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T1

Investigation of Neuromelanin-Sensitive MRI as an Index of Norepinephrine System Integrity in Healthy Aging, Mild Cognitive Impairment, and Alzheimer’s Disease

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Background: The norepinephrine system supports critical brain functions including arousal and memory. Norepinephrine neurons located in the locus coeruleus (LC) have been shown to gradually degenerate with normal aging and in neurodegenerative conditions. Pathology of the LC has been hypothesized to play a role in the onset of Alzheimer’s disease (AD). Recent studies have found that a novel type of MR imaging, neuromelanin-sensitive MRI (NM-MRI) is capable of capturing decline in LC integrity with age and in some neurodegenerative conditions. Here we collected NM-MRI images from individuals with AD, mild cognitive impairment (MCI), and cognitively normal older adults (CN) to test whether NM-MRI signal in the LC could differentiate the groups and track cognitive performance.

Methods: Study participants included 66 CN, 23 MCI, and 15 AD individuals. All participants underwent a cognitive assessment including the Mini-Mental State Exam (MMSE) and a neuroimaging session with a 3T Siemens Prisma scanner at the Montreal Neurological Institute (MNI). This session included a NM-MRI scan (turbo spin echo sequence with partial brain coverage, TR = 600 ms, TE = 10 ms, 7 averages, 20 slices 0.7 x 0.7 x 1.8 mm resolution) and a standard T1-weighted anatomical scan. The LC was segmented on raw NM-MRI images by identifying clusters of hyperintense voxels (k = 5 per side and slice) within an over-inclusive mask of the LC that had been warped from standardized MNI space to individual space using SPM12. Contrast-to-noise ratio for all LC voxels was calculated relative to a reference region with negligible NM content (the pons) and, in the final step, CNR values for all LC voxels were averaged to generate a measure of NM-MRI signal in the whole LC (LC-CNR). Statistical analyses tested relationships of LC-CNR to clinical group and cognitive performance using one-way ANOVA and Spearman correlations respectively.

Results: Comparing LC-CNR across the three groups found a significant main effect of group (F2,103 = 3.27, P = 0.042, 1-way ANOVA). Post-hoc testing showed NM-MRI signal was significantly lower in the MCI group compared to the CN group (Pcorrected = 0.032, t-test with Bonferroni correction) but no significant difference was observed between CN and AD groups (Pcorrected = 0.70). Cognitive scores on the MMSE were not correlated to LC-CNR in CN (ρ = −0.03, Spearman correlation) or AD groups (ρ = −0.07) but these factors were significantly negatively correlated in the MCI group (ρ = −0.51, p = 0.018, n = 21). Secondary analyses found LC-CNR to be higher on the left side of the LC compared to the right side in 65 of 66 CN participants (t65 = 19.4, P = 10-28, one-sided t-test of the difference LCleft-CNR − LCright-CNR). Following up on this evidence of lateralization in LC-CNR, we repeated the above analyses on unilateral measures of LC-CNR and found that LCright-CNR had the stronger relationship to both MCI status (tb7 = 2.63, p = 0.010) and MMSE score (ρ = −0.60, P = 0.004).

Conclusions: This preliminary evidence found that NM-MRI signal in the locus coeruleus (LC-CNR) tends to be lower in individuals with MCI but, similar to other recent reports, not in individuals with AD. This finding is consistent with the possibility that MCI individuals with low LC-CNR may follow an alternative trajectory that does not lead to AD. Our finding that low LC-CNR correlates to better performance on a standard cognitive test used in AD is also consistent with this possibility. If these results are confirmed in larger datasets, NM-MRI signal could show promise as a supplemental screening tool to predict conversion to AD.

Keywords: Neuromelanin-Sensitive MRI, Locus Coeruleus, Alzheimer’s Disease, Cognitive Decline, Norepinephrine

Disclosure: Nothing to disclose.

T2

Contributions of Normal Aging to Tau Pathology in Transentorhinal Cortex

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Background: Intracellular inclusions comprised of hyperphosphorylated tau appear in the transentorhinal cortex as early as middle-age and may comprise a normal feature of human brain aging. However, abnormal tau phosphorylation is posited to promote self-aggregation associated with altered protein conformation and solubility that culminates in the formation of neurotoxic neurofibrillary tangles, a hallmark of Alzheimer’s disease (AD). While transgenic models developed from rare disease-associated mutations are useful to study the molecular interactions that govern neurofibrillary tangle formation, few studies have evaluated the contribution of normal aging to the regionally selective and anatomically directed progression of AD tau pathology. Given that a host of psychiatric conditions and...
cognitive complaints occur within the context of advancing age, there is an important need to model tau pathology in aging transentorhinal neurons in order to determine downstream cognitive and affective consequences.

Methods: All experiments used male, Fischer 344 × Brown Norway F1 rats at ages of 6 months (young adult) or 24 months (aged). In Experiment 1, expression of naturally occurring tau isoforms was measured in area 35 of the perirhinal cortex and lateral entorhinal cortex (the rat homolog of human transentorhinal cortex; n = 6/age). Tissue was separated into TBS-soluble and urea/SDS soluble fractions before quantifying levels of total (phosphorylated and non-phosphorylated) or phosphorylated (detected with 7F2 antibody which recognizes phosphorylation at Ser202 and Thr205 residues) tau using SDS-PAGE and immunoblotting procedures. In Experiment 2, young and aged rats (n = 5–6/group) received stereotaxic delivery of rAAV1 containing constructs to drive expression of human wild-type 0N4R tau (hWTtau) fused to an eGFP reporter molecule or eGFP alone (n = 5–6/age/virus). Two months after surgery, brains were harvested for analysis using procedures that were identical to those applied in Experiment 1. Expression of hWTtau was verified using the CP27 antibody, which detects an epitope that is specific to human tau and not present in tau naturally expressed in the rodent brain.

Results: In Experiment 1, young and aged rats were found to express four distinct tau isoforms of 49, 52, 56 and 62 kDa. Expression of the 56 kDa isoform, likely a composite of 1N3R and 1N4R tau isoforms, was expressed at higher levels than other isoforms and also showed a comparatively greater rate of phosphorylation. Expression and phosphorylation ratios were similar between TBS- and SDS/urea-soluble fractions for all isoforms. Critically, aging did not change the relative expression of these tau isoforms, phosphorylation status or differentially influence tau solubility. In Experiment 2, young and aged rats injected with AAV-hWTtau expressed a CP27+ (human tau-specific) 79 kDa tau isoform, which corresponds to the predicted molecular weight of 0N4R human tau fused to eGFP. Expression of hWTtau was similar between young and aged rats, suggesting equivalent efficiency of viral transduction across age groups. Interestingly the expression of hWTtau induced a significant increase in TBS-soluble levels of the naturally occurring 56 kDa rat tau isoform in young adults but a decrease in expression of the same isoform and fraction in aged rats. Importantly, other isoforms in the same fraction were not altered and changes in TBS-soluble fraction did not affiliate with differences in the SDS/urea-soluble fraction.

Conclusions: The present findings indicate that, when challenged by the introduction of a human-derived tau variant, expression of naturally occurring tau isoforms in area 35 of the perirhinal and lateral entorhinal cortices varies between young and aging rats. This differential response is not a normal feature of rat brain aging as non-manipulated young and aged rats show no differences in expression, phosphorylation or aggregation of tau in this same brain area. Consequently, these findings are consistent with the notion that aging is a modulator of tau biochemistry and support further use of these methods to investigate the manner in which aging differentially modulates susceptibility to tau pathology. Ongoing studies will determine if this AAV-mediated increase in total and phosphorylated tau is associated with conformational changes (i.e. Ab250 or Phi25), whether aging exacerbates the propagation of abnormally phosphorylated tau to interconnected brain regions, and, most importantly, how increasing tau burden in aging individuals endangers cognitive and affective functions that depend on the medial temporal lobe.

Keywords: Alzheimer’s Disease, Medial Temporal Lobe, Neurofibrillary Tangle

Disclosure: Nothing to disclose.
Endocannabinoid Regulation of Fear-Extinction Neural Circuitry in Youth: Effects of FAAH Genotype

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Background: The endocannabinoid system modulates emotion-related behavior and is implicated in fear-based disorders, such as anxiety and posttraumatic stress disorder. Neuroimaging studies in adults have linked genetic variation in endocannabinoid signaling with variation in fear-related neural circuitry. For example, individuals carrying the A allele of the fatty acid amide hydrolase (FAAH) gene (rs324420) – associated with lower enzymatic degradation and higher brain endocannabinoid levels – demonstrate increased resting-state functional connectivity between the amygdala and the ventromedial prefrontal cortex (vmPFC). Previous studies link higher amygdala-vmPFC connectivity to lower anxiety and better extinction recall ability, suggesting that the endocannabinoid system is involved in fear-extinction learning. However, despite evidence that fear-based disorders frequently begin in childhood and adolescence, no studies to date have examined the impact of the endocannabinoid system on fear-extinction neural circuitry in youth.

Methods: 48 youth (23 female, ages 6-17 years) completed a novel, validated fear-extinction paradigm in virtual reality. Twenty-four hours later, functional magnetic resonance imaging (fMRI) and skin conductance response (SCR) data were collected while completing a test of extinction recall, and during a resting-state condition. Genetic data were collected from saliva for the FAAH rs324420 variant by Taqman Genotyping.

Results: During extinction recall, youth carrying the A allele demonstrated lower neural response in the dorsomedial prefrontal cortex, a region involved in conditioned fear responding, relative to youth with the CC genotype. Relative to youth with the CC genotype, youth carrying the A allele also demonstrated lower SCRs during extinction recall, but this effect did not reach significance (p = 0.098). Compared to youth with the CC genotype, youth carrying the A allele demonstrated higher amygdala-vmPFC resting-state functional connectivity.

Conclusions: These findings provide the first evidence that genetic variation in endocannabinoid signaling alters fear-extinction related neural circuitry in youth. The endocannabinoid system may play a role in susceptibility to fear-based disorders during childhood and adolescence and may serve as a promising target for innovative interventions.

Keywords: Endocannabinoids, Fear Extinction, Pediatric PTSD, FAAH, Adolescent Anxiety

Disclosure: Nothing to disclose.

T5

Don't Be Scared of That: Neural Network Activity During Experiential and Instructed Extinction Learning and Recall

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Background: Fear conditioning and extinction learning are laboratory models for fear-related disorders and exposure therapy, respectively. Fear and safety learning can take place via personal experience, observation of others, and instruction. During fear conditioning, a neutral cue (conditioned stimulus, CS+) is repeatedly paired with an aversive stimulus (unconditioned stimulus, US) until a fear response is elicited in response to the CS+ even in the absence of the US. This fear response is reflected in increased activity in the salience network—dorsal anterior insula, dorsal anterior cingulate cortex, and the amygdala. During extinction learning, repeated exposure to the CS+ without concurrent presentation of the US leads to the decay of the fear response. A new memory is formed: that in the current spatial, temporal, or social context, the CS+ no longer predicts the US, leading to extinction of the fear response. The hippocampus, which is involved in context processing and memory, and medial prefrontal cortex, which is key for inhibition of fear responses, are involved in extinction learning. While research on extinction learning and recall in humans often only includes the experiential component of safety learning and physical context, in the clinic exposure therapy includes both instructional and experiential aspects of safety learning; our understanding of the combined effects of instruction and experience in extinction learning and its neurobiology is very limited. In this neuroimaging study, we hypothesized that safety instruction would have additive effects on experiential extinction learning, via enhanced recruitment of the ventromedial prefrontal cortex (vmPFC). We also predicted that the hippocampus would be involved in instructed extinction learning and recall.

Methods: 21 participants (12F, mean age 23) completed a two-day fMRI study. Day one consisted of habituation, conditioning, and extinction learning; extinction recall took place on day two. The conditioning session included two CS+ stimuli which were paired with the US, a white noise burst, and one CS− which was not paired with the US. CSSs were differently colored lamps presented in one context (ex. an office) on day one, and another context (ex. a living room) on day two. Prior to extinction, all participants were informed that they would not hear the loud noise US when presented with one of the CS+–s, (instructed CS; CS+I). No information was given about the second CS+–s (uninstructed CS+; CS+U). Prior to recall, half of the participants were re-informed of the safety information; the other half were not. Regions of interest included bilateral amygdala, insula, hippocampus, parahippocampus, cingulum, and mPFC. A CS−&gt;CS+–U contrast was created to examine the effect of instruction during extinction learning; to examine the impact of removal of safety instruction vs its repetition during recall, an independent samples t-test was used to compared the re-instructed and non-re-instructed groups using CS−&gt;CS+–U regressors for the early phase (first six out of twelve trials) of extinction recall. Monte Carlo (MC) alpha probability simulation was used as a correction for multiple comparisons. To assess the network profiles of the distinct brain regions which showed significant activation during extinction and recall, psychophysiological interaction (PPI) analyses were employed. For extinction learning, the left vmPFC was selected as a seed region (-6, 58, 2); for extinction recall, the left insula (-38, -18, 11) and right parahippocampus (26, 0, -34) were selected as seed regions. Significant clusters in ROIs representing differential PPI connectivity were identified using MC correction as described above. Data were evaluated at p ≤ 0.05.

Results: During the early phase of extinction learning, activation in the vmPFC (MC-corrected p < 0.001) was identified for the CS−&gt;CS+–U. A seed was then created using this cluster, and PPI analysis revealed significant correlation between activity in the dmPFC, hippocampus, parahippocampus, & amygdala and the seed region, for the CS−&gt;CS+–U contrast, MC-corrected p < 0.05. For the early phase of recall, significantly greater activation in the left insula and right parahippocampal gyrus was observed for the re-instructed group compared to the non-re-instructed group, MC-corrected p < 0.001. These clusters were then used to define
seeds for PPI. PPI indicated significant correlation between activity in the parahippocampus and hippocampus and the insula seed region, and significant correlation between activity in the amygdala, prefrontal cortex, insula, and hippocampus and the parahippocampus seed region, MC-corrected p < 0.05.

Conclusions: These preliminary findings in healthy individuals indicate that instruction yields greater activation of emotional regulation and context processing networks than experience alone during extinction learning and extinction recall. Since the extinction learning paradigm is commonly used to better understand exposure therapy as well as measure the impacts of exposure therapy in clinical populations, it is important to build a laboratory model that can yield accurate models of the neural networks engaged when cognitive context is provided. Using this model in anxiety disorders and PTSD can help identify those who may more or less benefit from instruction and identify target brain regions for neuromodulation interventions.

Keywords: Extinction Learning, Extinction Recall, Instructed Safety Learning, Anxiety & PTSD, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

T6
Dynamic Chromatin Remodeling and Sex-Specific Risk for Anxiety and Depression

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Background: Anxiety and depression are the most frequent psychiatric disorders and are about two times more prevalent in women than in men. Biological factors certainly play a critical role in this sex disparity, and, among those, natural fluctuation in sex hormone levels in females is likely one of the major contributors. Numerous clinical findings indicate that natural hormonal shifts are associated with the increased risk for anxiety and depression in women of the reproductive age. Consistent with this, we have found that a physiological drop in estrogen in female mice results in higher anxiety levels, both compared to their high-estrogenic estrous cycle phase and to males. The major aim of this study is to reveal the molecular mechanisms through which exposure therapy as well as measure the impacts of exposure therapy in clinical populations, it is important to build a laboratory model that can yield accurate models of the neural networks engaged when cognitive context is provided. Using this model in anxiety disorders and PTSD can help identify those who may more or less benefit from instruction and identify target brain regions for neuromodulation interventions.

Methods: We examined female mice in two phases of the estrous cycle: early diestrus (low estrogen; mimicking the luteal phase in humans) and proestrus (high estrogen; mimicking the follicular phase), previously shown to exhibit high and low anxiety levels, respectively. From 6-8 weeks of age, the estrous cycle stage was checked daily by vaginal cytological analysis and hormone levels were later confirmed by measuring sex hormone levels in serum and the hippocampus. Females placed in diestrus and proestrus groups were examined with age-matched males (11 weeks of age). We first assessed the effects of the estrous cycle and sex on neuronal chromatin organization, three-dimensional chromatin interactions, and gene expression (N = 6/group) in the ventral hippocampus, an area strongly implicated in anxiety-related behavior. We performed the following unbiased genome-wide assays: the assay for transposase-accessible chromatin using sequencing (ATAC-seq), chromosome conformation capture (Hi-C), and nuclear RNA sequencing (nucRNA-seq) on FACS-purified neuronal nuclei isolated from the ventral hippocampus from each of the three groups. We performed the gene ontology, KEGG pathway and motif analyses on each genome-wide assay, and integrated the data to reveal sex- and estrous cycle-specific gene regulatory mechanisms. Finally, we performed AAV-mediated overexpression of a candidate, estrogen-responsive chromatin regulator, Egr1, in ventral hippocampal neurons in vivo, in order to examine the causal role of Egr1 in the regulation of chromatin organization (ATAC-seq, N = 6/group) and anxiety behavior (open-field test, elevated plus maze, N = 12/group) across the estrous cycle and sex.

Results: We show that neuronal chromatin structure significantly varies with the estrous cycle and differs between sexes. In particular, within-female and between-sex variation in chromatin organization are of similar magnitudes (around 30% of all genomic regions), indicating that hippocampal chromatin is not only sex-specific but undergoes significant re-organization during the estrous cycle in females. These chromatin organization changes were associated with the transcriptional activity of genes essential for neuronal function including neuronal excitability, neurotransmission, and synapse formation. Furthermore, we find an enrichment of anxiety risk genes with concomitant changes in chromatin structure and gene expression across the estrous cycle, exemplifying how fluctuating hormone levels may mediate female-specific vulnerability to anxiety through cyclic changes in gene expression. Remarkably, we also find that the expression of chromatin remodeling factors varies with the estrous cycle, indicating the importance of dynamic chromatin regulation for female brain function. Our findings reveal an estrogen-responsive transcription factor, Egr1, as an upstream regulator of the estrous cycle-dependent chromatin and transcriptional changes, and highlight it as a plausible candidate for the sex-specific regulation of anxiety behavior.

Conclusions: This study reveals a novel, sex-specific epigenetic mechanism through which cycling sex hormones dynamically regulate neuronal gene expression, and links these chromatin dynamics to variation in anxiety-related behavior. These findings provide a mechanistic insight into female-specific risk for anxiety and depression and facilitate the development of sex-specific approaches to treat these disorders.

Keywords: Epigenetic, Anxiety, Depression, Sex Hormones, Sex Difference

Disclosure: Nothing to disclose.

T7
Neuroanatomical Correlates of Acute Traumatic Stress Reactivity Revealed by Multimodal Magnetic Resonance Imaging

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Background: Individuals exposed to traumatic events often initially suffer acute, adverse effects on cognitive and affective function. Importantly, acute traumatic stress reactivity is linked to the later development of chronic, debilitating dysfunction in the form of posttraumatic stress disorder (PTSD). However, individuals vary both in acute traumatic stress reactivity and in susceptibility to PTSD. Thus, identifying the mechanisms that underlie stress reactivity acutely following trauma may be important for understanding PTSD susceptibility. Although prior research has
suggested the structural morphology of the brain may partially underlie dysfunction in - and susceptibility to - PTSD, limited research has investigated brain structure acutely following trauma and less still have utilized multimodal, multivariate approaches to understand brain morphology in PTSD in general. Therefore, in the present study we utilized multimodal magnetic resonance imaging (MRI) to investigate brain structure in recently traumatized individuals tied to acute traumatic stress reactivity.

