analyze individual change by task difficulty (easy, hard) across drug sessions (placebo, 10 mg, 20 mg)

Results: Subjects reported feeling alert with increased systolic blood pressure at both doses. Desctroamphetamine significantly sped hit reaction time (RT) [$F(2,78)=4.123$, $p = 0.02$] and increased overall performance as measured by D-prime [$F(2,75)=6.628$, $p = 0.002$], without an interaction with task difficulty. There was also a significant main effect of drug on P3a amplitude [$F(2,78)=5.047$, $p = 0.009$] at FCz during correct NoGo conditions, but no effect on N1, P2, N2 or P3b. Time frequency analyses found a significant drug x task difficulty interaction for response locked theta power during Go conditions [$F(2,78)=3.24$, $p = 0.045$]. Post hoc comparisons revealed a significant effect of drug only during the easy condition [$F(2,43)=4$, $p = 0.026$]. Individual differences in drug-induced change in P3b amplitude on hard hit trials was significantly correlated with drug-induced changes in hit rate ($p = 0.01$) and D-prime ($p = 0.05$)

Conclusions: Acute doses of amphetamine (10 and 20 mg) was well tolerated and bioactive in HP. Dextroamphetamine improved 5C-CPT performance by speeding hit reaction time and improving D-prime in both easy and hard task conditions, suggestive of enhanced attention and vigilance. Although there was no difference in response inhibition (correct rejections), dextroamphetamine enhanced frontal activation during correct NoGo conditions. These data support the premise that ERP measures are more sensitive to drug effects than behavioral measures. A see-saw effect on cognitive control was noted in the difficulty conditions, whereby dextroamphetamine significantly lowered frontal midline theta power during easy Go conditions but enhanced the relationship between P3b and performance on hard Go conditions. These hit-related neurophysiological responses suggest that dextroamphetamine contributes to a diminishment of control when the task is easy but a tighter link between brain and behavior function when the task is hard. Future studies will focus on using these ERP measures of cognitive control construct as biomarkers to predict amphetamine sensitivity in people with SZ.

Keywords: Attention, EEG Biomarkers, Amphetamine, Dopamine

Disclosure: Nothing to disclose.

M199. Interrogating the Complexity and Dynamics of Youth Mental Health Among a Cohort With Emerging Mental Disorders

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Background: Efforts to intervene among young people with emerging mood and psychotic disorders aim to interrupt paths to chronic illness and disability. Yet a challenge for effective early intervention is the heterogeneous pattern of illness and diverse needs of young people. This study aims to interrogate the dynamics between syndromes, functioning and comorbidities over time.

Methods: The cohort consists of 1962 individuals aged 12–30 years (57% female), followed up for 3 to 24 months. They presented to the Brain and Mind Centre's youth mental health clinics which include primary care services and more specialised services.

Results: This paper uses dynamic Bayesian networks, specifically probabilistic models represented by directed acyclic graphs (DAGs) to report on the dependence and causal structure of across five domains; social and occupational function; self-harm, suicidal thoughts and behaviour; alcohol or other substance misuse; physical health; and illness type. This work will demonstrate the putative mediators of the association between common clinical and functional outcomes among young people.

Conclusions: This paper improves our understanding about the complex interactions between common factors contributing to illness trajectories. This work has implications for the development of personalised and measurement-based mental health care that promotes targeted interventions and secondary prevention.

Keywords: Youth, Transdiagnostic, Bayesian Inference, Illness Trajectory, Longitudinal Study

Disclosure: Nothing to disclose.

M200. Transcriptomic Immaturity Inducible by Neural Hyperexcitation is Shared by Multiple Neuropsychiatric Disorders

Abstract not included.

M201. Nucleus Accumbens D2-Receptor-Expressing Neurons Regulate Reversal Learning in the Attentional Set Shifting Test

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Background: The ability to use environmental cues to guide advantageous behavior is critical for survival and is thought to be controlled within the basal ganglia neurocircuitry. In particular, medium spiny neurons (MSN) of the nucleus accumbens (NAc), which can largely be divided into two subpopulations expressing either dopamine D1 receptors (D1-MSNs) or D2 receptors (D2-MSNs), has been revealed to play a critical role in the acquisition of learned associations between environmental cues and the outcomes that they predict. Indeed, activity in NAc D1- and D2-MSNs has been shown to be necessary for the acquisition of appetitive and aversive Pavlovian conditioning, respectively. Interestingly, inhibition of activity in NAc D2-MSNs also associated with increased perseverative responding at previously rewarded locations during reversal stages of place learning tasks, suggesting that the NAc may also play an important role in behavioral flexibility. However, the potential roles that these D1- and D2-

MSNs play in guiding and switching attention between environmental cues with changing associated outcomes is less clear. Here we investigated whether activity in NAc D1- and D2-MSNs was necessary for attention shifting between environmental cues of similar or different modalities.

Methods: A reversible neurotransmission blocking (RNB) technique that incorporated a combination of a transgenic mouse line in which the release of neurotransmission-blocking tetanus toxin could be controlled to either NAc D1- or D2-MSNs by intra-NAc expression of AAV vectors was used to selectively and persistently inhibit signaling from either NAc D1-MSNs (D1-RNB) or D2-MSNs (D2-RNB). D1- and D2-RNB male mice, as well as WT controls ($n = 8$ each), were subjected to an attentional set shifting task (ASST) in which several varieties of two different dimensions (odors: nutmeg, coffee, rosemary, ginger; or platform materials: styrofoam, wire, cardboard, plastic) were paired with a food pellet reward buried in a bowl of digging material that could be acquired by using the dimension cues to discriminate the correct location and ignore a non-rewarded location. At each stage only one cue signaled the location of the reward, and trials were repeated until the mouse could consistently discriminate the rewarded location (6 consecutive correct trials). The amount of trials to reach criterion was recorded, and upon completion of the stage the associated cue (attentional set) was switched or reversed intradimensionally (different cue of the same dimension) or extradimensionally (different cue of a different dimension). This task was thus able to measure whether NAc D1- or D2-MSN activity plays a role in reversing (reversal stages), modifying (intradimensional switch), or updating (extradimensional switch) learnt strategies. Data were analyzed by 3-factor ANOVA. Post-hoc analyses of significant interactions within groups were performed using Bonferroni and Tukey's tests, with $p < 0.05$. All experimental protocols 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: D1-RNB, D2-RNB, and WT mice showed similar abilities to discriminate between environmental cues signaling a rewarded and a non-rewarded location during an initial discrimination stage. Additionally, while all mice showed an increase in the amount of trials taken to reach criteria in both intra- and extra-dimensional shifting stages, this did not significantly differ between D1-RNB, D2-RNB, and WT mice. Interestingly, while D1-RNB mice performed similarly to WT mice on reversal stages, D2-RNB mice were found to take a significantly greater amount of trials to reach criterion, indicating impaired reversal learning.

Conclusions: We have previously suggested that NAc D2-MSNs may play an important role in the suppression of learnt strategies that have now become obsolete, as their inhibition results in perseverative responding towards previously correct cues. The findings of the current study indicate that while NAc D2-MSNs play a critical role in suppressing responding towards specific learnt cues that are now associated with unfavorable outcomes (i.e. in reversal stages), they are not involved in the suppression more general learnt strategies (i.e. suppression of attention towards a whole modality). These findings also support a growing literature demonstrating the role of NAc D2-MSNs in inhibiting responses to learnt stimuli, but not in more general extinction learning.

Keywords: Attention, Nucleus Accumbens, Dopamine Receptor Type 2-Expressing Striatal Medium Spiny Neuron

Disclosure: Nothing to disclose.