Methods: Participants (n = 78) were recruited in a Level-I trauma center within 24 hours of trauma exposure and completed a series of questionnaires on demographics and past-trauma history. Participants returned to the lab to report on their PTSD symptom severity over the next year, completing the PTSD Symptom Scale (PSS) 1, 3, 6, and 12 months post-trauma. Participants also completed the Childhood Trauma Questionnaire, the Posttraumatic Diagnostic Scale, and the Peritraumatic Dissociative Experiences Questionnaire in the emergency department. MRI scans were completed within three weeks of the 1-month assessment to assess brain grey and white matter characteristics. Voxel-based morphometry (VBM) and cortical surface reconstruction of T1-weighted images were completed to estimate grey matter density, cortical thickness (CT), and pial surface area (PSA). Diffusion tensor analyses were completed with diffusion weighted images to estimate fractional anisotropy (FA), mean diffusivity (MD), and mode of the diffusion tensor (MO) of the white matter skeleton. The VBM, CT, PSA, FA, MD, and MO maps were used as features in a linked independent component analysis (ICA) to perform multimodal data fusion. ICA identifies multi-modal spatial covariance patterns that reflect variability in the sample, and a set of subject loadings for each pattern that reflect the strength of the pattern in each person. LICA was completed at several dimensionalities (L = 8, 9, 10, 11, 12) to evaluate the stability of components and signal-to-noise separation. Components of interest were selected as those whose subject loadings (L = 10) were associated with acute traumatic stress reactivity (i.e., PSS total scores at 1 month) via simple regressions (loadings as the dependent variable and PSS and PSS (1 mo) were followed-up with multiple regression analyses including age, gender, and occurrence of prior trauma, childhood trauma, and peritraumatic dissociation as covariates with PSS (1 mo) and PSS (1 mo) squared.

Results: Two components were identified that related to acute traumatic stress reactivity. The first component was a predominantly FA-weighted component (77%) that showed increased FA within the cingulum bundle, corpus callosum, uncinate fasciculus, and corticospinal tract. A multiple regression analysis revealed this component was negatively related to PSS total scores at 1 month [t(71) = −2.155, p = 0.035, β = −0.245]. Exploratory follow-up multiple regressions revealed this component was significantly related to re-experiencing symptoms [t(71) = −2.516, p = 0.014, β = −0.275], but not avoidance or arousal symptoms (p > 0.05). The second component included predominantly VBM (25%) and PSA (27%) features that reflected increased VBM and PSA within the visual cortex and anterior temporal lobe, and reduced VBM within the anterior cingulate cortex. The component was curvilinearly related to PSS total scores at 1 month [t(70) = 3.105, p = 0.003, β = 0.404] such that greater strength of this pattern was associated with greater PSS total scores at 1 month. Exploratory follow-up multiple regressions revealed this component was significantly related to re-experiencing [t(70) = 2.857, p = 0.006, β = 0.363], avoidance [t(70) = 2.661, p = 0.010, β = 0.462], and arousal symptoms [t(70) = 2.019, p = 0.047, β = 0.381].

Conclusions: Our results indicate that several multimodal structural components are related to acute traumatic stress reactivity. Specifically, reductions in white matter anisotropy and gray matter of the anterior cingulate cortex, as well as increased gray matter of the visual cortex and anterior temporal lobe, appear to be linked to individual variability in acute posttraumatic symptom expression. Thus, the present data-driven approach suggests that multimodal structural profiles linked to individual variability in posttraumatic stress reactivity are identifiable acutely following trauma. Further work will be necessary to establish the generalizability of these profiles to other samples and identify structural profiles tied to long-term PTSD symptoms. Together, the results of the present study provide an initial characterization of the variability in brain structure tied to posttraumatic stress symptom expression acutely following trauma exposure.

Keywords: PTSD, Multimodal Neuroimaging, Brain Structure, Acute Traumatic Stress

Disclosure: Nothing to disclose.

T8

Associations of Emotion Dysregulation and Complex PTSD With Heart Rate Variability in Trauma-Exposed African American Women With Type 2 Diabetes

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Background: Emotion dysregulation is a transdiagnostic risk factor for many trauma-related disorders, including posttraumatic stress disorder (PTSD) and complex PTSD. Complex PTSD is recognized by the International Classifications of Disease (ICD) as a distinct disorder from PTSD that is characterized by significant emotion dysregulation, negative self-concept, and interpersonal difficulties in addition to other PTSD symptoms. A key biological system involved in physiological arousal is the autonomic nervous system (ANS) and a critical component of emotion regulation is an individual’s ability to flexibly adjust physiological arousal. Heart rate variability (HRV), a measure of both parasympathetic and sympathetic arousal in the ANS, is an important potential biological marker of emotion regulation abilities, and poorer HRV has been previously implicated in PTSD as well as many medical conditions, including diabetes. African American women from low-income communities are at particularly high risk for the development of PTSD. Thus, understanding patterns between emotion regulation, PTSD, and HRV in the context of common chronic medical conditions is essential as we continue to make efforts toward reducing health disparities in low-income African American communities. The goals of the current study were to: 1) determine whether self-reported emotion dysregulation was associated with HRV in the context of an acute psychosocial stressor task and 2) examine whether HRV following the same acute stressor differed across PTSD and complex PTSD diagnoses among African American women with type 2 diabetes mellitus (T2DM).

Methods: We examined associations between emotion dysregulation, PTSD, complex PTSD, and HRV among 46 African American women with T2DM recruited from an urban hospital. Emotion dysregulation was measured using the Emotion Dysregulation Scale. PTSD and complex PTSD were measured using semi-structured clinical interviews. Child abuse and lifetime trauma load were also assessed using self-report measures. HRV was derived from electrocardiogram (ECG) activity collected using the Biopac system sampled at 1kHz. The measure of HRV used in this study was low frequency (LF; 0.04-0.12 Hz) to high frequency (HF; 0.12-0.40 Hz) HRV ratio showing sympathetic-vagal balance.

Disclosure: Nothing to disclose.
derived using spectral analysis with the Hamming windowing function. Higher scores on LF/HF indicate greater sympathetic over parasympathetic activity. The acute psychosocial stressor task conducted during this study was the Trier Social Stress Task (TSST), and HRV was assessed at two baseline time points as well as three time points post-stressor (at −15 min and 0 min [B1 and B2, respectively], and at +15, +30, and +45 min [T1, T2, and T3, respectively]). Patterns across the time points for variables of interest were assessed.

**Results:** Correlational analyses showed that emotion dysregulation was significantly associated with higher LF/HF at T2 and T3 post-TSST (p < 0.001), but not at baseline or T1 (p > 0.05). Childhood abuse (p > 0.05) and trauma exposure (p > 0.05) were not associated with HRV at any time point. In hierarchical linear regression models, emotion dysregulation was significantly associated with higher LF/HF at T2 and T3 (R² change = 0.22, p < 0.001 and R² change = 0.29, p < 0.001, respectively) independent of age and baseline HRV. Using repeated measures ANOVA, complex PTSD diagnosis was significantly associated with greater LF/HF compared to non-complex PTSD diagnosis (F = 3.17, p < 0.05) with elevations at T2 and T3. Current PTSD diagnosis was not significantly related to LF/HF at any time point (p > 0.05).

**Conclusions:** The current findings demonstrate that self-reported emotion dysregulation is related to biological indicators of ANS sympathetic dominance in the context of acute psychosocial stress, particularly after significant time has passed and individuals with more effective emotion regulation skills may have recovered. While complex PTSD diagnosis was associated with greater sympathetic dominance following stress exposure, PTSD diagnosis was not, suggesting that complex PTSD may show particular ANS dysfunction in the context of psychosocial stress among African American women with diabetes, which could increase risk for negative long-term health outcomes for these women. A major component of complex PTSD is emotion dysregulation, across both hyperactivation and deactivation patterns. As a consequence, because negative self-concept is pervasive in women with complex PTSD, interpersonal stressors may be particularly problematic. Emotion dysregulation is therefore a potentially valuable treatment target for at-risk women in medical settings where comorbid medical and psychiatric disorders often occur. In addition, LF/HF HRV may serve as a potential biomarker for treatment outcomes.

**Keywords:** Post Traumatic Stress Disorder, Trauma Exposure, Emotional Dysregulation, Heart Rate Variability

**Disclosure:** Nothing to disclose.

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**Methods:** qPCR and immunocytochemistry were used to quantify the RNA and protein levels of GABAergic markers in control and ASD group. Electrophysiology was used to measure functional synaptic transmission differences between groups.

**Results:** The qPCR and immunocytochemistry concomitantly suggest the increase of GABAergic neuronal markers in ASD organoids, such as DLX, vGAT and GAD67. Electrophysiological studies suggest an increase of GABAergic input while a decrease of glutamatergic input in ASD organoids.

**Conclusions:** These results suggest an excitation-inhibition neurotransmission imbalance may be one of the possible mechanisms that may underlie cellular alterations in ASD circuitry.

**Keywords:** Autism Spectrum Disorder and Related Syndromes, Brain Organoids, GABA

**Disclosure:** Nothing to disclose.

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**T9**

**Excitation-Inhibition Neurotransmission Imbalance in Cortical Organoids Derived From Children With Autism Spectrum Disorder**

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**Background:** Aberrant gamma-aminobutyric acid (GABA) system, the major inhibitory neurotransmitter in the CNS, is highly implicated in autism spectrum disorder (ASD). However, mechanisms are essentially unknown. To study this, we reprogram fibroblasts that are terminally differentiated back to pluripotent stem cells (iPSCs) allows the investigation of human neurodevelopment in vitro. The iPSCs are developed into three-dimensional organoids that resemble human brain tissue to compare synaptic transmission in control (non-affected) vs. ASD.

**Methods:** We administered subcutaneous injections of the selective TLR7 agonist imiquimod (IMQ, 5.0 mg/kg) or vehicle to timed-pregnant dams (C57BL/6J mice) on embryonic days (E) 12, 14, and 16. A subset of pregnant mice, 3 and 16 hours following injection. Co-inoculated with IMQ have normal baseline locomotor activity, but are hyperactive

**Results:** IMQ injection led to a delayed increase in interferon-alpha in the maternal serum at 16 hours (p < 0.05) but not 3 hours following injection. Co-inoculated with IMQ exhibits a behavioral phenotype characterized by decreases in anxiety-like behavior (p < 0.05), fragmentation of social behavior (p < 0.05), and alterations in USV production. This phenotype is readily distinguishable from those seen following prenatal activation of TLR3 and/or TLR4. On many of these measures there are significant sex differences. Additionally, mice exposed to prenatal IMQ have normal baseline locomotor activity, but are hyperactive

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in response to various types of stimuli including the presence of a social partner, circadian cues, or gonadal hormone fluctuations. This includes an increase in locomotor activity detectable only during a highly restricted period during the dark phase of estrus ($p < 0.01$). Prenatal IMQ exposure causes decreases in microglia density ($p < 0.05$) and increases in the number of microglial ramifications ($p < 0.05$). RNA-sequencing of the dorsal striatum revealed that prenatal IMQ exposure induces differential expression of hundreds of genes, especially those encoding synaptic components, cell adhesion molecules, and glial markers. However, there are dramatic sex differences, with virtually no overlap in differentially expressed genes between males and females.

**Conclusions:** Prenatal immune activation with a TLR7 agonist induces a type I interferon response in pregnant mice apparent in the maternal serum and placentas. The offspring of these treatment exhibit a behavioral phenotype and changes in microglia that are distinct from previous models that involve early activation of other TLR subtypes. Underlying this phenotype is a propensity for “conditional hyperactivity”, reflected by an exaggerated response to some types of internal and external stimuli. Further, genome-wide analysis of mRNA identified numerous molecular pathways affected by prenatal IMQ exposure, but demonstrated profound sex differences in the directions and patterns of expression. Considered with the existing literature, our findings suggest that early immune system activation can promote various—and sometimes even opposite—developmental trajectories, depending on the type and/or pattern of TLRs activated.

**Keywords:** Maternal Immune Activation, Toll-Like Receptors (TLRs), Telemetry

**Disclosure:** Nothing to disclose.

T11

**Development of a Humanized Mouse Model to Investigate Role of the Maternal Microbiome on Offspring Neurodevelopment**

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**Background:** The microbiome has emerged as a key regulator of metabolism, immunity, brain function and behavior in health and disease. The microbiome of an adult reflects both environmental factors and the history of exposure to microbiota, suggesting that early life represents a discrete window during which the immune system and the developing brain are responsive to microbiobial instruction. However, much of our knowledge regarding microbial colonization, immunity, neurodevelopment and disease risk has been informed by studies in germ-free animals that are devoid of microorganisms. Far less is known about the environmental factors that shape these microbial communities and the subsequent programmatic effects on offspring. Using our mouse model of early prenatal stress, in which exposed males, but not females, exhibit lasting changes to metabolism, stress responsivity and cognition, we examined the hypothesis that stress alterations to the maternal microbiome mediates the effects of prenatal stress. We developed a novel transplantation method in which mouse pups were delivered by cesarean section, thereby preventing natural colonization, and then transplanted with vaginal microbiota via orogastric gavage. Transplantation of vaginal microbiota from stressed dams into naive pups delivered by cesarean section recapitulated the prenatal stress phenotype, including reduced body weight, increased corticosterone response to acute stressors, and upregulation of immune and inflammatory gene signatures in the hypothalamus. However, transplantation of control vaginal microbiota into prenatally stressed pups delivered by cesarean section failed to rescue the prenatal stress phenotype. The inability to rescue the prenatal stress phenotype was dependent on transcriptional reprogramming to pathways involved in the regulation of innate immunity in the fetal gut and brain. Further, these results suggest that an important interaction between the maternal microbiome and immune development exert a direct effect on neurodevelopmental reprogramming. Thus, we examined the hypothesis that sex-specific brain development is influenced by the maternal microbiome in both mice and a humanized mouse model.

**Methods:** We used a combination of genomic, flow cytometric, mass cytometric and pharmacological manipulations to assess the neurodevelopmental programming via the maternal gut microbiome. Reconstitution experiments were used to examine the casual contribution of maternal gut microbiome to rescue offspring phenotypic aspects in adulthood. To establish translational relevance of our mouse model, vaginal microbiota samples were collected from women over the course of pregnancy. We selected two distinct human vaginal microbial communities based on population-level factors, such as stress and adversity in the environment, and immune parameters, such as differences in the risk for infection and ability to stimulate distinct arms of the immune system. Postnatal colonization experiments were conducted with these two distinct human vaginal microbial communities that were transplanted into C-sectioned mice at embryonic day 18.5. Validation and control experiments were conducted to confirm specificity and rule out environmental contamination.

**Results:** Maternal stress exposure increased presence of inflammation-associated microbiota and disrupted microbiota production of metabolites necessary for brain development. Comparison of metabolites from maternal and fetal tissues demonstrated stress-mediated decreases in key metabolites in maternal cecum and fetal brain. As these metabolites regulate innate immune development, we next determined how altered metabolite availability impacts the fetal brain immune compartment using high dimensional single-cell mapping and multicolor flow cytometry. Analysis revealed sex-specific changes in the frequency and activation patterns of resident and infiltrating immune cells in the brain. In our humanized mouse model, we validate colonization by human microbiota into mice by sequencing the neonate gut 48 hours following inoculation by 16S rRNA marker gene sequencing. Lasting effects on post-pubertal growth, stress axis responsivity and transcriptional changes in the hypothalamus were assessed.

**Conclusions:** Our translational approach demonstrates that the maternal microbiome is a putative biomarker of lasting neurodevelopmental risk in offspring. The mouse model has identified key mechanistic points whereby changes in inflammation and metabolites produced by the maternal gut microbiota impact fetal brain development and exert lasting outcomes on offspring physiology and behavior and metabolic function. Validation of a humanized mouse model highlights a significant translational advance in the ability to transplant microbiota from human donors into C-section mice and conduct mechanistic studies that cannot be readily conducted in humans where confounding variables, including mode of delivery, diet, and antibiotic exposure are impossible to control. The studies in the humanized mouse model show that colonization by distinct human microbial communities can phenocopy microbial patterns in a newborn mouse, thereby providing a novel approach to investigate the role of human microbial communities on the development of immune system and brain.

**Keywords:** Microbiome, Neurodevelopment, Sex Differences, Neuroimmunology

**Disclosure:** Nothing to disclose.
DGC8R Expression Predicts Cortical Morphometric Patterns in 22q11.2 Deletion Syndrome

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Background: 22q11.2 deletion syndrome (22q11DS) arises due to misalignment of low copy repeats (LCR) during non-allelic homologous recombination. It is associated with broad phenotype that includes widespread alterations in brain morphology that likely contribute to the psychiatric and developmental phenotypes observed in 22q11DS. As the typical deletion spans a gene-rich ~2.6 Mb region, identifying the specific genes underlying these neuroanatomic patterns remains a challenge.

Methods: As part of the 22q11.2 ENIGMA Working Group, we first characterized neuroanatomic alterations in the largest sample of 22q11DS individuals with molecularly confirmed deletions spanning the typical LCR A-D region (22q11DS-AD; n = 232), compared to demographically matched controls (n = 290). We then used transcriptional data from the Allen Human Brain Atlas to examine associations between the spatial expression pattern of each protein-coding 22q11.2 gene and regional surface area (SA) or cortical thickness (CT) deviation found in 22q11DS-AD.

Results: 22q11DS-AD patients showed significantly reduced total SA relative to control subjects, with prominent SA reductions in midline posterior and lateral association regions. CT was thicker overall in 22q11DS-AD, with focal thinning in the superior temporal cortex, parahippocampus, and caudal anterior cingulate. DGC8R and AFM3 regional expression patterns were significantly associated with deviation in SA in 22q11DS-AD, with DGC8 expression predicting regional SA changes at each age range examined. P2RX6 expression was also associated with deviation in CT.

Conclusions: Results highlight DGC8R as a key candidate driver of neuroanatomic abnormalities in 22q11DS-AD and demonstrate the utility of combining neuroanatomic and publicly available transcriptomic datasets to derive mechanistic insights into complex traits.

Keywords: 22q11.2 CNV, Structural MRI, Gene Expression

Disclosure: Nothing to disclose.

T13

Structural Hyperconnectivity of a Corticostriatal Circuit Involved in Habitual Decision-Making in Anorexia Nervosa and its Associations With Compulsive Behaviors

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Background: Behavioral symptoms in anorexia nervosa (dieting, over-exercising) are among the most challenging symptoms in psychiatry, and existing treatments have limited efficacy. The intractable nature of many anorexic behaviors causes anorexia to have the highest mortality rate among mental illnesses. Evidence suggests that many behavioral symptoms in anorexia might be primarily habitual in nature. We aimed to examine the structural connectivity of the white matter circuits involved in habitual and goal-directed behavior in anorexia nervosa. We hypothesized that anorexia, particularly its compulsions symptom severity, is associated with an abnormal hyperconnectivity, defined by higher number, volume and integrity of tracts in habit, and/or impairment (hypoconnectivity defined by lower number, volume and integrity of tracts) in goal-directed, decision-making corticostrital circuits.

Methods: We applied deterministic tractography to measure characteristics of white matter tracts: volume, number of tracts, and diffusion properties (fractional anisotropy – FA) involved in corticostrital circuits mediating habit and goal-directed behaviors. Diffusion weighted imaging data was acquired from weight-restored adult participants with restricting anorexia nervosa (AN; n = 20) and healthy controls (HC, n = 30). Tracts in the habit behavior circuit were defined by white matter connections between pre-supplementary/premotor motor area (preSMA/SMa) and postero-lateral putamen (Put) bilaterally (preSMA/SMa-Put tract). Tracts in the goal-directed circuit were defined by white matter connection between anterior caudate (Cau) and ventromedial prefrontal cortex (vmPFC) bilaterally (Cau-vmPFC). We performed deterministic tractography implemented in DSI studio (http://dsi-studio.labsolver.org/).

Results: Total bilateral preSMA/SMa-Put tracts in the habit circuit had a significantly higher volume in the anorexia group compared to the healthy controls (AN = 5241.25 ± 886.7 mm³; HC: 3331.83 ± 425.4 mm³; P = 0.003; effect size = 0.58). The total bilateral number of preSMA/SMa-Put tract streamlines was higher in the anorexia group compared to the healthy controls, at trend level (AN = 13.6 ± 2.4; HC = 8.9 ± 1.3; P = 0.07; effect size = 0.31). The average FA value in the bilateral preSMA/SMa-Put tracts was not significantly different between AN and HC groups (AN = 0.45 ± 0.041; HC = 0.47 ± 0.048; P = 0.21; effect size = 0.44). None of the characteristics of the goal-directed tracts (Cau-vmPFC) was significantly different between AN and HC groups. To examine associations with clinical variables, we performed post hoc correlation analyses, focusing on obsessions and compulsions from the Yale-Brown-Cornell Eating Disorder Scale (YBC-EDS) because of their relevance to habitual behaviors, which were measured in a subset of AN subjects (n = 14). We found that the compulsion subscore (compulsion symptom severity) was significantly correlated with the total bilateral volume of preSMA/SMa-Put tracts (r = 0.54; P = 0.021) as well as with the total bilateral number of preSMA/SMa-Put tract streamlines (r = 0.53; P = 0.026).

Conclusions: Our findings showed that anorexia nervosa is associated with abnormal structural hyperconnectivity of a habitual decision-making circuit in the brain. This hyperconnectivity can be either caused by, or the result of, overuse (overactivation) of the habit circuit which can reinforce anorexic compulsive behaviors. This may have implications for a potential novel circuit-based treatment target in anorexia.

Keywords: Anorexia, DTI, Habitual Decision-Making, Corticostriatal, Compulsivity

Disclosure: Nothing to disclose.

T14

Top Down Cortical Control of Conditioned Overconsumption

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Background: The ability to molecularly define cell types controlling complex behaviors would greatly enhance our ability to study these behaviors and their underlying circuitry. Feeding is a complex goal-directed behavior in anorexia nervosa. We hypothesized that anorexia, particularly its compulsions symptom severity, is associated with an abnormal hyperconnectivity, defined by higher number, volume and integrity of tracts in habit, and/or impairment (hypoconnectivity defined by lower number, volume and integrity of tracts) in goal-directed, decision-making corticostrital circuits.
motivated behavior that is controlled not just by metabolic and homeostatic factors, but also by environmental factors such as emotion and the hedonic nature of the food itself. Yet, little is known about how brain regions involved in cognition and emotion might contribute to overeating, and therefore, obesity. In order to probe this neural circuitry, we recently developed and validated a simple and rapid task in which cues associated with food availability can later lead to increased food consumption in sated mice (Stern et al. Molecular Psychiatry 2018). We then utilized this task in order to describe the mechanisms by which the brain coordinates conditioned overconsumption.

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

Results: In the conditioned overconsumption task, sated mice reliably consume more in the context previously paired with food than in the unpaired context. We found that the insular cortex and central amygdala, among others, are activated in sated mice following the consumption test. Furthermore, we find that the insular cortex, and specifically, the insular cortex → CeA projection, is required for overconsumption, but not for homeostatic feeding measured over 24 hours. Using retro-TRAP, we then identified neuronal nitric oxide synthase 1 (nos1) and vesicular glutamate transporter 2 (slc17a6) as markers for this projection. Chemogenetic inhibition of insular cortex Nos1 neurons also prevented cue-mediated overconsumption, which occurs through suppression of homeostatic satiety signals within the CeA.

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

Keywords: Obesity, Amygdala, Insular Cortex, Eating Disorders, Molecular Profiling

Disclosure: Nothing to disclose.

T15

A Neuroeconomic Approach to Quantify the Subjective Cost of Self-Control and its Modulatory Factors: Stress, Risk and Ambiguity

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Background: The failure to use self-control to guide goal-directed behavior is a central problem for both healthy individuals and those with clinical disorders marked by pathological choice behavior (e.g., substance use disorder, excessive gambling and obesity). Emerging theoretical work suggests that deviations from goal-directed behavior may emerge from a decision-making process that weights the costs of exerting cognitively demanding control against its perceived benefits. These ‘control costs’ are thought to stem from the limited cognitive resources available to support the demands of self-control. Here, we aimed to (1) develop an econometric approach to quantify the subjective cost of exercising self-control each individual, (2) to measure how these costs are modulated by changes in affective state, and (3) identify whether these costs are sensitive to different forms of uncertainty (risk and ambiguity).

Methods: Healthy, hungry (male and female) dieters first provided subjective ratings for food items, allowing us to identify a highly tempting food for each individual. Both before food exposure and at regular intervals after exposure, participants reported their willingness-to-pay in dollars to remove the tempting food for the remainder of the experimental period, effectively “pricing” their subjective cost for exercising self-control (Study 1: N = 32). We then measured how these costs differed in an independent cohort of dieters who first underwent exposure to an acute stressor (cold-pressor task), which is widely thought to compromise the use of self-control (Study 2: N = 31). Finally, in Study 3 (N = 38), dieters made a series of binary choices between spending a predictable amount of time with a highly-tempting food reward (certain option) or a lottery option, for which they could be required to spend a greater amount of time with this food (5-60 minutes; higher control costs), or no time at all (0 minutes; no control cost). Critically, the probability of each option was either stated explicitly (risk) or with some degree of uncertainty (ambiguity). We measured how much dieters were willing-to-pay to avoid temptation as an index of self-control costs (Study 1-2) and the proportion of lottery choices participants were willing to accept as an index of participants’ tolerance for risk and ambiguity when making decisions regarding self-control (Study 3).

Results: Across Studies 1 and 2, we found evidence that individuals were willing to pay to avoid exposure to temptation, confirming that we can measure subjective control costs in units of “dollars” humans. Specifically, control participants paid ~17% of their $10 endowment (DOLLARS/min) to restrict exposure to tempting foods, while stressed participants paid significantly more to avoid temptation (~34%; DOLLARS/min; independent t-test: t(61) = 2.65, p < 0.01), suggesting that stress-related deficits in behavioral control may stem from higher subjective costs of control after stress exposure. In Study 3, participants revealed a marked aversion to uncertainty, such that they were less likely to choose lottery choices when the cost of self-control was not predictable. Specifically, participants chose fewer ambiguous lottery choices than risky ones (paired t-test: t(37) = 3.44, p < 0.01), suggesting they were averse to choice environments in which they could not fully predict the cognitive costs of self-control.

Conclusions: Consistent with an emerging framework viewing goal-directed control as a cost-benefit decision-making process, these data suggest that the subjective cost of self-control can be quantified in humans and that these costs are highly sensitive to changes in affective states. Our findings also suggest that tolerance for risk and ambiguity play a role in when choosers are willing to engage in self-control processes, pointing to new avenues of research that use these decision preferences to predict when individuals will use self-control.

Keywords: Self-Control, Decision-Making, Stress, Computational Psychiatry, Uncertainty

Disclosure: Nothing to disclose.
Decoding Impulsive Decision-Making From Rat Cortical-Striatal Oscillations

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Background: Impulsive decision-making is observed in many psychiatric disorders, from ADHD and bipolar to substance use disorders. Variation in impulsive decision-making can be evaluated in both pre-clinical and clinical populations using specific tasks (e.g., the delay discounting task). In patients, measures of impulsive decision-making relate prospectively to problematic behaviors—gambling, substance use, violence, and suicide—and also predicts non-response to treatment and risk of relapse. We hypothesize that treatments that normalize the dysregulated brain activity that underlies impulsive decision-making could meaningfully impact an array of neuropsychiatric conditions. In order to develop new treatments that target systems-level brain activity, a better understanding of the relationship between brain activity and behavior is needed. Prior work has shown that lesioning and modulation of nodes within the cortical-striatal network can alter performance on the delay discounting task. Thus, neural activity recorded from the cortical-striatal system should have some relationship to delay discounting performance. This study investigated the relationship between oscillatory activity (local field potentials - LFPs) recorded from the cortical-striatal network and performance on the delay discounting task to understand the relationship at different timescales - from trait through state and down to trial.

Methods: Male and female Sprague-Dawley rats were trained in the delay discounting task with ascending delays across blocks (12 trials per block) from 0 to 32 seconds. After stable behavior was achieved, animals were implanted bilaterally with electrodes targeting the nucleus accumbens shell and core as well as the infralimbic and orbitofrontal cortex. Local field potentials (LFPs) paired with video were recorded (Plexon) during free behavior in a neutral environment over 3 sessions (used for trait-based analysis). Animals were then recorded over multiple sessions during the performance of the delay discounting task (used for state and trial-to-trial analysis). For trait and state-based analysis, impulsivity was quantified using the area under the delay choice curve. Custom code written in Matlab was used to extract LFP features of power and coherence (connectivity between brain regions) across 6 established frequency bands (delta, theta, alpha, beta, low gamma, and high gamma) from 1-90 Hz. The LFP features were then used as predictors in machine learning (Lasso) models to classify delay discounting performance (average performance across sessions - trait; session to session performance - state; or trial-to-trial choices). Secondary analysis also evaluated the predictability of within block decision flexibility. To determine if models were significantly outperforming chance, all analyses were performed on permutations of the data. The distributions (real and permuted) of model performance were described with (±95%) confidence intervals and differences between the distributions was quantified using a z-score. If models built from all LFP features outperformed the permuted models, then an exhaustive evaluation of each LFP feature (logistic regression) was carried out to determine the relative information attributable to each LFP feature.

Results: LFP features recorded from regions within the cortical-striatal network during free behavior were able to predict trait impulsivity. This was determined by the accuracy of classifying if an animal's average area under the delay choice curve was above or below the median split (mean accuracy = 88 ± 0.5%; permuted accuracy = 35 ± 5%; z = 4.6). Similarly, at the state level, when each session from every animal was pooled and divided by median split, the model performed with an AUC equal to 0.80 ± 0.02 (permuted model = 0.52 ± 0.03). Interestingly, when trying to predict the area under the delay choice curves for every session, the model did not outperform our permutation tests (real error = 57 ± 2 and permuted error = 84 ± 3; z = -0.44). To determine if information about trial to trial choices (delay versus immediate) could be predicted, LFP features extracted from the 5 seconds before the choice was made were used as predictors. A generalized model from all animals performed with an AUC of 0.58 ± 0.02 on left out data (permuted AUC = 0.51 ± 0.01, z = 2.18). When individualized models were built to predict left out data from an individual rat (across multiple sessions) the AUCs varied from 0.88 ± 0.02 down to 0.51 ± 0.01 and this variation was not related to sample size. Surprisingly, LFP data from the 5 seconds before a choice was made could predict if that trial belonged to a block in which the animal made consistent decisions across the block (>80% delay or immediate choices) versus blocks in which decisions were flexible (between 40–60%)(real AUC = 0.67 ± 0.01, permuted AUC = 0.5 ± 0.01, z = 4.82).

Conclusions: These data show that rat cortical-striatal oscillations contain information that can classify high and low trait and state impulsive decision-making as well as predict trial to trial choices in a delay discounting task. Interestingly, the information about trait and state impulsivity resided mostly in measures of connectivity between brain regions, whereas the information about trial-to-trial decision making was reflected in power variation within regions. Overall, this data supports the utility of neural oscillations as a marker of impulsive decision-making and its variation across different time-scales.

Keywords: Delay Discounting, Machine Learning, Local Field Potentials, Impulsivity

Disclosure Nothing to disclose.
clear. Moreover, whether therapeutically-relevant manipulations can be used to augment motivation and how they affect dopamine function is poorly defined.

**Methods:** Male and female C57 mice (n = 11) were trained on a trial-based, chain schedule of reinforcement that spatially and temporally dissociated the seeking and taking phases of sucrose reinforcement. Fast-scan cyclic voltammetry (FSCV) was used to monitor NAc dopamine release during these distinct phases of reward-directed behavior. In a separate group of male and female mice (n = 19), we used a counter-balanced, within-subject design to assess whether chronic (6 day), low dose (8 mg/kg, i.p.) administration of the endocannabinoid (eCB) degradation inhibitor JZL-184 – which we have previously used to acutely rectify motivational deficits in mice – supports stable increases in motivation and dopamine function compared to vehicle control.

**Results:** NAc dopamine release diurnally encoded cues indicating the expected and experienced cost (lever presses) of sucrose reinforcement (P < 0.001). Chronic inhibition of eCB degradation produced stable increases in reward seeking by increasing the cost mice will overcome to gain reward (P < 0.001), decreasing the latency to initiate reward seeking (P < 0.001), and increasing rate of responding (P < 0.001).

**Conclusions:** Our findings clarify how NAc dopamine release is controlled by effortful cost during reward seeking and supports eCB agonism as a viable treatment option for rectifying diminished motivation and dopamine function.

**Keywords:** Dopamine, Motivation, Endocannabinoids, Fast Scan Cyclic Voltammetry, Reinforcement

**Disclosure:** Nothing to disclose.

**T20**

**Stress Resilience is Promoted by a Zfp189-Driven Transcriptional Network in Prefrontal Cortex**

Abstract not included.

**T21**

**Ketamine is Not an Opioid, but Requires Opioid System, for Anti-Depressive Actions**

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**Background:** The slow response to standard treatment for depression increases suffering and risk of suicide. Ketamine can rapidly alleviate depressive symptoms and reduce suicidality, possibly by decreasing hyperactivity in the lateral habenula (LHb). Unlike other drugs currently used for treatment of depression, ketamine displays high affinity for, and inhibits, the NMDA receptor (NMDAR). However, studies have called into question whether NMDARs mediate the antidepressant effects of ketamine. Other NMDAR antagonists don't produce an antidepressant response and ketamine also binds to a variety of other targets, including the mu opioid receptor, albeit with significantly lower affinity. A recent clinical study suggested that ketamine may act through opioid receptors to achieve an antidepressant effect, however subsequent studies have not replicated these findings. These disparate results highlight the need for a more complete understanding of the mechanism of action of ketamine, particularly in regard to the opioid system, as the use of ketamine in the community becomes more widespread.

**Methods:** We used behavioral assays, and neuronal calcium imaging in a rat model of different aspects of human depression to test the behavioral and cellular effects of co-administration of ketamine and naltrexone.

**Results:** We find that opioid receptors and NMDA receptors are both required for ketamine to reduce the depression-like behavioral and LHb hyperactive cellular phenotypes in helpless rats. An antidepressant dose of ketamine rapidly improves performance in the forced swim test and reduces LHb activity, but a hedonic dose of morphine is not sufficient to produce these effects. Importantly, a specific NMDAR antagonist both mimics and occludes the effect of ketamine on the LHb activity, confirming that the cellular effects of ketamine are mediated through the NMDAR. Finally, both the behavioral and cellular effects of ketamine are blocked by the opioid antagonist naltrexone indicating that the opioid system is permissive but does not mediate the actions of ketamine.

**Conclusions:** An antidepressant dose of ketamine likely does not act as an opiate, rather its cellular and behavioral effects are primarily mediated through the NMDAR. However, these effects require intact NMDA and opiate receptor signaling, suggesting that these two neurotransmitter systems may interact to mediate the antidepressant effect.

**Keywords:** Ketamine, Lateral Habenula, Opioid System

**Disclosure** Nothing to disclose.
The Role of Microglia in the Sculpting of Developing Stress Circuits by Early-Life Adversity

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

Methods: To interrogate microglial function, we employed male and female dual-reporter transgenic mice with visible CRH neurons and microglia and two-photon time-lapse imaging in acute slices of the PVN. We obtained these hypothalamic slices from P8 mice that were reared in LBN or control cages from P2 to P8. We then visualized live microglial process dynamics and their from P8 mice that were reared in LBN or control cages from P2 to acute slices of the PVN. We obtained these hypothalamic slices male and female dual-reporter transgenic mice with visible CRH and that adverse early-life experiences interfere with this function. Normal synapse reduction on CRH neurons in the developing PVN, remain unknown. We hypothesize that microglia contribute to normal synapse reduction on CRH neurons in the developing PVN, and that adverse early-life experiences interfere with this function.

Results: Early-life adversity augmented the number of vGlut2+/PSD95+ excitatory synapses onto CRH neurons by the end of the LBN experience (P10) in both males and females, without altering the number of CRH neurons or microglia in the PVN at P4, P8, or P10. However, microglial processes overlapped more substantially with CRH neurons at P8 than P4, potentially indicating a period of greater microglial-neuronal interaction. Pharmacological inhibition of microglia increased the density of excitatory synapses onto CRH+ neurons, phenocopying early-life adversity, and supporting the idea that microglia regulate synapse number in the developing PVN. Live-imaging revealed that microglial process dynamics were diminished in the PVN of P8 LBN mice, concomitant with decreased microglial engulfment of vGlut2+ presynaptic terminals. Characterization of whether DREADD-mediated microglial re-activation can prevent the adversity-induced microglial dysfunction and synapse excess onto CRH neurons is currently ongoing.

Conclusions: Microglia are potential contributors to early-life experience-dependent sculpting of stress-sensitive circuits. Ongoing studies include manipulation of microglial function during development aiming to prevent stress-related emotional disorders in adulthood, thereby providing novel targets for therapeutics or preventative interventions.

Keywords: Microglia, Synapse Growth-Pruning, Early-Life Adversity, Corticotropin-Releasing Hormone, Paraventricular Nucleus of the Hypothalamus

Disclosure: Nothing to disclose.
The Topography of GABA/Glutamate Co-Release in the Rodent and Primate Lateral Habenula

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Background: The lateral habenula (LHb) responds to aversive stimuli, and its hyperactivity is hypothesized to contribute to depression. Recent studies in rodents showed that inputs from the basal ganglia and ventral tegmental area to the lateral habenula co-release GABA with glutamate; the balance of GABA and glutamate at these synapses may regulate LHb responses to aversive stimuli and mood. In this study, we investigated whether the magnitude and topography of co-release of GABA and glutamate in the LHb is evolutionarily conserved in primates.

Methods: We co-labeled for glutamic acid decarboxylase (GAD; the synthesizing enzyme for GABA) and the vesicular glutamate transporter (Vglut2) in 30 micron sections from mice, monkeys, and humans. We then constructed high magnification images of the habenula and surrounding tissue and quantified the amount of GAD/Vglut2 co-labeling in tiled regions that together comprised each image.

Results: Our data indicate substantial co-labeling of GAD and VGLUT2 in synaptic terminals in the monkey and human LHb, consistent with co-release of GABA and glutamate from individual terminals onto primate LHb neurons. However, preliminary data suggest that there are differences in the topography of co-release in the primate and rodent LHb, perhaps due to expansion of the LHb in primates.

Conclusions: Although structural differences between the rodent and primate lateral habenula appear to exist, co-release of GABA with glutamate may be a conserved mechanism for regulation of LHb activity and mood in rodents and primates.

Keywords: Lateral Habenula, Neurotransmitter Co-Release, Depression

Disclosure: Nothing to disclose.

The Association Between Synaptic Density and mGluR5 in Depression: A Dual-Tracer PET Study

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Background: Converging evidence implicates a loss of synaptic connections in depression. A related pathophysiological mechanism is glutamate dysfunction, and the metabotropic glutamate receptor mGluR5, which modulates synaptic plasticity, has been specifically implicated in symptoms of depression. Using Positron Emission Tomography (PET) and [11C]UCB-J, a novel radioligand that binds to the presynaptic vesicle protein SV2A (which is dispersed throughout all the vesicles in the brain and is a marker of synaptic density), we recently demonstrated lower synaptic density in individuals with high severity depression. In a separate group of individuals, we showed that modulation of mGluR5 (measured using the radioligand [18F]FPEB) can alleviate depressive symptoms. However, the relationship between synaptic density and the glutamatergic system has not been investigated in vivo. Here, we used [11C]UCB-J and [18F]FPEB in the same individuals to examine the association between the densities of presynaptic SV2A and postsynaptic mGluR5 in relation to depression.

Methods: Twelve healthy controls (mean[SD] = 44[16]yrs; 5 women) and 20 individuals with major depressive disorder (MDD; 40[11]yrs; 16 unmedicated; 10 women) participated in one [11C]UCB-J and one [18F]FPEB scan. For both PET scans, our outcome measure was volume of distribution (VT), which is proportional to SV2A and mGluR5 density. We report VT in three regions important for mood regulation — the dorsolateral prefrontal cortex (dPFC), anterior cingulate cortex (ACC) and hippocampus. Depressed individuals were stratified according to depression severity (using a HAMD score of >14; moderate severity), and regional differences across severity and HC groups were assessed using independent-samples t-tests. Correlations between [11C]UCB-J and [18F]FPEB VT were assessed using Pearson’s r and differences between correlations were assessed using Fisher’s r to z transformation.

Results: In line with our previous findings in a larger sample of unmedicated individuals, stratifying MDD individuals according to severity led to an observation of lower synaptic density in the high severity vs. HC groups in dPFC (p = 0.047, Cohen’s d = 0.86) and ACC (p = 0.049; d = 0.85), but not hippocampus (p = 0.11, d = 0.67). We observed no significant differences in mGluR5 availability across groups (all p’s > 0.5, all d’s < 0.3). When assessing the relationship between [11C]UCB-J and [18F]FPEB VT, we observed a significant correlation between SV2A and mGluR5 in the dPFC (r = 0.60, p = 0.04) in the HC’s. Conversely, in individuals with MDD, there were no associations between SV2A and mGluR5 in any ROIs (all r’s < 0.1). The difference in correlations between HC and MDD groups was significant in the dPFC (p = 0.034).

Conclusions: We demonstrate a disconnect between synaptic density and glutamatergic tone in individuals with MDD. The significant correlation between SV2A and mGluR5 in the dPFC of healthy controls is in line with research indicating a relationship between mGluR5 and synaptic plasticity. However, there was no such association in MDD. This lack of association could reflect a disconnect between pre- and postsynaptic function in the dPFC of depressed individuals. Further, it could reflect an aberrant association between mGluR5 and synaptic plasticity in depression. The observed disconnect could be indicative of a loss of synaptic plasticity in the dPFC, which could in turn reflect reduced top-down control over emotional processing. Further work should evaluate whether synaptogenic treatments such as ketamine ameliorate coupling between synaptic markers and mGluR5 density.

Keywords: Depression, Synapse, Glutamate, PET

Disclosure: Nothing to disclose.

Amygdala Activity to Threat Mediates Depression Outcomes of Integrated Collaborative Care for Comorbid Depression and Obesity

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Background: Depression and obesity are prevalent, often comorbid and each associated with lower quality of life, disability, and lost productivity. An integrated collaborative care intervention incorporating a 12-month behavioral weight loss treatment with problem-solving therapy, is effective at treating depression in those with comorbid obesity. However, the neural targets underlying these improvements in depression remain unknown. Several lines of evidence suggest that the negative affective circuit, which includes the amygdala and prefrontal cortex (PFC), may be one such target. First, depression is associated with dysfunction in negative affective circuit. Second, the negative affective circuit has been shown to mediate the response to several pharmacological and psychosocial treatments for depression. Third, the negative affective circuit is hypothesized to play a prominent role in the regulation of emotion, an explicit target of problem-solving therapy. Here we test whether the negative affective circuit may represent a response biomarker and mechanistic target mediating depressive symptom improvements following an integrated collaborative care intervention with problem-solving therapy.

Methods: We report on a subsample of participants (n = 108; 70% female) from the RAINBOW trial who enrolled in the ENGAGE neuroimaging sub-study with comorbid depression and obesity. Participants were randomly assigned to either 12-month integrated collaborative care intervention (I-CARE) that included problem-solving therapy with as-needed antidepressant medication for depression and a video-based behavioral weight loss treatment or to usual care. Depressive symptoms were measured using the 20-item Depression Symptom Checklist (SCL-20) at baseline, 6, and 12 months. Negative affective circuit function was also assessed using an established emotional face task in fMRI at baseline, 2, 6, and 12 months. Regions of interest (ROIs) included bilateral amygdala and the medial PFC (mPFC). Kraemer’s mediation analysis approach was used to examine whether early changes in the negative affective network (baseline to 2 months) mediated SCL-20 outcomes at 6 and 12 months.

Results: The amygdala nodes of the negative affective circuit were response biomarkers to the integrated intervention. Specifically, compared with usual care, the intervention group had a relative decrease in reactivity to masked threatening faces in both left and right amygdalae (Left: t = 2.00, p = 0.049; Right: t = 2.18, p = 0.033) from baseline to 2 months. Changes in amygdala reactivity mediated corresponding changes in SCL-20 symptom scores at 6 months for both groups such that larger decreases in amygdala activation from baseline to 2 months was associated with a greater reduction in depressive symptoms (Left: beta = 0.18, t = 2.36; p = 0.022; Right: beta = 0.28, t = 2.63; p = 0.011). Further, the relationship between changes in depressive symptoms and changes in right amygdala reactivity was dependent on treatment assignment, such that the same degree of changes in amygdala reactivity led to relatively larger changes in depressive symptoms in those who received usual care than those who received I-CARE (beta = −0.25, t = −2.13, p = 0.038). No effects were found for the mPFC at 6 or 12 months (all p’s > 0.05). No mediation effects were found for either region for SCL-20 at 6 months (all p’s > 0.05).

Conclusions: These findings add to a growing body of literature supporting the importance of the amygdala, a core node of the negative affective circuit, in the pathophysiology and treatment of depression. Specifically, our results suggest that the amygdala may be a neural target and mediator of an integrated collaborative care intervention with problem-solving therapy for depression comorbid with obesity.

Keywords: Brain Based Markers for Depression, Treatment Mechanisms, Amygdala, fMRI Negative Affective Stimuli, Integrative Health

Disclosure: Nothing to disclose.

T27

Microbial Metabolites: Tryptophan, Kynurenine, and Serotonin at the Intersection of the Microbiota-Gut-Brain Axis and Perinatal Mood and Anxiety Disorders

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Background: Intestinal microbes modify, create, and promote creation of critical metabolites such as serotonin. Key metabolites interact with the immune system and other components of the microbiota-gut-brain axis, increasingly identified as related to depression and anxiety. Perinatal Mood and Anxiety Disorders (PMAD) have significant impacts on mother and child and require novel pathways for intervention. Currently Selective Serotonin Reuptake Inhibitors (SSRIs) are the medication treatment of choice highlighting the importance of the serotonin system. There is variation in serotonin serum levels and in effectiveness of SSRIs in neuro-development populations. For example, a subset of individuals with treatment with a SSRI had lower serum serotonin. There is a foundation of literature that shows changes in tryptophan and its metabolites (i.e., kynurenine and serotonin) are associated with PMAD. The role of the microbiota in relation to PMAD and the tryptophan metabolites needs to be elicited. The purpose of this pilot study is to assess tryptophan, kynurenine, and serotonin in relation to microbial composition in a cohort of perinatal women enriched for women with psychiatric history of depression and/or anxiety.

Methods: Thirty women were recruited in the first or second trimester, characterized by a number of factors including mental health history, and followed into the postpartum period. Anxiety and distress were assessed by the Edinburgh Postnatal Depression Scale (EPDS) and the Generalized Anxiety Disorders-7 (GAD-7). Microbial composition was analyzed through 16S sequencing of fecal samples. Microbial composition alpha-diversity was measured with Chao1, Shannon, PD whole tree, and observed species methods. Beta-diversity was determined by weighted UniFrac. LC-MS was used to analyze serum for tryptophan metabolites.

Results: Sixty percent had a psychiatric history. Those with a history of anxiety have lower alpha-diversity (p-value 0.01) (less rich and less even community of bacteria) and significant differences in beta-diversity (uniquely different communities of bacteria) compared to women without a history of depression or anxiety. While only four women in the cohort were taking SSRIs, two subjects taking SSRIs had much lower levels of serotonin and lower alpha-diversity than all other study participants at all visits and were therefore excluded from analyses as outliers. EPDS and GAD-7 scores were not associated with tryptophan metabolites but there were only three participants with GAD-7 scores ten or greater and only two with EPDS scores greater than 13. On average, across all subjects included for analysis, tryptophan decreased in the third trimester and then increased postpartum, kynurenine increased across the perinatal period, and serotonin increased most from the third trimester to the postpartum period. Alpha-diversity was negatively associated with the change in serotonin from third trimester to postpartum (p-values ranging from 0.007 to 0.04). Alpha-diversity at the first visit was positively associated with change in kynurenine from earlier in pregnancy to the third trimester (p-values 0.02, 0.04). Alpha-diversity was negatively associated with change in tryptophan from third trimester to postpartum (p-values 0.02, 0.04). Beta-diversity also was associated with the change in tryptophan from third trimester to postpartum (p-value 0.01).

Conclusions: Our pilot study results are the first to demonstrate an association between alpha and beta diversity and tryptophan metabolites in perinatal women. The microbiota may be a missing link in understanding serotonin levels and PMAD response to SSRI treatment. Serotonin across subjects increased, but further study is needed to determine if this is secondary to a cohort enriched for those with history of anxiety and/or depression (but a very small percent taking psychotropics). The two excluded subjects with consistently very low levels of serotonin as well as very low diversity may support that this variation in response to SSRIs that has previously also been shown may be due to antimicrobial effects and the microbiota-gut-brain axis. Changes in tryptophan, kynurenine, and serotonin need to be considered in relation to tryptophan being obtained from the diet and then metabolized to the kynurenine pathway versus to serotonin. Factors such as inflammation determine which pathway. It is unclear if the patterns seen here such as greater breakdown of tryptophan in the third trimester is unique to this enriched cohort where some had slight increases in EPDS and GAD-7 scores but also had very few develop PMAD. This may suggest resilience factors such as from diet and further study of the microbiota may inform resilience factors and areas for intervention. Larger samples are required to confirm these novel findings, and a larger replication study is currently underway. Further research of the tryptophan, kynurenine, and serotonin is needed to provide better understanding of these critical pathways during the perinatal period and inclusion of the microbiota and host immune responses may be important missing links that associate with and even drive changes in healthy pregnancies versus when PMAD develop. Future research will also need to focus on greater numbers taking SSRIs and will inform who may most benefit from SSRIs for PMAD.

Keywords: Perinatal Depression, Perinatal Anxiety, Gut Microbiome, Serotonin, Selective Serotonin Reuptake Inhibitors (SSRIs)

Disclosure: Sage Therapeutics, Honoraria, UpToDate, Royalties.

T28

Precision Psychiatry With Immunological and Cognitive Biomarkers: A Multi-Domain Prediction for the Diagnosis of Bipolar Disorder or Schizophrenia Using Machine Learning

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Background: Precision Psychiatry is attracting increasing attention lately as a recognized priority. One of the goals of precision psychiatry is to develop tools capable of aiding a clinically informed psychiatric diagnosis objectively. Cognitive, inflammatory and immunological factors are altered in both bipolar disorder and schizophrenia, however, most of these alterations do not respect diagnostic boundaries from a phenomenological perspective and possess considerable variability in different individuals with the same phenotypic diagnosis and, consequently, none so far has proven to have the ability of reliably aiding in the differential diagnosis of bipolar disorder (BD) and schizophrenia (SZ). We also wanted to verify if a multi-domain approach considering both the immune blood biomarkers and the cognitive biomarkers would produce an algorithm with better diagnostic properties than one with each of these domains in isolation.

Methods: We developed a probabilistic multi-domain data integration model consisting of immune and inflammatory biomarkers in peripheral blood and cognitive biomarkers using machine learning to predict the diagnosis of BD and SZ. In this study, 102 blood-based biomarkers and 19 cognitive biomarkers were initially selected for analysis. Among them, any biomarker that had more than 30% of missing values were removed and, through this process, 27 blood biomarkers and 19 cognitive biomarkers were selected for final analysis. Within each selected biomarkers, subjects with any missing biomarker were also removed. This filtering resulted in a total of 416 participants, being 323, 372, and 279 subjects for blood, cognition, and combined biomarkers analysis, respectively.

The analysis was performed in two stages: 1) principal components analysis (PCA) for unsupervised analysis of the full dataset, aimed at determining whether a multivariate signal was present; 2) partial least squares discriminant analysis (PLS-DA) to help determine the identity of the biomarkers responsible for the separation. We built linear discriminant (LD) binary classifiers for three diagnosis pairs: BD vs. controls, SZ vs. controls, and BD vs. SZ. We used the 10-fold cross-validation strategy. We reported the area under the curve (AUC) value as the predictive measure of the final model. Receiver Operating Characteristic (ROC) plots were derived from linear discriminative analysis (LDA) based on the top 6 biomarkers from the PLS-DA approach. Three different models according to different domains were developed using selected biomarkers from blood (single immune-inflammatory factors in the peripheral blood domain), cognition (single cognitive domain) and their combined pool (multi-domain composed of immune-inflammatory factors in peripheral blood and cognitive biomarkers). The summary index of the optimal sensitivity and specificity for the three models were determined by the ROC curve and the AUC.

Results: A total of 416 participants, being 323, 372, and 279 subjects for blood, cognition, and combined biomarkers analysis, respectively. For the immune blood-based biomarkers, IgG1, IgG2, IgG3 and anti-cardiolipin antibodies A (ACA A) showed the best properties for discriminating bipolar disorder from controls, and Cytomegalovirus (CMV), herpes simplex virus 2 (HSV2), and Toxoplasma Gondii for SZ from controls. For the cognitive biomarkers, WAIS deterioration showed the best discriminant properties for BD from controls, and CVLT Total number of correct answers during list A Short Delay Cued Recall (CVLT SDCR) and CVLT Total Number of Correct Answers during List A Long Delay Cued Recall (CVLT LDRC) for SZ from controls. WAIS Digit Symbol Coding Score (WAIS DSC S) also showed significant discriminant capacity, however less than the others described above. When considering the ability of both domains together in separating BD from controls and SZ from controls, the peripheral blood-based biomarkers domain showed better-discriminating properties for BD, and the cognitive domain showed better discrimination for SZ. For the multi-domain model the blood-based biomarkers selected were IgG1, Toxoplasma Gondii IgG, and anti-cardiolipin antibodies; the cognitive biomarkers for the multi-domain model were subtasks of the WAIS, CVLT, and NART33. Our multi-domain model performances for the BD vs. control (sensitivity 80% and specificity 71%) and for the SZ vs. control (sensitivity 84% and specificity 81%) pairs were high in general, however, our multi-domain model had only moderate performance for the differential diagnosis of BD and SZ (sensitivity 71% and specificity 73%).

Conclusions: Our results show that the diagnosis of BD and SZ, and that the differential diagnosis of BD and SZ can be predicted with possible clinical utility by a computational machine learning algorithm employing blood and cognitive biomarkers, and that their integration in a multi-domain outperforms algorithms based in only one domain. The fact that all blood-based biomarkers selected are already implemented in clinical laboratories, and that the cognitive batteries selected can be done by trained personnel, would facilitate implementation. Independent studies are needed to validate such findings, particularly studies with longitudinal and
for the SKI RFR model included kurtosis of the right limb of the superior fronto-occipital faciculus; mean, axial, and radial diffusivity of the anterior corona radiata; and correlations between the sensory motor network (SMN) and the posterior default mode network (DMN), central executive (CEN), right fronto-parietal and salience networks; and the correlation between the visual network and posterior DMN. Stronger inverse correlation between resting state networks was widely associated with more robust antidepressant response following SKI, however, higher correlation between the visual network and DMN was predictive of better treatment outcomes. The SKI RFR model was not a significant predictor of TSD ($r = 0.21$, $t = 0.83$, $df = 15$, $p > 0.05$) or ECT-related ($r = -0.38$, $t = -1.68$, $df = 16$, $p > 0.05$) QIDS-SR changes.

**Conclusions:** This preliminary study compared treatment-response biomarkers across several rapidly-acting antidepressant interventions with unique access to the central nervous system: ECT, SKI, and TSD. Data collection is ongoing and the current sample sizes are relatively small for machine learning approaches. Nevertheless, in the largest of the three treatment groups, SKI, we identified promising multimodal predictors of treatment response. The SKI RFR model demonstrated numerically promising, though statistically non-significant, predictive value in the TSD cohort. Future iterations of this project that include larger TSD and ECT cohorts may identify more robust transmodal biomarkers of therapeutic response.

**Keywords:** Major Depressive Disorder (MDD), Rapid-Acting Antidepressant, Biomarker, Machine Learning, Multimodal Neuroimaging

**Disclosure:** Nothing to disclose.

**T30**

**Alterations in Dynamic Network Modularity in Major Depressive Disorder**


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**Background:** Depression often is characterized by a lack of flexibility, including perseverative thinking and problems with cognitive control. Improving the identification of biomarkers of inflexibility in depression could lead to more targeted treatments to improve functional outcomes. Prior work in depression has demonstrated elevated resting-state connectivity within the default mode network (which is active during self-focused thought) and less connectivity within the frontoparietal network (a system critical for problem-solving and executive functioning). However, recent innovations in dynamic functional connectivity have enabled the exploration of how network patterns fluctuate over time, which might provide more nuanced insight into the role of these networks in depression. We explored connectivity dynamics within the default mode and frontoparietal networks among individuals with major depressive disorder (MDD) and healthy comparison participants (HCs). We hypothesized that within the default mode network, individuals with MDD would have greater dwelling time (representing greater time spent in its community), less flexibility (fewer changes in community allegiance) and less promiscuity (interacting with fewer communities) relative to HCs. Given the novelty of this approach, analyses within the frontoparietal network were exploratory.

**Methods:** Data were collected at an urban community medical center. Participants (Mean age = 26; 69% female) were 251 young adults with MDD and 164 HCs. Participants completed an eight-
minute, eyes-open resting-state fMRI scan. We compared MDD and HC groups on indices of network modularity in regions within the default mode and cognitive control networks. Just as social networks can be divided into cliques that describe modes of association (family, school, etc.), the brain can be divided into modules or communities based on functional networks. Several steps were taken to generate dynamic connectomes and estimate community structure before computing network modularity indices. Instantaneous phase synchrony analysis was used to create person-specific dynamic connectomes (based on the 32 network nodes defined by the CONN toolbox), representing the extent to which each node was functionally connected to each other node at the next time point. To generate estimates of community structure, we used probability-associated community estimation, which utilizes an iterative procedure to compute global-to-local bifurcating trees representing collections of nodes that form communities. For network nodes within the default mode and frontoparietal networks, we then computed the following indices of network modularity: dwelling time, flexibility, and promiscuity.

**Results:** Preliminary results suggest that relative to controls, individuals with MDD had greater dwelling time, less flexibility and less promiscuity (ps < .05, ds = 0.20-0.23) within a left lateral parietal node within the default mode network, consistent with the hypothesis of perseveration within this network in depression. Individuals with MDD also had greater dwelling time with a left lateral prefrontal cortical node within the frontoparietal network (p < 0.01, d = 0.26), and less promiscuity with a left posterior parietal cortex node also within the frontoparietal network (p = 0.01, d = 0.23). Planned analyses also will examine how network modularity indices may distinguish a subset of individuals with MDD who have a history of a suicide attempt.

**Conclusions:** These preliminary findings indicate that individuals with MDD show distinct patterns of dynamic network modularity during resting state. With the default mode network, individuals with MDD demonstrated elevated dwelling time and less interaction with other communities, supporting the hypothesis of the network's involvement in perseverative cognition and excessive self-focus. Elevated dwelling time and less flexibility with the frontoparietal network in MDD might reflect compensatory effort of this network to overcome difficulties with cognitive control. These analyses reflect a promising method for examining dynamic network modularity to gain insight into the functioning of intrinsic networks over time. Future work should examine how network modularity dynamics correspond with phenotypes such as perseveration and inflexibility, and how these modularity indices may be altered with tools such as neuromodulation.

**Keywords:** Functional MRI (fMRI), Dynamic Connectivity, Major Depression Disorder, Neural Flexibility

**Disclosure:** Nothing to disclose.

**T32**

Cortical Activity Motifs Increase the Variability of Cortical Sensory Responses After Stress

Abstract not included.

**T33**

Acute Effects of Cannabinoids on Symptoms of Obsessive-Compulsive Disorder: A Human Laboratory Study

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**Background:** Preclinical data suggest the involvement of the endocannabinoid system (ECS) in the pathology underlying obsessive-compulsive disorder (OCD). OCD symptoms have been linked to increased cannabis use, possibly as a coping strategy. There has been increased marketing of cannabis products for therapeutic use to treat anxiety and other OCD-related symptoms. Yet, few studies have tested the effects of cannabis on psychiatric symptoms in humans.
Methods: We recruited 12 adults with OCD and prior experience using cannabis to complete a randomized, placebo-controlled, within-subject human laboratory study assessing the effects of cannabis containing varying concentrations of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) on OCD symptoms. Participants completed three laboratory sessions during which they smoked either THC (7.0% THC/0.18% CBD), CBD (0.4% THC/10.4% CBD) or placebo (0% THC/0% CBD) cannabis varieties in random order. Following baseline measures, acute changes in drug-related effects, cardiovascular measures, OCD symptoms (via the Yale-Brown Obsessive-Compulsive Challenge Scale, YBOCCS, and OCD Visual Analogue Scale, OCD-VAS), and state anxiety (via the Spielberger State-Trait Anxiety Scale, State Scale, STAI-S) were assessed as a function of cannabis condition.

Results: THC increased heart rate, blood pressure, and intoxication compared to CBD and placebo. A linear mixed model revealed that self-reported OCD symptoms and anxiety decreased as a function of time in all three conditions (YBOCCS, $F_{1,6,10} = 4.31$, $p = 0.001$; OCD-VAS, $F = 8.93$, $df = 6$, 10; $p < 0.001$; STAI-S, $F = 7.00$, $df = 6$, 10; $p < 0.001$) reflecting either expectancy or time-within-session effects. OCD symptoms did not vary as a function of cannabis condition. However, state anxiety was significantly higher immediately after administration of both the THC and CBD varietals relative to placebo (THC, mean difference in STAI-S = 4.31, $SE = 1.34$, $p = 0.001$; CBD, mean difference in STAI-S = 3.85, $SE = 1.34$, $p = 0.004$). Post-hoc analyses revealed that participants who had significantly lower STAI-S scores 20 and 40 minutes after placebo administration relative to both THC and CBD.

Conclusions: This study, the first placebo-controlled investigation of cannabis in OCD, suggests that smoked cannabis, whether containing primarily THC or CBD, has little acute impact on OCD symptoms and may transiently increase anxiety in patients with OCD.

Keywords: Cannabinoids, Obsessive-Compulsive Disorder (OCD), Anxiety, Human Laboratory Study, Delta9-tetrahydrocannabinol, Cannabidiol

Disclosure: Nothing to disclose.

T34

Whole-Exome Sequencing Study of Parent-Child Trios With Trichotillomania

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Background: Trichotillomania (also known as hair-pulling disorder) is an impairing psychiatric condition characterized by recurrent hair removal that leads to noticeable hair loss despite attempts to stop. Approximately 1% of the population is impacted by trichotillomania, and studies generally suggest that it is more common in women than men. The natural history of trichotillomania is characterized by a typical onset at 11–13 years old followed by a chronic course of waxing-and-waning symptom severity throughout the lifetime with elevated rates of developing psychiatric comorbidities. Studies of pharmacological treatments for trichotillomania show mixed results, and currently there are no clear first-line agents or FDA approved medications. Hence, there is an urgent need for a better understanding of the biological mechanisms of trichotillomania in order to develop new effective treatments for patients and families. Previous research suggests that genetic factors are important in the development of trichotillomania, but progress has been slow in identifying reproducible risk genes. Currently, there are no published genome-wide studies focused on trichotillomania. Whole-exome sequencing of parent-child trios with de novo variant detection has proven to be a powerful approach for finding risk genes associated with related childhood-onset neuropsychiatric conditions, including autism, Tourette disorder, and obsessive-compulsive disorder. Here, we present results from the first whole-exome sequencing study of parent-child trios impacted by trichotillomania.

Methods: Whole-exome sequencing was performed on 27 parent-child trios (81 individuals total) where the child had a diagnosis of trichotillomania, and the majority of parents (98%) were unaffected. These families were recruited through the TLC Foundation for Body-Focused Repetitive Behaviors (BFRBs) and completed questionnaires about their medical history and symptoms. Exome capture was performed using the IDT xGen Exome Panel, followed by sequencing on the Illumina NovaSeq with approximately 75x coverage. After quality control, we analyzed 25 trios for de novo single nucleotide variants and indels using the GATK pipeline. We focused on variants that were rare in reference databases and that were either likely gene disrupting (including stop codons, splice sites, and frameshift indels) or missense variants that were predicted to be damaging based on PolyPhen-2. Rates of de novo variants were compared to 225 previously sequenced control trios from the Simmons Simplex Collection.

Results: De novo variant analysis identified 5 likely gene disrupting and 9 predicted damaging missense variants in these first 25 parent-child trios that were sequenced. These de novo variants occur in genes expressed in the brain, and overlap with gene sets of other psychiatric conditions, including autism and Tourette disorder. Preliminary pathway analyses using Ingenuity (IPA) suggest that the top canonical pathways are glutamate receptor signaling ($p = 0.0007$) and synaptic long-term depression ($p = 0.006$). Given these promising preliminary results, we are currently sequencing an additional 25 parent-child trios impacted by trichotillomania recruited from a previous clinical trial and from the TLC Foundation for BFRBs. We will plan to present these combined sequencing results, examining de novo and rare inherited variants, in at least 50 parent-child trios with trichotillomania at ACNP.

Conclusions: Whole-exome sequencing can identify de novo damaging DNA coding mutations in 25 parent-child trios with trichotillomania. We are currently sequencing an additional 25 parent-child trios and are continuing our recruitment efforts to hopefully reach a goal of over 100 trios impacted by trichotillomania. By increasing our sample size, we hope to identify the first high-confidence risk genes associated with trichotillomania and relevant biologic pathways. Trichotillomania remains an impairing, understudied condition with limited treatment options. This study is a first step to close this gap by using an approach that is known to be powerful in related conditions in order to shed light on the underlying biological mechanisms of trichotillomania.

Keywords: Trichotillomania, Human Genetics, Whole Exome Sequencing

Disclosure: Nothing to disclose.

T35

Analysis of Mitochondria Genetic Variants in Obsessive-Compulsive Disorder

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Background: Obsessive-compulsive disorder (OCD) is a severe neuropsychiatric disorder of unknown etiology that is exacerbated by stress. OCD and stress-related disorders may result from deficits...
in mitochondrial efficiency. These organelles are the main source of energy for neurons, and are involved in neurogenesis, synaptic plasticity and neuronal transmission, which are important parameters for successful adaptation to stressful conditions. The mitochondrial system is bigenic, involving genes encoded by both the nuclear genome (>1,000) and mitochondrial DNA (mtDNA, 37 genes). Our objective was to examine the association between a subset of the nuclear-encoded mitochondrial genes, i.e. those in the oxidative phosphorylation system, as well as mtDNA genes with OCD risk and subphenotypes.

**Methods:** For nuclear-encoded mitochondrial genes: a total of 59 SNPs were analyzed in 477 (54.7% females) OCD subjects. Logistic regression was used for OCD risk analysis and linear regression was used to test association with age at onset (AAO) and the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) symptom dimensions derived from the Y-BOCS symptom checklist using the principal components analysis (SPSS version 20). For mtDNA genes, we used genotyping data available for OCD samples in the Psychiatric Genomics Consortium database (N = 220 SNPs, 1,196 European OCD individuals). We tested the association using logistic regression in PLINK. For analysis of OCD risk, we downloaded 1000 Genomes data and selected the European subjects (N = 503) as healthy controls for our analysis.

**Results:** From case-control analysis, we observed nominally significant association for the SNPs rs4011457 in the NDUFS7 gene and OCD risk (N = 856, p = 0.004). Also, nominally significant evidence for association was observed for the SNP rs3820189 in the 5’ region of the MFN2 gene and YBOCS total score (N = 346; p = 0.002) and for the SNP rs42464944 in the PP1F gene and Sex/Religion factor (N = 371; p = 0.002) (all p-values uncorrected). A permutation-based test of all 59 SNPs jointly showed significant association with OCD (P(perm) = 0.003). For mtDNA, only common SNPs (N = 84, minor allele frequency > 1%) were included in our statistical model. We found two SNPs significantly associated with OCD risk, rs41534044 in the MT-CO2 (P = 0.002), and rs41531144 (P = 0.02) in the mtDNA control region (all p-values corrected).

**Conclusions:** To the best of our knowledge, this is the first study to date to show evidence that mitochondrial genes influence OCD risk. The results are limited by the small sample size. The subphenotypes of OCD used here may help in the design of future studies investigating the details of the obsessive-compulsive diagnosis and spectrum disorders.

**Keywords:** Mitochondrial Genetics, Obsessive-Compulsive Disorder (OCD), Mitochondria

**Disclosure:** Nothing to disclose.

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**T36**

**Brain-Periphery Inflammatory Markers in Individuals at Clinical High Risk for Psychosis**

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**Background:** Brain immune cells, in particular microglia, and immunoregulatory proteins (e.g. cytokines and C-reactive protein, CRP) are proposed as two of the main players of neuroinflammatory responses in the pathophysiology of psychosis. However, there is a lack of data on the link between these two components in the literature. In the current study, we investigated the association between translocator protein 18kDa (TSPO), as a marker for inflammation in the brain, and peripheral cytokines and CRP in a sample of individuals at clinical high risk for psychosis (CHR).

**Methods:** Thirty-nine CHR and 20 healthy volunteers underwent positron emission tomography of brain for TSPO using [18F]FEPPE. Also, CRP and cytokines (interleukins, IL, including: IL-1beta, IL-2, IL-6, IL-8, IL-10, IL-12; interferon-gamma; tumor necrosis factor-alpha, TNF-alpha) levels were measured in the blood samples of the participants.

**Results:** Except for the IL-8 levels that were significantly higher in CHR than healthy volunteers (F = 5.2, p = 0.03), there were not significant differences between groups with regard to [18F]FEPPE VT in the brain or CRP/cytokines levels in the periphery. Mixed-effects regression models that were used to investigate the effects of each blood marker on [18F]FEPPE VT revealed that while CRP levels were negatively associated with [18F]FEPPE VT (F = 4.78, p = 0.03, 95% CI = −4.55, −0.19), IL-1beta levels were positively associated with [18F]FEPPE VT (F = 4.54, p = 0.04, 95% CI = 0.05, 1.66). A mixed-effects model that was used to assess which peripheral markers can predict [18F]FEPPE VT revealed CRP (F = 7.01, p = 0.01, 95% CI = −4.58, −0.62), IL-2 (F = 4.69, p = 0.04, 95% CI = 0.02, 0.72) and TNF-alpha (F = 4.65, p = 0.04, 95% CI = −0.7, −0.02) as the best predictors.

**Conclusions:** The results of this study, while preliminary, suggest a link between peripheral and central markers of inflammation.

**Keywords:** Clinical High Risk for Psychosis, Neuroinflammation, Activated Microglia, Positron Emission Tomography Imaging, Pro-inflammatory Cytokines

**Disclosure:** Nothing to disclose.

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**T37**

**Multivariate Relationships Between Blood Cytokine Alterations and Clinical, Cognitive, Brain Structural and Functional Connectivity Measures in Psychosis: Moving Towards an Inflammatory Subtype of Psychosis**

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**Background:** Identifying key cytokine signatures within blood has the potential to predict clinical, structural and functional alterations in psychosis and may identify individuals who might respond to anti-inflammatory interventions. It is well established that a set of cytokines are elevated in the blood of people with schizophrenia and bipolar disorder and few attempts have been made at identifying subgroups determined by their cytokine status. Furthermore, cytokine levels have been associated with symptom, cognition, and brain structural/functional changes. However, since cytokine, cognitive, and brain phenotypes vary in severity, the relationship of specific cytokine levels to the aforementioned phenotypes is less certain. The purpose of this study was to define the relationships between cytokines and symptoms, cognition, brain structure or function using a multivariate data-driven approach in psychosis probands and healthy controls.

**Methods:** We compared 18 cytokines (IL1beta, IL2, IL4, IL6, IL8, IL10, IL12.23, IL12p70, CRP, C4A, TNFalpha, TNFbeta, IFNgamma, VEGFA, VEGFC, VEGFD, sFlt-1, TGFbeta1) focusing on inflammatory, immune, neurotrophic, and angiogenic pathways from the plasma of 140 psychosis probands (Schizophrenia/Schizoaffective, SZ, n = 79; Psychotic Bipolar, BPP, n = 61) and 60 healthy controls (HC) recruited from the Bipolar-Schizophrenia Intermediate Phenotype (B-SNIP1) study, Chicago site. We also used a subset
of neurobiologically defined biotypes (BT1 n = 17, BT2 n = 28, and BT3 n = 58, HC n = 56). Cytokine data was transformed, winsorised and adjusted for storage days, batch, and hemolysis score. Multivariate linear regression was used to perform pairwise contrasts for each cytokine while controlling for age, sex and ancestry. We used an empirical, data-driven approach for feature selection and dimensionality reduction to identify a linear combination of cytokine features that correlate with distinct clinical (PANS5, YMRS, MADRS), cognitive (BACS), brain structural (T1 MRI) or functional (rsfMRI) combinations. Canonical correlation analyses for each cytokine while controlling for age, sex and adjusted for storage days, batch, and hemolysis score. Multivariate linear regression was used to perform pairwise and adjusted for storage days, batch, and hemolysis score.

**Results:** IL6, CRP and VEGFA levels were significantly increased in the SZ group (d = 0.6, 0.5 and 0.4, respectively; p < 0.05) compared to HC. TNFalpha was increased in the BPP group (d = 0.4, p < 0.05) compared to HC. IL6 and CRP was increased in the SZ group (d = 0.4 and 0.4 respectively; p < 0.05) compared to BPP. BT1 had significantly greater IL6 levels (d = 0.7, p < 0.05), while BT3 had greater IL4, IL10 and TNFalpha levels (d = 0.5, 0.5, 0.4; p < 0.05) compared to HC. In BT1, IL2 (d = −0.6, p < 0.05) and TNFalpha (d = −0.8, p < 0.05) were lower, while IL6 (d = 0.5, p < 0.05) was higher compared to BT3. TNFalpha was lower in BT1 compared to BT2 (d = −0.7, p < 0.05). No other analytes were different between the groups. Worse affective symptoms were associated with greater IL1beta, IL6, IL12.23 and TNFbeta levels and lower C4A. Poorer general cognition correlated with greater IL1beta and IL6 and lower IL8, IL12.23 and sFlt-1. Smaller volumes in frontal-temporal-occipital-cerebellar regions were associated with larger CRP and IL6, as well as lower TNFbeta levels. Lastly, greater functional connectivity in auditory, visual and subcortical-cerebellar networks was correlated with greater CRP, IL1beta, and IL6, and lower TNFbeta. We summed the cytokines that consistently associated with behavioral and imaging phenotypes (IL1beta, IL6, IL12.23, CRP and TNFbeta and demonstrated that a higher cytokine score was significantly associated with worse global (beta = −1.7, p < 0.05) and social functioning scores (beta = −3.3, p < 0.001) in the overall group.

**Conclusions:** In this study, we demonstrated that elevations in a subgroup of cytokines were associated with worse affective symptoms, poorer cognition, smaller volumes in frontal-cerebellar regions and greater functional connectivity in auditory and visual networks. We suggest that cytokine signatures and associated phenotypes may define a subgroup of psychotic disorders that could be targeted through anti-inflammatory mediated interventions. The outcomes from our study may advance our understanding of differences between the mechanisms associated with cytokine alterations and their relationship with behavior, as well as brain structure and function.

**Keywords:** Inflammation, Cognition, Functional and Structural MRI, Psychosis Continuum

**Disclosure:** Nothing to disclose.

**T38**

**Changes in School Environment, Family Stress, and Screen Time Predict Increased Psychotic-Like Experiences in Children: An Adolescent Brain Cognitive Development Study-Based Longitudinal Analysis**

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**Background:** Although the onset of psychotic disorders such as schizophrenia does not typically occur until adolescence and young adulthood, psychotic-like experiences (PLEs; e.g. hallucinations) may occur as early as childhood. Indeed, these experiences are common, affecting 10–20% of children. Children with these symptoms are also thought to be more likely to develop a psychotic illness. Accordingly, understanding the relationships between these symptoms and environmental factors during development is of major importance. Here we analyzed data from Adolescent Brain Cognitive Development (ABCD) Study Release 2.0 to examine relationships between changes in school environment, neighborhood safety, screen time, and family stress with change in self-reported PLEs over a one-year period.

**Methods:** Self-reported changes in PLE severity score using the Prodromal Questionnaire Brief Child version (PQ-BC) from baseline to one-year follow-up were available for 4950 children ages 8–12 (2370 female, 2580 male) from the ABCD dataset. Stepwise regression was performed using number of PQ-BC “yes” responses and PLE severity as dependent variables and changes in family environment (e.g. violence in the household), neighborhood safety, school risk and protective factors (e.g. classroom safety, satisfaction with school and teachers), and screen time as well as age and sex as potential predictors with F(p) < 0.05 as the criterion for entry.

**Results:** All predictors examined showed only modest collinearity as evidenced by variance inflation factor scores of no more than 1.005 for all variables. For number of PQ-BC “yes” responses, worsening family environment and increased screen time significantly predicted increased number of responses (family environment: standardized β = 0.08, t = 5.64, p < 0.001; screen time: standardized β = 0.04, t = 3.11, p = 0.002; final model: F (2, 4928) = 21.04, p < 0.001). For PQ-BC severity score, worsening school risk and protective factors, worsening family environment, and increased screen time significantly predicted higher score (school risk and protective factors: standardized β = 0.06, t = 4.15, p < 0.001: family environment: standardized β = 0.04, t = 3.11, p < 0.002; screen time: standardized β = 0.05, t = 3.43, p = 0.001; final model: F(3, 4928) = 13.31, p < 0.001).

**Conclusions:** The results of this preliminary analysis suggest that changes in school environment, stress in the family, and screen time are significantly and independently associated with increased PLEs in school-aged children. As ABCD Release 2.0 only contained one-half of the planned one-year follow-up sample, the dataset will be re-analyzed next year when it becomes fully available. Within-site and family (e.g. twin and sibling) associations will also be accounted for in future analyses.

**Keywords:** Children, Psychotic-Like Experiences, Screen Time

**Disclosure:** Nothing to disclose.
nearly nothing is known about the neural mechanisms and the computations for this regulation.

We previously demonstrated that pOxr1+ excitatory cells in the medial entorhinal cortex layer III (pOxr1+ cells) project to the hippocampal CA1 pyramidal cells and are necessary for TFC (Kitamura et al., Science, 2014; Kitamura et al., Neuron, 2015). On the other hand, CalB+ excitatory cells in MECII (CalB+ cells) project to GABAergic neurons in hippocampal CA1, suppress the MECIII input into the CA1 pyramidal cells through the feed-forward inhibition, and inhibit TFC. These findings lead us to propose a disinhibition model to regulate TFC, driving TFC by pOxr1+ cells and regulating TFC by CalB+ cells. The central hypothesis of our model is that successful TFC depends on disinhibition of hippocampal CA1 pyramidal cells through the reduction of feed-forward inhibition mediated by CalB+ cells. Activation of dopamine receptors is thought to be crucial for TFC. Since our data showed that CalB+ cells specifically express dopamine D1 receptors (D1R), we further propose that dopaminergic input into CalB+ cells via D1R inhibits the neural activity of CalB+ cells during TFC and is crucial for TFC. To test these hypotheses, we examined following experiments.

Methods: 1) To define the roles of pOxr1+ and CalB+ cell activity for TFC, we examined the neural activity of pOxr1+ and CalB+ cells during trace fear conditioning (TFC, the association of tone and aversive footshock with 20 sec temporal gap, 3 trials) by specifically expressing GCaMP6f, a calcium indicator, into pOxr1+ cells or CalB+ cells in MEC through the miniature fluorescent microscope. N = 3-4 mice each group. Second, to test whether the activity of pOxr1+ or CalB+ cells is crucial for successful TFC, we optogenetically activated/inactivated the neural activity of CalB+ or pOxr1+ cells during TFC (N = 9-10 mice each group).

2) To determine the role of dopamine D1 receptors (D1R) in CalB+ cells for TFC, we examined the fluorescent double in situ hybridization in the MEC (N = 5 mice). Second, we generated MECII CalB+ specific D1R knockout mice, and then examined the effect on TFC (N = 10 mice each groups). Finally, we examined whether the activation of D1R in CalB+ cells induces learning-dependent activity reduction of CalB+ cells during TFC by using c-fos immunohistochemistry (N = 4-5 mice each). 15-20 weeks old male mice were used. Experimenters were blinded with respect to the genotype of the mice and the experimental designs were fully counterbalanced. Equal numbers of animals from each experimental group (i.e. genotype, age) was assigned. Statistical analysis: Comparisons between two-group were analyzed by unpaired Student’s t test or paired t-test, if normally distributed, otherwise by Wilcoxon rank sum or signed-rank test. Multiple group comparisons were assessed using a one-way, two-way, or repeated-measures analysis of variance, followed by the post-hoc test when significant main effects are detected. The null hypothesis was rejected at the P < 0.05 level. Estimated animal numbers were based on power analysis calculations and previous studies (Kitamura et al., Science, 2017; Kitamura et al., Neuron, 2015; Kitamura et al., Science, 2014; Kitamura et al., Cell, 2009).

Results: 1) We found the population calcium activity of pOxr1+ cells significantly increased by tone stimulus, and even after tone cessation, remained elevated in trace periods through all 3 trials (P < 0.05). While the population calcium activity of CalB+ cells also significantly increased by tone stimulus and kept maintained in trace periods in 1st trial compared to base level (P < 0.05), the population activity of CalB+ cells became insensitive to tone stimulus in 3rd trial compared to that in 1st trial (P < 0.05). Optogenetic inactivation/activation of pOxr1+ cells or CalB+ cells showed that pOxr1+ cells is crucial for bridging temporal gap while CalB+ cells is essential for separating two temporally segregated events as different episodes.

2) First, we found that CalB cells superficially express D1R in the EC. We also found that VTA and LC neurons directly project to CalB+ cells. Second, we found that ECII CalB+ specific D1R knockout mice showed deficit in trace fear conditioning compared to control group (P < 0.05). Third, by using c-Fos immunohistochemistry we found that the activation of D1R in CalB+ cells in EC is crucial for the learning-dependent activity reduction of CalB+ cells during TFC that is essential for successful TFC.

Conclusions: We identified that pOxr1+ cells are necessary for TFC, while CalB+ cells regulate TFC by suppressing the MECIII input into the CA1 pyramidal cells through the feed-forward inhibition. The gating activity of CalB+ cells during TFC is controlled by dopaminergic inputs via the activation of D1R in CalB+ cells. The circuit mechanism can be a pharmaceutical new target for preventing inadequate memory formation.

Keywords: Hippocampus, Learning and Memory, Entorhinal Cortex, In Vivo Calcium Imaging, Optogenetics

Disclosure: Nothing to disclose.

T40

Memantine Effects on EEG Measures of Putative Cortical Excitatory / Inhibitory (E/I) Balance in Schizophrenia

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Background: Persistent neurocognitive deficits in schizophrenia are refractory to many common antipsychotic regimens and contribute to functional impairment. Deficits in early auditory processing in schizophrenia are thought to mediate both neurocognitive and functional impairment, and appear to be normalized by acute treatment with the NMDA antagonist, memantine. Abnormalities in cortical E/I balance in schizophrenia have been identified and linked to many core cognitive disturbances in this disorder. The aperiodic 1/f component of the neural power spectra is thought to index relative E/I balance. The present study assessed the impact of acute memantine administration on EEG measures of putative E/I balance in schizophrenia patients and healthy comparison subjects.

Methods: Thirty-six well-characterized subjects with a diagnosis of schizophrenia (SZ; M:F = 24:12; mean age (range, y) = 36 (19-48)) and 31 healthy adult comparison subjects (HCS; M:F = 24:7; mean age (range, y) = 31 (19-45)) were assessed using 64 channel EEG on measures of early auditory information processing from the passive auditory oddball task. Subjects were tested on two days about a week apart, after ingesting either placebo or 10 or 20 mg MEM po, in a double-blind, within-subject cross-over design. EEG power spectral densities (PSD) were estimated from individual event trials using Welch’s method. The aperiodic component of the EEG power spectra was calculated from individual PSDs using a robust linear regression algorithm and averaged across all electrodes.

Results: Analysis revealed a significant effect of diagnosis (p < 0.02), dose (p < 0.005), and a significant dose x diagnosis interaction (p < 0.0001). The main effect of diagnosis indicated elevated aperiodic signals in SZ patients compared to HCS (p < 0.01), suggestive of a reduced E/I ratio. This deviation was confirmed using a robust linear regression algorithm and averaged across all electrodes.

Results: Analysis revealed a significant effect of diagnosis (p < 0.02), dose (p < 0.005), and a significant dose x diagnosis interaction (p < 0.0001). The main effect of diagnosis indicated elevated aperiodic signals in SZ patients compared to HCS (p < 0.01), suggestive of a reduced E/I ratio. This deviation was confirmed using a robust linear regression algorithm and averaged across all electrodes.
Conclusions: Findings confirmed deficient E/I balance in antipsychotic-medicated SZ patients that were normalized by acute administration of memantine (20 mg). Future studies may identify symptomatic or neurocognitive profiles predicting greatest memantine E/I sensitivity; conceivably, such a predictive metric may distinguish subgroups of patients who will most benefit from memantine augmentation therapy.

Keywords: Excitation-Inhibition Balance, Schizophrenia Novel Treatment, EEG Biomarkers

Disclosure: Nothing to disclose.

T41

EEG Resting-State Attentional Networks Abnormalities are Associated With Negative Symptoms and Cognitive Deficits in Ultra High-Risk Syndrome

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Background: The brain is continuously active, even in resting state conditions, in order to prepare the system for the arrival of new internal or external stimuli. Microstate resting-state analysis is relevant for translational purposes due to the consistency of its findings and the brevity and simplicity of its registration. In brief, it consists of the analysis of four transient electrical topographies that are related to four resting-state networks: A-phonological, B-visual, C-salience and D-attentional. For statistical purposes, each microstate can be described in detail according to its occurrence (1/s), duration (ms), coverage (%) and global explained variance (GEV). Previously, microstate resting-state abnormalities have been reported in schizophrenia (SZ; Koeing et al., 1998) and 22q11 deletion syndrome subjects (Tomescu et al., 2015), which is thought to be a genetic risk group for SZ. Specifically, decreased microstate D (attentional networks) and increased microstate C (saliency) parameters, have been widely replicated in SZ microstate studies. However, little is known about this topic in ultra-high-risk (UHR) syndromes (Fusar Poli et al., 2013) and its relationship with clinical symptoms.

Methods: A four-minute EEG recording microstate resting-state analysis was performed in 23 UHR and 29 age and sex matched controls (CNT). UHR group was also assessed using the Structured Interview for Prodromal Syndromes (SIPS) for clinical symptoms and the MATRICS Consensus Battery (MCCB) for cognitive symptoms. UHR subjects were recruited from the first UHR cohort of Chile (Gaspar et al., 2018). 78.26% of UHR subjects were taking atypical antipsychotics. EEG recordings were pre-processed and analyzed in an open source microstate EEGLab toolbox for MATLAB (Poulsen et al., 2018). Finally, an unpaired t-test was performed to evaluate differences of each microstate statistic (duration [ms], coverage [%], occurrence [1/s] and GEV) between both groups. Pearson’s correlation was used to detect interactions between microstate statistics and SIPS/MCCB scores. Spearman’s correlation analysis was performed to discard any antipsychotic influence (measured as chlorpromazine equivalents [mg]) in microstate results. All subjects included in the analysis signed an informed consent and the research was approved by the local ethics committee.

Results: Obtained microstate topographies were similar to those found in previous studies (A, B, C, D; Lehmann et al., 2005). UHR subjects presented a decreased microstate D duration (ms) (p < 0.001), coverage (%) (p = 0.005) and GEV (p = 0.001). Also, increased microstate B coverage (%) (p < 0.003) and GEV (p < 0.001) were found. Microstate C abnormalities were not found in this study. No correlations were shown between microstate statistics and antipsychotic treatment. Additionally, microstate D coverage (%) was negatively correlated with total negative symptoms score in UHR subjects (p = 0.027; r = −0.460), and microstate D duration (ms) was negatively correlated with the speed of processing MCCB domain (p = 0.034; r = −0.445) and Total MCCB score (p = 0.018; r = −0.522). Microstate B coverage (%), however, was positively correlated with the problem-solving MCCB domain (p = 0.036; r = 0.439).

Conclusions: Microstate D abnormalities previously seen in SZ were replicated in this UHR study, suggesting abnormal resting-state connectivity in core attentional areas in psychotic disorders. Negative correlations between resting-state abnormalities, negative symptoms, and Total MCCB score support this statement. Thus, this finding could represent an early SZ trait marker of potential clinical utility. On the other hand, increased coverage (%) and GEV of microstate B, related to visual networks, represents a more inconsistent finding (only noted in one previous SZ study [Kikuchi et al., 2007]), though not less interesting given its correlation with cognitive problem-solving functions. Although microstate C has also been widely reported in SZ studies, it did not show significant differences in this study. This could potentially be explained by ethnic differences or later neurodevelopmental emergence.

Keywords: Cortical Circuit Function, Schizophrenia, EEG Biomarkers, Ultra High-Risk Youth, Cognitive Functioning, Negative Symptoms

Disclosure: Nothing to disclose.

T42

Sensitive Periods for Activity-Dependent Refinement of PFC Parvalbumin Interneuron Connectivity and Behavior

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

Methods: We used slice electrophysiology to record from PFC PV interneurons from MIA and control offspring at different time points during development and in adulthood. We then utilized a viral and genetic approach to express the pharmacogenetic receptor, hM4D, in developing PV interneurons and administered the agonist for hM4D, clozapine-N-oxide (CNO), during the postnatal window (P14-P50) to mimic the physiological changes in activity we observed in MIA offspring during development. Forty days following the end of this transient pharmacogenetically-induced reduction in activity, we assayed whether this manipulation persistently effects the connectivity of PFC PV cells onto pyramidal neurons in adulthood (P90).
using optogenetics combined with slice electrophysiology. We also examined persistent effects on adult attentional set shifting behavior as well as underlying prefrontal activity recorded using stereotrodes in behaving animals. Both male and female mice were used for this study, but the numbers were not large enough to examine effects of sex.

Results: We discovered that PFC PV interneurons in MIA offspring show decreased intrinsic excitability transiently during early peripubertal and adolescent development, corresponding to a window during which extensive refinement of their synaptic connections normally occurs. We found that pharmacogenetically mimicking this transient suppression of PFC PV activity between P14 and P50 resulted in persistent effects on PFC PV interneuron functional connectivity in adulthood (P90), similar to the decreased PFC PV interneuron functional connectivity found in adult MIA offspring. We found that this transient manipulation in excitability was also sufficient to persistently impair adult set shifting behavior and associated prefrontal activity.

Conclusions: Our results indicate a sensitive developmental period during which transient alterations in PFC PV interneuron activity are able to affect the strength of PFC PV functional connectivity, set-shifting behavior and associated neural activity. Current work is investigating whether this same manipulation delivered in late adolescence or adulthood would result in similarly persistent effects. These findings suggest early dysfunction in PFC PV interneurons can result in persistent changes in prefrontal cortical function and may give insight into disease etiology. From a therapeutic perspective, these findings may indicate a developmental period during which the brain would be particularly susceptible to interventions that engage the prefrontal cortex and thereby naturally enhance PV interneuron activity.

Keywords: Medial Prefrontal Cortex, Parvalbumin Fast-Spiking GABAergic Interneurons, Adolescence, Critical Periods

Disclosure: Nothing to disclose.

T43

Lateral Hypothalamus Opposes Learning About Neutral Information: Implications for Schizophrenia

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Background: While the lateral hypothalamus has traditionally been viewed as a switch to control food consumption, recent work has demonstrated more complex encoding of stimuli associated with rewards. For example, we have shown that the lateral hypothalamus is necessary for rats to associate cues with rewards. Here, we tested whether GABAergic neurons in the lateral hypothalamus would be important for learning to associate two neutral cues.

Methods: To do this, we infused AAV5-EF1a-DIO-eNpHR3.0-eYFP (experimental group; n = 8) or AAV5-EF1a-DIO-eYFP (control group; n = 12) into the lateral hypothalamus of male and female GAD-Cre rats. Following surgery, we exposed rats to four auditory stimuli, presented in pairs as A1-A2 and B1-B2, where each 10s stimulus was followed by a short delay between its associate to inhibit GABAergic neurons in our experimental group during this phase for 11s beginning 500ms before the onset of A1, mirroring our previous study with cues and rewards. Presenting stimulus A2 with food allowed us to selectively assess the efficacy of the A1-A2 association formed in the first phase by examining how likely rats were to enter the food port when cue A1 was presented. If an effective association between A1 and A2 had been formed, presentation of A1 should evoke a representation of food and motivate rats to go to the food cup.

Results: Using a repeated-measures, mixed design ANOVA, we found that all subjects spent more time in the food port during presentation of A1 relative to the B1 stimulus (stimulus: F(1, 18) = 15.438, p = 0.001). However, our experimental group showed an enhanced response to the A1 stimulus over the B1 stimulus (stimulus × group: F(1, 18) = 5.961, p = 0.028). Analyses showed this interaction was due to a significant difference between A1 and A2 in the experimental group (F(1, 18) = 16.615, p = 0.001), that was completely absent when the control group was analysed in isolation (F(1, 18) = 1.489, p = 0.238).

Conclusions: These results demonstrate that inhibition of GABAergic neurons in the lateral hypothalamus during A1-A2 pairings enhanced learning of this association during the first phase. Taken together with our previous findings that inactivation of these neurons impairs learning about cues and rewards, we believe this suggests lateral hypothalamus generally opposes learning about neutral information in favor of learning about cues directly paired with something motivationally significant (like rewards). Thus, when you inhibit GABAergic neurons in the lateral hypothalamus you enhance learning about neutral cues as you release the opposition of learning usually exerted by this nucleus. This dissociation in learning is striking as it resembles the learning dissociation seen in patients with schizophrenia; poor learning about reward information, with an increase in learning about stimuli that are irrelevant to current goals. We discuss these results in the context of a developing framework of how lateral hypothalamus modulates dopamine prediction errors during learning, which may have relevance for changes in prediction-error signalling seen in schizophrenia.

Keywords: Lateral Hypothalamus, Dopamine, Schizophrenia-like Behavior, Reward Learning, GABA

Disclosure: Nothing to disclose.

T44

Mefloquine Impairs Sleep-Dependent Declarative Memory Consolidation in Humans

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Background: Sleep has been shown to benefit memories. This occurs through the repeated reactivation of memory traces encoded during prior wakefulness. When rats explore a novel environment place cells in the hippocampus develop place specific firing patterns that have been argued to be the physiological substrate of declarative memory. After learning, these place cell firing patterns have been shown to re-emerge during subsequent sleep. Sharp-wave/ripples have been identified as the electrophysiological correlate of this process and their disruption via electrical or optogenetic methods impairs the consolidation of hippocampus-dependent memories. As gap-junctions, i.e., direct electrical connections between neurons, are necessary to generate sharp-wave/ripples, we hypothesized that blocking them would impair declarative memory consolidation during sleep.

Methods: We used 250 mg mefloquine to block gap junctions in three placebo-controlled, balanced, cross-over experiments...
with a total $N = 44$ male participants. In the afternoon, participants learned a declarative word-pair task and a procedural finger sequence tapping task. In experiments 1 and 2 they received the drug at 18:00 to achieve plasma maximum approximately during the first half of the subsequent night. In experiments 1 and 3, participants were allowed to sleep during the night, while participants in experiment 2 were sleep-deprived. Participants in experiment 3 received the drug in the morning to achieve plasma maximum at retrieval, during the afternoon.

**Results:** We found that mefloquine impaired declarative memory retention significantly only in the group that received it before being allowed to sleep. Unexpectedly, it improved retention of the procedural memory task over all experiments.

**Conclusions:** We conclude that gap junctions may play an important role for sleep-dependent consolidation that is likely mediated by their role in generating sharp-wave/ripples in the hippocampus. The involvement of gap-junctions in this process may explain previous findings of unperturbed consolidation after blocking glutamatergic neurotransmission. The effect on procedural memory can be explained by an overall disengagement of the hippocampus caused by mefloquine, as the hippocampus has been shown to interfere with consolidation of this type of memory. Of note, mefloquine also has non-gap junction related effects in the brain that cannot be completely ruled out to have contributed to the effects reported here.

**Keywords:** Sleep, Memory and Learning, Neurotransmission

**Disclosure:** Nothing to disclose.

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**T45**

**Role of Orbitofrontal Cortex in Incubation of Oxycodone Craving in Male and Female Rats**

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**Background:** Drug seeking progressively increases after withdrawal from drug self-administration (incubation of drug craving). Previous studies have shown that this incubation generalizes across different drug classes in both rats and humans. Here, we examine neural mechanisms underlying incubation of craving to oxycodone, a commonly abused prescription opioid, and we focus on orbitofrontal cortex (OFC), a brain region previously implicated in incubation of heroin craving.

**Methods:** We trained male and female adult Sprague Dawley rats to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d for 10 d). Next, we either tested (Relapse-test) or did not test (No-test) rats for oxycodone seeking (2-h) under extinction condition on withdrawal day 1 or withdrawal day 15. Immediately following the tests, we perfused rats for immunohistochemistry to measure c-Fos, a neuronal activity marker, in OFC.

**Results:** We found that rats escalated oxycodone intake during training, and exhibited higher oxycodone seeking on withdrawal day 15 than on withdrawal day 1. We observed no sex differences either during the self-administration training or relapse test. Furthermore, c-Fos expression increased in the Relapse-test group compared with the No-test group on withdrawal day 15.

**Conclusions:** Results demonstrate that incubation of oxycodone craving occurs in both adult male and female rats after withdrawal day 15 from oxycodone self-administration and is associated with neuronal activation in OFC. Studies are underway to examine the causal role of OFC in incubation of oxycodone craving by pharmacological and chemogenetic approaches.

**Keywords:** Orbitofrontal Cortex (OFC), Incubation of Drug Craving, Oxycodone

**Disclosure:** Nothing to disclose.

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**T46**

**β3 Integrin and Focal Adhesion Kinase as a Signaling Pathway for MMP-9 Induction of Transient Synaptic Plasticity in D1 Vs D2-MSN in Cocaine Relapse**

Abstract not included.

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**T47**

**In Vivo Imaging of 11β-Hsd1 With [18f]As2471907 In Alcohol Use Disorder and in Trauma-Exposed Individuals: Methodology and Preliminary Implications for Alcohol Use and Stress**

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**Background:** Stress is a primary mechanism underlying the maintenance of and relapse to alcohol use. Stress is also a potent activator of the hypothalamic-pituitary-adrenal (HPA) axis, initiating the release of glucocorticoid hormones. Levels of glucocorticoids (e.g., cortisol, cortisone) present in the brain are dependent on the enzyme 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), which catalyzes the conversion of cortisol to cortisone and amplifies the action of glucocorticoids in the brain. Glucocorticoids bind to either mineralocorticoid receptors (MR) or glucocorticoid receptors (GR). GRs become activated when stress hormone levels are high, such as after a stressful event. These receptors and 11β-HSD1 are located in brain regions critical in the negative feedback of glucocorticoids and in alcohol addiction, including the amygdala and prefrontal cortex (PFC). Thus, high brain glucocorticoid levels, driven by 11β-HSD1 and induced by stress, may contribute to problem alcohol use. We used positron emission tomography (PET) imaging with the 11β-HSD1 specific radioligand [18F]AS2471907 to assess 11β-HSD1 expression in participants with alcohol use disorder (AUD) and those with a history of trauma exposure.

**Methods:** To date, we have imaged 5 individuals with moderate to severe AUD (n = 3 men, n = 2 women; mean age = 40.6 years) and 12 healthy controls (n = 7 men, n = 5 women; mean age = 31.3 years). Additional imaging data has been collected for 18 trauma-exposed individuals (n = 11 men, n = 7 women), with or without posttraumatic stress disorder (PTSD; n = 1 risky drinker, n = 1 with severe AUD). Participants received $95 ± 13$ MBq [18F]AS2471907 as a bolus injection at high specific activity and were imaged for 180 minutes on the High-Resolution Research Tomograph (HRRT; 2.3 mm resolution). 11β-HSD1 availability was quantified by [18F]AS2471907 volume of distribution (VT; mL/cm³), an equilibrium ratio of [18F]AS2471907 in tissue to un-metabolized [18F]AS2471907 in arterial plasma. A priori regions of interest included amygdala, hippocampus, ventromedial PFC and caudate, as these corticolimbic regions are involved in HPA axis regulation and stress pathophysiology. Individuals were required to be overnight abstinent from drinking. For exploratory analyses, levels of 11β-HSD1 were correlated with alcohol use on the Timeline Followback and stress measures (i.e., childhood trauma, mood, anxiety, depression).

**Keywords:** Alcohol Use Disorder and in Trauma-Exposed Individuals: Methodology and Preliminary Implications for Alcohol Use and Stress

**Disclosure:** Nothing to disclose.
Results: [18F]AS2471907 exhibits suitable kinetic properties for quantification of 11β-HSD1 in the human brain and [18F] AS2471907 VT values agree with 11β-HSD1 mRNA expression from the Allen Brain Atlas in cortical brain regions. Current data are highly preliminary but suggest that 11β-HSD1 levels may be elevated in amygdala, hippocampus, ventromedial PFC, and caudate in individuals with moderate to severe AUD compared to healthy controls. Exploratory analyses in trauma-exposed individuals indicate positive associations of 11β-HSD1 levels in the caudate with drinks per week (p = 0.02; mean = 11.04, SD = 30.94) and average drinks per drinking day (p = 0.04; mean = 2.50, SD = 4.65) during the month prior to study participation. For stress-related outcomes, exploratory analyses indicate a positive association of 11β-HSD1 levels in the caudate, hippocampus, and ventromedial PFC with childhood physical abuse (p = 0.01-0.03).

Conclusions: This is the first in vivo examination of the relationship between 11β-HSD1 levels and drinking behavior and in individuals with AUD compared to healthy controls. These preliminary findings suggest a possible role for 11β-HSD1 in problematic alcohol use. Exploratory analyses also suggest a role for 11β-HSD1 in early trauma exposure. These results are consistent with work suggesting that exposure to early life stress is related to heightened stress reactivity in adulthood, and may have mechanistic implications for the high rate of trauma in individuals with AUD. Future studies will attempt to further validate [18F]AS2471907 as a marker of 11β-HSD1-mediated HPA-axis reactivity (e.g., brain cortisol regulation) in relation to alcohol use and stress exposure, including stress-related drinking. Consideration of 11β-HSD1 inhibitors as a target for problematic alcohol use or stress-related disorders may be a relevant future pharmacotherapeutic avenue.

Keywords: PET Imaging, Alcohol Use Disorder, Trauma Exposure, Cortisol, Glucocorticoid

Disclosure: Nothing to disclose.

T48

A Computational Psychiatry Approach to Addiction Using Translational Neuroeconomics: Species-Specific Similarities and Differences Among Humans and Mice

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Background: Individuals who desire to recover from addiction often make poor decisions and relapse despite this desire. Impairments in this conflict could arise from dysfunctions in distinct neural circuits. It is recognized that valuations involved in obtaining a reward are fundamentally different from those involved in its hedonic appreciation. These processes can be dissociated by carefully measuring complex behaviors before vs. after reward consumption, particularly within an economic framework. Improving our current understanding of addiction requires resolving how such heterogeneous valuation processes might uniquely malfunction. In order to improve clinical practice, our ability to translate findings across models of addiction depends on understanding to what extent such maladaptations, and thus unique circuit-specific dysfunctions, are or are not preserved across species.

Methods: We used a translational neuroeconomic task to dissociate separable valuation algorithms using complex behavioral analyses equally applicable to rodents and humans.

We trained 32 C57BL/6J male mice on the Restaurant Row task to spend time from a limited budget (60 min) foraging for food of four different flavors (indicated by visual cues). Reward costs (delays ranging 1-30s) were cued by the pitch of a tone that, if accepted, descended in pitch. Thus, mice were tasked to accept or reject serial offers. During each trial, mice were able to cancel accepted offers not yet earned. Separately measurable hedonic behavior was operationalized as within-trial place preference measured as time spent lingering at feeding sites after consuming rewards before advancing to the next restaurant. Well-trained mice were then exposed to 12 daily intraperitoneal injections of saline (n = 10), cocaine (n = 7), or morphine (n = 10) and tested during abstinence for two weeks after final drug exposures.

We also recruited 12 human non-treatment seeking cocaine users (mean age: 39.7 [s.e. 3.0], 22.2% female, 55.6% white) and 9 healthy matched controls (mean age: 35.9 [s.e. 2.9], 16.6% female, 58.3% white) from online advertisements. Participants were tested on the computer-based Web-Surf task designed to match the Restaurant Row task. Participants spent time from a limited budget (20 min) to earn natural rewards, i.e., entertaining video clips (4s in playback length) among four unique video genres (indicated by an icon). Rewards were earned after varying costs (delays ranging 1-30s, cued by a download bar and text). Participants were tasked to accept or reject serial offers and then immediately rate the hedonic value of consumed videos on a scale of 1-4 stars on each trial. All participants filled out drug use history questionnaires and were compensated $40.

Subjective flavor or video preferences were ranked based on total earnings in each restaurant or genre.

Results: All mice performed the Restaurant Row task by reliably treating randomly presented cued offer lengths differently in each restaurant (i.e., as a stable function of cost and subjective preferences). However, when encountering unique offers in preferred restaurants with a delay above one’s willingness to wait, cocaine-exposed mice were less likely to appropriately reject economically disadvantageous offers. Furthermore, these mice did so despite spending more time deliberating between future options. In contrast, morphine-exposed mice demonstrated distinct impairments when given the opportunity to correct past mistakes, a process we previously demonstrated was uniquely sensitive to specific alterations in strength of synaptic connectivity of the infralimbic-accumbens shell circuit in mice. With regard to hedonic post-consumption place preference behaviors, all mice lingered longer at feeding sites in more preferred restaurants. This relationship was exaggerated in cocaine-exposed mice, and while weakened across trials in cocaine-exposed mice, was intact, was down-shifted in morphine-exposed mice. Furthermore, post-consumption place preference behaviors measured as a function of time, while stable across trials in control mice, weakened across trials in cocaine-exposed mice and conversely strengthened across trials in morphine-exposed mice.

All humans were capable of performing the Web-Surf task reliably. Like cocaine-exposed but not morphine-exposed mice, human cocaine users displayed impairments in the ability to reject economically disadvantageous offers despite deliberating. Additionally, post-consumption ratings measured as a function of time weakened across trials in human cocaine users compared to stable healthy controls, similar to the cocaine but not morphine findings in mice. However, human cocaine users, unlike cocaine-exposed or morphine-exposed mice, inverted the relationship between genre preference and post-consumption hedonic valuations (video ratings) compared to controls. That is, after consuming videos of more preferred genres, human cocaine users rated these videos with fewer stars compared to consumed videos of less preferred genres.

Conclusions: These data elucidate neuroeconomic facets of addiction both shared and different between humans and non-human animals. Our translational approach can help shed light on conserved pathophysiological mechanisms underlying...
impairments in dissociable aspects of decision making, informing both models of and models for addiction. In doing so, we can better characterize circuit-computation-specific processes in order to identify novel diagnostic parameters and targets for intervention.

**Keywords:** Translational Neuroscience, Neuroeconomics, Computational Psychiatry, Addiction, Cross Species

**Disclosure:** Nothing to disclose.

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**T49**

**Alcohol Consumption Interacts With the Glucagon-Like Peptide-1 (GLP-1) System: A Novel Therapeutic Target for Alcohol Use Disorder?**

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**Background:** Physiological processes that regulate food intake and metabolism considerably overlap with those involved in the neurobiology of addiction. This notion is particularly relevant for alcohol because not only is alcohol a drug with pharmacological actions in the central nervous system (CNS) but it also has palatable properties and is a direct source of calorie. Increasing evidence suggests that the glucagon-like peptide-1 (GLP-1) system modulates biobehavioral mechanisms underlying acquisition, maintenance, and progression of addictive behaviors. In rodents, administration of GLP-1 or a GLP-1 receptor analog reduces alcohol-induced accumbal dopamine release, conditioned place preference for alcohol, and operant self-administration of alcohol. In humans, GLP-1 receptor has begun to be investigated as a potential therapeutic target for alcohol use disorder. Therefore, it is crucial understand whether and how excessive alcohol drinking may affect different components of the endogenous GLP-1 system.

**Methods:** First, we conducted a series of analyses on human laboratory experiments to examine the effect of alcohol administration on peripheral blood GLP-1 concentrations in heavy-drinking individuals. Specifically, four separate alcohol administration sessions were conducted: oral self-administration (variable dose), oral fixed dose, intravenous self-administration (variable dose), and intravenous fixed dose. Repeated blood samples were obtained during each session and GLP-1 concentrations were measured via a bead-based multiplex enzyme-linked immunosorbent assay (ELISA). Next, we looked at the GLP-1 receptor gene expression in postmortem brain tissue from patients with alcohol use disorder and healthy controls (New South Wales Tissue Resource Centre, University of Sydney). GLP-1 receptor mRNA was extracted from five brain regions (i.e., prefrontal cortex, ventral tegmental area, nucleus accumbens, amygdala, and hippocampus), and real-time quantitative polymerase chain reaction (PCR) with TaqMan gene expression assay was run.

**Results:** Analysis of peripheral GLP-1 concentrations showed that, in all four human laboratory experiments, alcohol administration consistently resulted in significant reduction of blood GLP-1 levels ($p < 0.001$). Analysis of postmortem brain tissue data showed that fold change in GLP-1 receptor mRNA in the hippocampus was significantly higher in patients with alcohol use disorder compared to healthy controls ($p = 0.01$). Finally, GLP-1 receptor gene expression levels were negatively correlated with age of onset of alcohol drinking ($p = 0.05$) and positively correlated with pack years of cigarette smoking ($p = 0.04$).

**Conclusions:** These data indicate that exposure to alcohol influences the GLP-1 system, both in the periphery and in the brain. Specifically, our results suggest that alcohol intake reduces peripheral GLP-1 levels and increases central expression of the GLP-1 receptor. Future studies should investigate whether targeting the GLP-1 system may represent a novel and effective pharmacological approach to treat alcohol use disorder.

**Keywords:** Addiction, Alcohol, GLP-1, GLP-1 Receptor

**Disclosure:** Nothing to disclose.

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**T50**

**Insular Cortex Dopamine D3 Receptor Involvement in Oxycodone Seeking and Analgesia**

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**Background:** Opioid abuse continues to be a major public health crisis with massive personal and economic costs. Prescription opioids, including oxycodone, are critical in pain management but are often misused or diverted. Identifying non-opioid-based pharmacotherapies for the treatment of opioid abuse that do not interfere with analgesia is vital. We recently discovered that highly selective dopamine D3 receptor (D3R) antagonists decrease oxycodone self-administration and augment analgesia in rats. However, the neural substrates mediating these seemingly paradoxical effects of D3R antagonism are unknown. Both drug abuse and pain share overlapping structural and functional changes in the insular cortex (IC), which receives dopaminergic projections from the ventral tegmental area and expresses a high density of µ opioid receptors. Here we sought to determine whether D3R in the IC mediates oxycodone seeking and analgesia. We employed molecular and pharmacological approaches: first characterizing expression of D3R on glutamate and GABA neurons throughout the IC, then examining the impact of local D3R blockade in anterior IC on oxycodone self-administration, reinstatement, and hot plate analgesia.

**Methods:** First, RNAseq in situ hybridization was used to characterize D3R expression on IC glutamate and GABA neurons. Fresh frozen coronal sections were obtained from drug-naïve mouse brains (n = 3) and were labeled using probes for VGluT1 (SLC17A7, cortical glutamate marker), GAD1 (GABA marker), and D3R (DRD3) messenger RNA, according to manufacturer-provided protocols. Fluorescent images were captured at 20x magnification using a Keyence BX-X800 microscope and quantified using Keyence software. Then, adult male Long-Evans rats (n = 24) were implanted with intravenous jugular catheters and bilateral cannula in the anterior IC (AP +2.8, ML ±4.5, DV -5.0). Rats were allowed to self-administer oxycodone under an FR2 schedule during 3-hour sessions (12.5, 25, and 50 µg/kg/infusion), followed by a progressive ratio (PR) reinforcement schedule (12.5 or 50 µg/kg/infusion). After stable responding was achieved under each schedule and oxycodone dose, rats received anterior IC microinjections of vehicle or SB-277,011A (D3R antagonist, 1 or 3 µg/side, counterbalanced) 5 min prior to the self-administration session. All rats then underwent response extinction training until active lever responding was <15% of the self-administration (FR2) baseline, followed by cue-induced or oxycodone-primed (1 mg/kg, i.p.) reinstatement in the presence of vehicle or SB-277,011A (3 µg/side) IC microinjections. Nociception (latency to paw withdrawal) was measured one week later using the hot plate test following combinations of either vehicle or SB-277,011A anterior IC.
microinjections (3 µg/side) and systemic oxycodone pre-treatment (1 mg/kg, i.p.).

Results: RNAseq revealed D3R mRNA colocalization with both VGluT1 and GAD1 throughout the anterior and posterior IC. D3R more strongly colocalized with VGluT1 than with GAD1 across the IC (P < 0.001). There were no significant differences in D3R expression between the agranular, dysgranular, and granular layers, except that less D3R labeling and more D3R + VGluT1 colocalization was observed in the granular compared to agranular layers (P < 0.05). SB-277,011A microinjections into anterior IC did not alter oxycodone seeking or intake under the FR2 schedule compared to vehicle. However, SB-277,011A significantly reduced PR breakpoints and active lever responding for 50 µg/kg/infusion oxycodone (P < 0.05), but not for 12.5 µg/kg/infusion oxycodone. During reinstatement, SB-277,011A had no significant effect on cue-induced oxycodone-seeking, but blocked oxycodone-primed reinstatement compared to vehicle (P < 0.05). Microinjection of SB-277,011A into the IC modestly increased nociceptive response latencies on the hot plate test, but was not statistically different from vehicle treatment.

Conclusions: The D3R is highly expressed on IC glutamatergic neurons as well as on a subset of IC GABA neurons. Blockade of D3R in the anterior IC reduces the motivation to earn a high, but not a low dose of oxycodone, and attenuates the ability of oxycodone to induce oxycodone seeking during reinstatement testing. However, D3R blockade in the anterior IC does not appear to affect oxycodone intake under “easy” reinforcement (FR2) or low reward (PR responding for a low oxycodone dose) conditions, and marginally improves oxycodone analgesia. Taken together, these results suggest that the anterior IC partially mediates the effects of D3R antagonism in reducing opioid reward and enhancing analgesia, but additional neural substrates likely also play a role. Ongoing studies are investigating the role of VTA→IC dopaminergic projections, as well as IC glutamate outputs, in mediating opioid abuse and pain.

Keywords: Opioid Abuse, Dopamine (D2, D3) Receptors, Insular Cortex

Disclosure: Nothing to disclose.

T52
Adolescent Vulnerability to Methamphetamine Use: Rodent and Human Findings

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Background: Methamphetamine (meth) is an urgent problem with its usage ranking 2nd most prevalent of all the illicit substances in the world. Adolescents and young adults form the dominant users. We have established a powerful rat model of adolescent vulnerability to meth use. This model shows that meth self-administration during adolescence leads to deficits in inhibition of a meth-associated cue that causes a greater cue induced relapse of meth-seeking relative to adults. It also shows that meth self-administration in adolescence leads to an escalated meth intake when the dose is increased. These data suggest that adolescent meth use alters subsequent addiction behaviors and related brain regions more dramatically compared to adult meth use.

Methods: Genome-wide transcriptome analysis (RNAseq) of the dorsal striatum in adolescent and adult rats following comparable acquisition of self-administration. In healthy controls and people with current meth use disorder, cognitive tests and cue extinction followed by genotyping.

Results: RNAseq revealed that compared to saline, meth self-administration in adolescence (but not in adulthood) reduces SLC18A1 expression in the dorsal striatum. SLC18A1 codes for vesicular monoamine transporter 1 (VMAT1) protein that is critical for cytosolic monoamine uptake and storage. In humans, adolescent-onset of meth use leads to deficits in inhibitory control and cue extinction compared to adult-onset of meth use. Single-nucleotide polymorphisms in SLC18A1 significantly differ between adolescent- vs adult-onset of meth use in people with meth use disorder.

Conclusions: Our central hypothesis is that SLC18A1 mediates vulnerability to meth addiction, with an early age of onset of meth use potentiating such vulnerability. We are currently investigating the role of SLC18A1 in human adolescence.

Disclosure: Nothing to disclose.
Cannabinoid Exposure in Adolescence Dysregulates Genes That Orchestrate Dopamine Development and Alters Cocaine-Motivated Behavior

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Background: Cannabis is the most commonly abused illicit drug among adolescents, and excessive use in this population is associated with the development of psychiatric conditions, including drug addiction. Adolescence is a critical period for the refinement and organization of neuronal connectivity, especially within the mesocorticolimbic dopamine circuitry. In particular, dysregulation of the guidance cue receptor, DCC, in ventral tegmental area (VTA) dopamine neurons disrupts targeting of dopamine axons to the nucleus accumbens (NAC), inducing their ectopic growth to the medial prefrontal cortex (mPFC). We have previously demonstrated that exposure to a cannabinoid-1/2 receptor agonist in adolescence alters in drug-motivated behaviors and in dopamine function in adulthood.

Methods: Male and female mice (n = 10/group) were treated daily with WIN-55,212-2 during early adolescence (PND 21-32) and sacrificed on PND 38 to assess Dcc mRNA and Netrin-1 protein expression. Additional mice (n = 10/group) were allowed to grow into adulthood and trained to self-administer cocaine during simultaneous sub-second dopamine recordings in the NAc and the mPFC.

Results: Preliminary findings demonstrate that adolescent exposure to WIN downregulates Dcc mRNA expression in the VTA and its ligand, Netrin-1, in the NAC and mPFC, suggesting disruption of pre- and postsynaptic components of mesocorticolimbic dopamine circuitry. Additionally, WIN-treated mice display aberrant responding for cocaine as well as potentiated cocaine-mediated anxiety. Ongoing experiments will elucidate functional changes in cocaine-evoked phasic dopamine release in the NAc and mPFC.

Conclusions: Overall, these findings support that repeated exposure to a cannabinoid-1/2 receptor agonist in adolescence impacts mesocorticolimbic dopamine system maturation and may have important implications for dopamine-mediated learning and psychostimulant-motivated behavior later in life.

Keywords: Cannabinoid, Dopamine, Adolescence, Cocaine

Disclosure: Nothing to disclose.

T54

Nucleus Accumbens Neuronal Ensembles in Cue-Induced Reward Seeking

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Background: Unmanaged reward seeking is a shared central feature of eating and substance use disorders. Recent research shows that rewarding drug-related experiences induce synchronized activation of a discrete number of neurons in the nucleus accumbens (NAcc) that are causally linked to reward-related contexts. These results suggest a finely tuned specificity of ensembles. Here we characterized the neuronal ensemble that is built through drug experience and codes for drug seeking. We additionally address the question of whether or not addictive drugs usurp circuitry used by natural rewards or involve distinct circuitry mechanisms by evaluating the segregation between cocaine- and sucrose-related ensembles within the same animal.

Methods: We used targeted recombination in active populations (TRAP) strategy, specifically FosCreERT2/+ /Ai14 (cFos-TRAP) transgenic mice to tag cells as potentially encoding these behaviors. To define and compare different reward-specific ensembles within the same animal, we developed a dual cocaine and sucrose self-administration (SA) paradigm in mice, where each reward is associated to a different discrete cue.

Results: Using this paradigm, we were able to assess the neurons included in the cocaine or sucrose ensembles, and to quantify the overlap between the two populations within the same animal exposed to both types of reward. Moreover, we used RNAscope to determine the cell types included in the seeking ensembles. We tagged with tdTomato the small number of neurons in the NAc core activated during repeated cued-induced seeking to cocaine or sucrose. The tdTomato cells were specifically activated during seeking, and not during extinction behavior or after animals remained in the home cage. Moreover, we validated the dual cocaine and sucrose SA paradigm, and were able to compare the overlap of reward-specific seeking ensembles in animals previously exposed to both rewards.

Conclusions: The data obtained here sheds new light on the complexes in the NAcc sustaining maladaptive drug-oriented seeking behaviors and how it compares to natural rewards responses.

Keywords: Addiction, Reward Network, Reward Self-Administration

Disclosure: Nothing to disclose.

T55

Distinct Fos-Expressing Neuronal Ensembles in Rat Ventromedial Prefrontal Cortex Mediate Self-Administration of Food and Cocaine

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Background: Cocaine and palatable food are strong motivators. Neuronal ensembles in the vmPFC play a role in both cocaine-
seeking and food-seeking. It is unknown whether the neuronal ensembles mediating food- and cocaine-seeking overlap.

**Methods:** We used the Daun02 inactivation procedure to test the specificity of neuronal ensembles in mediating either food-seeking or cocaine-seeking. We trained male and female Fos-LacZ rats to self-administer palatable food pellets and cocaine on alternating days for 18 days. We then exposed the rats to a brief test of either food- or cocaine-seeking to induce Fos and β-gal associated with recall of food- or cocaine-seeking. We then infused Daun02 into the vmPFC to permanently inactivate either the food- or cocaine-seeking ensemble. Two days later we tested each rats’ recall of food- or cocaine-seeking.

**Results:** We found that inactivation of the food-seeking ensemble reduced food-seeking, but not cocaine-seeking. Conversely, we found that inactivation of the cocaine-seeking ensemble reduced cocaine-seeking, but not food-seeking.

**Conclusions:** Taken together, these results suggest that neuronal ensembles mediating food- and cocaine-seeking are highly specific to their associated behavior. This may enable us to design strategies to target specific learned associations, without influencing unrelated associations.

**Keywords:** c-Fos-Expressing Ensembles, Cocaine Seeking, Palatable Food, vmPFC

**Disclosure:** Nothing to disclose.

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**T56**

**Paraventricular Thalamus Provides a Synaptic Brake on Limbic CRF Neurons to Blunt Risky Alcohol Drinking**

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**Background:** Alcohol use disorder (AUD) and other neuropsychiatric diseases including as anxiety and mood disorders are debilitating diseases that are highly coexpressed, with twice the risk for this comorbidity in women compared to men. Binge alcohol drinking is a primary risk factor for these diseases, and neurons in the bed nucleus of the stria terminals (BNST) that synthesize and release the stress neuropeptide corticotropin-releasing factor (CRF) modulate drive the expression of this risky behavior, which is also expressed at higher levels in females. We hypothesize that BNSTCRF neurons are more excitable in females than males, possibly due to increased excitatory synaptic input.

**Methods:** Here, we used a combination of in vivo and ex vivo approaches, including chemogenetics during behavior (binge alcohol and sucrose drinking, anxiety behavior), slice electrophysiology combined with optogenetics, and neural circuit tracing to characterize sex differences in the excitability and synaptic transmission of BNSTCRF neurons, identify robust excitatory synaptic inputs to these neurons, and determine the role of these excitatory circuits in binge drinking behavior.

**Results:** We showed that female mice binge drink more than males and have greater BNSTCRF neuron excitability at the population level. We identified a glutamatergic synaptic input from the paraventricular thalamus (PVT) that is denser than previously described glutamate projections in both sexes and releases glutamate directly onto BNSTCRF neurons; however, the PVT-BNST projection also engaged a large local interneuron population to ultimately provide a net inhibition of BNSTCRF neurons; these synaptic effects were more robust in females than males. Further, chemogenetic manipulation of the PVT-BNST pathway showed that the activity of this pathway suppresses binge drinking, in contrast to manipulation of the entire PVT glutamate neuron population that played an opposing role. Lastly, we showed that repeated binge drinking produced a female-like phenotype in the male PVT-BNSTCRF excitatory synapse and led to a loss of net inhibition in females, without altering the function of PVTBNST neurons per se.

**Conclusions:** Our data describe a unique behavioral role of the feedforward inhibitory PVT-BNSTCRF glutamatergic circuit that is more robust in females and undergoes alcohol-induced sex-dependent plasticity. These findings implicate the PVT-BNSTCRF synapse as a critical node of a circuit mechanism regulating binge drinking behavior and underlying plasticity associated with the development of alcohol dependence and co-expressed phenotypes.

**Keywords:** Alcohol Use Disorder, Sex Differences, Corticotropin-Releasing Factor, Paraventricular Nucleus of the Thalamus, Bed Nucleus of the Stria Terminalis

**Disclosure:** Nothing to disclose.

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**T57**

**Nuclear HDAC5 in the Rat Nucleus Accumbens Suppresses Cued and Drug-Primed Reinstatement of Heroin Seeking in a Cell Type-Specific Manner**

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**Background:** Overexpression of a dephosphorylated, nuclear-localized form of histone deacetylase 5 (HDAC5) in the adult rodent nucleus accumbens (NAC) limits multiple addiction-related behaviors, including tone/light cued and drug-primed reinstatement of drug seeking in animals with a history of cocaine self-administration (SA). However, there remain a number of important unanswered questions, including: Does nuclear HDAC5 limit seeking of distinct classes of abused drugs like heroin and/or non-drug rewards (sucrose)? At what stage of drug SA is nuclear HDAC5 exerting its effects? In which medium spiny neuron (MSN) cell type(s) does nuclear HDAC5 function to suppress drug seeking? Finally, what are the cellular and molecular mechanisms behind HDAC5’s ability to limit reinstatement behavior?

**Methods:** To address these gaps in knowledge, we first infused an adeno-associated virus that expressed a dephospho-mutant HDAC5 (AAV2-HDAC5-3SA) in the NAC. Three weeks later, rats self-administered heroin for 2-3 weeks followed by abstinence and extinction training. We then measured heroin seeking in response to light/tone cues and heroin-prime. Next, we evaluated sucrose taking and seeking using a similar study design. We next altered our study design to allowed rats to self-administer heroin for 3 weeks before undergoing NAC nuclear HDAC5 overexpression. We then examined abstinence, extinction, and reinstatement behavior. To evaluate potential cell type-specific roles for nuclear HDAC5 in heroin SA, we used a cre-dependent virus (AAV2-DIO-HDAC5-3SA) in the NAc of dopamine D1 receptor (D1R)-cre or dopamine D2 receptor (D2R)-cre transgenic rats (NIDA Transgenic Rat Project) and performed the same heroin SA procedures as above. Finally, NAc D1 and D2-MSNs were examined for changes in nuclear versus cytoplasmic HDAC5 shuttling, AMPA/NMDA currents, intrinsic excitability, and paired pulse ratio following overexpression of nuclear HDAC5. Animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.
Results: Similar to cocaine, HDACS-3SA in the NAc did not alter active drug taking, but it reduced light/tone cue and drug-primed heroin seeking after abstinence and extinction training. Sucrose taking or seeking was unaltered. Nuclear HDAC5 had no effect if expressed during the abstinence phase following active heroin SA. Interestingly, HDACS-3SA overexpressed selectively in NAC D1R-MSNs reduced cued, but not heroin-primed, reinstatement of heroin seeking, while HDACS-3SA in D2R-MSNs selectively reduced heroin-primed, but not tone/light cued, seeking. Finally, HDACS-3SA altered nuclear vs cytoplasmic localization and electrophysiological properties in D1 and D2-MSNs.

Conclusions: Our data demonstrate that enhanced nuclear HDAC5 limits relapse-like heroin and cocaine seeking, suggesting an influence of a relapse subcircuit that is shared by both opioids and stimulants, but without altering natural reward seeking. Our data also demonstrate that nuclear HDAC5 functions to block future heroin seeking by its actions during active drug taking. As such, HDAC5 might disrupt or destabilize the formation long-lasting memories that link external and internal cues with drug reward experiences. As an epigenetic factor, HDAC5 likely alters the chromatin landscape and dysregulates the expression of genes critical for typical neuronal plasticity necessary for drug-cue memory formation. Finally, our data also demonstrate that nuclear HDAC5 suppresses cued heroin seeking via its function in D1R-MSNs, whereas nuclear HDAC5 suppresses drug-primed seeking through D2R-MSNs, suggesting that HDAC5’s ability to suppress external and internal cue reactivity occurs through actions in distinct cell populations in the nucleus accumbens.

Keywords: Epigenetics, Heroin, Nucleus Accumbens, Histone Deacetylase, D1/D2

Disclosure: Nothing to disclose.

TS8

Endocannabinoid Regulation of Stress-Induced Escalation of Cocaine Intake in Rats

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Background: Clinical evidence has identified stress as an important contributing factor to substance use disorder (SUD). This is particularly problematic as stress is unavoidable in daily life. Therefore, understanding the neurobiological mechanisms that underlie the contribution of stress to SUD is critical. One characteristic of SUD is a loss of control over drug intake that is modeled, in the rat, by conditions that result in escalating patterns of drug self-administration (SA). Repeated daily stress during the 5 min drug-free period (n = 9-12/group), and to an injection of the alpha-2 adrenergic receptor antagonist yohimbine (0, 1.25, 2.5 mg/kg, i.p; n = 14-17/group, p < 0.05). Stress-induced escalation of cocaine intake is regulated by the recruitment of endocannabinoid signaling as systemic administration of AM251 prior to SA attenuates cocaine intake only in stress-escalated rats (n = 7-8/group, p < 0.05). We have localized this effect to the NAc shell and VTA as direct administration of AM251 into either region prior to SA attenuates cocaine intake in stress-escalated rats (n = 6-8/group, p < 0.05). Interestingly, cocaine SA increases CB1R binding in the VTA with or without a history of stress (n = 11-14/group, p < 0.05) but has no effect on CB1R binding in the NAc shell (n = 11-14/group). Furthermore, the recruitment of eCB signaling to influence drug-related behavior is long-lasting as systemic administration of AM251 prior to reinstatement attenuates cocaine-primed reinstatement only in rats with a prior history of stress at the time of SA (n = 8-9/group, p < 0.05).

Conclusions: Chronic stress induces a glucocorticoid-dependent escalation of cocaine intake that is the result of persistent neuroadaptations. These neuroadaptations likely result in long-lasting changes in the endocannabinoid system as repeated stress recruits endocannabinoid signaling in the NAc shell and VTA to drive drug use. The recruitment of endocannabinoid signaling in the VTA likely involves, in part, increased CB1R expression and binding following cocaine experience. However, neuroadaptations in the endocannabinoid system that are selective for the stress and cocaine interaction are still unknown though they may involve regulation of endocannabinoid mobilization. Additionally, these stress-induced neuroadaptations are long-lasting and likely also occur in regions critical for drug-seeking behavior. Understanding the unique mechanisms by which stress can drive drug use has implications for identifying and treating sub-populations of patients with SUD in whom stress is a contributing factor.

Keywords: Acute and Chronic Stress, Drug Abuse, Endocannabinoids, Ventral Terminal Area (VTA), Nucleus Accumbens Shell

Disclosure: Nothing to disclose.


ACNP 58th Annual Meeting: Poster Session II
Serotonin and Norepinephrine Signaling in Dorsal CA1 Drive Sex Differences in Persistent Cocaine-Seeking

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Background: Numerous reports indicate that women advance from casual drug use to dependence more rapidly, struggle more when trying to quit, and maintain shorter periods of abstinence that are aggravated by stress, compared to men. In particular, the initiation of abstinence from a chronically self-administered drug is a stressful event during which time drug cravings may predict difficulty in long-term abstinence success. Female rats show greater cocaine-seeking behavior on the first day of the absence of expected drug (extinction day 1, ED1) compared to males. Our prior reports show that ED1 cocaine-seeking is reduced by blocking serotonin (5-HT) signaling in dorsal hippocampus (dHPC) in both male and female rats. In addition, inhibition of β-adrenergic signaling in dHPC is effective in reducing ED1 cocaine-seeking in females only. We hypothesized that the experience of ED1 can substantially influence later relapse behavior, and that dorsal raphe serotonin (DR 5-HT) and/or locus coeruleus norepinephrine (LC-NE) projections to dHPC may be involved in a sex-dependent manner.

Methods: Adult male and female Sprague-Dawley rats were trained to self-administer cocaine on an FR-1 schedule for 10 days. On the 11th day, rats were placed in the self-administration chamber and tested for lever pressing behavior in the absence of cocaine-cues or rewards (ED1). Rats returned to their homecage for 14 days, and on day 15 of withdrawal were retested for lever pressing behavior in absence of cocaine-cues or rewards to indicate cocaine-seeking persistence. Serotonin. We inhibited 5-HT1A/1B receptors (via WAY100,635 plus GR127935), or DR input (via DREADDs), in dHPC during the ED1 session and tested the effects on cocaine-seeking persistence 2wk later (n/group = 6-10). In other rats, we inhibited 5-HT1A or 5-HT1B receptors in dHPC during conditioned place preference (CPP) for cocaine, to examine mechanisms involved in persistent effects of ED1 manipulations (n/group = 8). Norepinephrine. We inhibited β1 and β2 receptors (via betaxolol plus ICI-118 551), or LC input (PR5x8 promoter driven DREADD), in dHPC during the ED1 session and tested the effects on cocaine-seeking persistence 2wk later (n/group = 6-8). hM3Dq and hM4Di DREADDs were microinfused bilaterally into LC to selectively excite or inhibit LC-NE neurons that project to dHPC, respectively.

Results: Serotonin. Inhibition of DR, or of 5-HT1A/1B signaling in dHPC, during ED1 testing significantly decreased drug-seeking on ED1 as expected, and persistently decreased cocaine-seeking 2wk later, confirming that 5-HT signaling in dHPC is involved in persistent drug-seeking after abstinence. The protein synthesis inhibitor anisomycin, given immediately after ED1 testing, blocked the effects of 5-HT inhibition on cocaine-seeking persistence, indicating that these effects are protein synthesis dependent. Administration of a 5-HT1B antagonist alone during the ED1 session transiently decreased drug-associated memory performance in CPP, whereas administration of a 5-HT1A antagonist had no effect on memory but blocked CPP on a test 24 h later. Norepinephrine. Inhibition of β1 and β2 receptors in dHPC during the ED1 session significantly decreased ED1 cocaine-seeking persistently in females only. DREADD-mediated inhibition of LC-NE to dHPC signaling decreased cocaine-seeking behavior on ED1 and ED2 in females only, while exciting this pathway significantly increased cocaine-seeking behavior on ED1 in males.

Conclusions: Our data demonstrate that inhibition and excitation of LC-NE signaling in dHPC has sex-specific effects on cocaine-seeking behavior and persistence of seeking, while inhibition of 5-HT1A/1B receptors has sex-shared effects on cocaine-seeking behavior and persistence. DR inputs to dHPC during ED1 augments recall of the drug-associated context, and facilitates drug seeking via 5-HT1B receptors. Memory consolidation of the updated non-drug context is facilitated by antagonism of 5-HT1A receptors and is protein synthesis dependent. In females only, NE signaling also contributes to the magnitude of cocaine-seeking behavior and persistence. Excitation of LC to dHPC signaling in males on ED1 induces seeking behavior similar to that of females, indicating that LC-NE signaling to dHPC may drive the observed sex difference in seeking behavior. The implications of our results further support the necessity for sex-specific and sex-shared treatments for drug-seeking in humans, potentially through manipulation of the LC-NE to dHPC pathway and through 5-HT1A/1B receptors in the dHPC. Supported by PHS awards R01-006214 and DA016511 to GAJ and T32ES007148 and K99DA045758 to ASK.

Keywords: Cocaine Addiction, Sex Differences, Serotonin, Norepinephrine, Dorsal Hippocampus

Disclosure: Nothing to disclose.
Naltrexone was titrated to 50 mg during the week before the quit date and then maintained at this dose for 12 weeks. For the first 4 weeks after the quit date, participants also received nicotine patch (21 mg x 2 weeks, 14 mg x 1 week, 7 mg x 1 week) to mitigate tobacco withdrawal as well as cognitive-behavioral smoking cessation counseling. During the week prior to the designated quit date, n = 254 participants provided a saliva sample which was assayed by liquid chromatography-mass spectrometry at the University of Toronto labs for cotinine and 3HC. The main outcome measures for this study were smoking abstinence at 4 and 12 weeks, weight gain at 4 and 12 weeks, and number of heavy drinking days (4 or more drinks for women or 5 or more drinks for men) at 12 weeks.

**Results:** Independent of medication group, NMR failed to predict abstinence rates at week 4 or 12 (main effect of NMR, ps > 0.27), weight gain at week 4 or 12 (ps > 0.36), and heavy drinking days at week 12 (p = 0.98). At week 4 of the trial, participants in the naltrexone group with a higher NMR (i.e., faster nicotine metabolism) were significantly less likely than those with a lower NMR to achieve abstinence (medication group x NMR, b = −3.279, p < 0.05; OR = 0.038, 95%CI: 0.002–0.870); NMR did not affect abstinence rates in the placebo group at this same time point. However, NMR did not moderate the effects of medication on smoking abstinence at week 12 (medication group x NMR, p = 0.33), and NMR was not associated with the effects of medication on weight gain at weeks 4 and 12 (ps > 0.44) or number of heavy drinking days at week 12 (p = 0.22). In an exploratory survival analysis, time to relapse (i.e., days to first cigarette) was independently assessed in the entire sample within two separate time frames: weeks 1–4, during which time participants received both pill and nicotine patch, and then weeks 5–12, during which participants only received pill. Within each time frame, NMR was not predictive of relapse rates on its own (main effect of NMR, ps > 0.56) nor was it related to response to medication (medication group x NMR, ps > 0.59).

**Conclusions:** Despite some evidence of NMR as an early predictor of smoking cessation in the presence of naltrexone plus NRT, the results of this study as a whole did not support a role of NMR as a predictor of ability to quit smoking on its own or as a moderator of the treatment effects of naltrexone in smoking cessation. In contrast to the observed results, most, but not all, prior studies have found that NMR moderated response to approved, effective medications (i.e., NRT, bupropion, and varenicline). While naltrexone is not approved as a treatment for smoking cessation, in this study, it did produce favorable outcomes in terms of increasing abstinence rates and reducing cigarette consumption. Despite this potential study limitation, the results from this study suggest more research is needed into the role of nicotine metabolism in smoking cessation.

**Keywords:** Smoking Cessation, Nicotine Metabolism, Naltrexone

**Disclosure:** Nothing to disclose.

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**T61**

**Exploring the Thalamocortical Neural Engram in Ethanol Drinking and Dependence**

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**Background:** Despite knowing the negative legal, health, and social consequences, individuals with an alcohol use disorder (AUD) continue to consume excessive amounts of alcohol. Adaptations in corticostriatal networks produced by excessive alcohol drinking are thought to enhance incentive salience of alcohol-associated cue and diminish engagement in behaviors related to alternative non-alcohol rewards. However, there is a substantial gap in our understanding of the mechanisms and neurocircuitry underlying this cortically driven inflexible behavior. One input that has remained largely unexplored in the context of alcohol-induced loss of flexible decision-making is the mediodorsal thalamus (MD). The MD is a higher-order thalamic nucleus that serves as a hub for integration of cortical and subcortical signaling. The MD has reciprocal glutamatergic projections with the prefrontal cortex and is innervated by reward-processing mesolimbic structures. Through its extensive cortical projections, the MD plays a critical role in adaptive goal-directed choice behavior and higher-order cognitive flexibility, making it perfectly situated to play a central role in driving cortical deficits in AUD.

**Methods:** This study used a combination of alcohol drinking and dependence models coupled with ex vivo slice electrophysiology, immunolabeling, and confocal imaging in C57BL/6J mice to study activated thalamocortical neurons. Use of the newly developed Targeted Recombination in Active Populations (TRAP) mouse line (FosTRAP) that allows tamoxifen-inducible Cre expression of tdTomato in Fos-expressing cells in a temporally controlled manner with slice electrophysiology revealed functional adaptations in neuronal ensembles activated in response to intermittent ethanol (EtOH) drinking behavior. FosTRAP mice were administered 4-hydroxytamoxifen (25 mg/kg, IP) 2 h into a 24 h EtOH drinking session or immediately after a 2 h drinking session in an effort to capture cells activated by EtOH consumption and used for slice electrophysiology or tract-tracing studies, respectively.

**Results:** Male and female FosTRAP mice increased their EtOH consumption during the first week before reaching a baseline level of intake that averaged ~15 g/kg/day. At the completion of the study, imaging revealed tdTomato+ cells in the IL, anterior cingulate, insular, and orbitofrontal cortices, as well as the MD thalamus. Patch-clamp electrophysiological recordings demonstrated that tdTomato+ IL neurons in layer II/III, which receives dense innervation from the MD, fired more evoked action potentials than adjacent non-activated neurons. In EtOH-dependent C57BL/6J mice, the number of cFos+ neurons was increased in infralimbic (IL) cortex and MD thalamus. Electrophysiological analysis of intrinsic excitability showed that MD neurons from EtOH dependent mice fired more evoked action potentials than controls (F(1, 6) = 6.91, p < 0.05, N = 15-19 cells). The adaptation in intrinsic excitability was largely driven by increased burst firing following the rebound from hyperpolarizing steps, suggesting a dependence-induced change in T-type low-voltage activated calcium channels.

**Conclusions:** Findings from these studies using newly developed technology in mice identified stable and specific subsets of neural populations in thalamocortical circuits that are activated by alcohol drinking and dependence. The functional assessment revealed neural mechanisms and functional signatures underlying ethanol-induced plasticity that may drive heavy drinking and loss of reward-based flexible behaviors in individuals with AUD.

**Keywords:** Alcohol Dependence, c-Fos-Expressing Ensembles, Thalamo-Cortical Connectivity, Mediodorsal Thalamus, Medial Prefrontal Cortex

**Disclosure:** Nothing to disclose.
T62

A Sex-Biased Breathing Pattern Influences Functional Connectivity MRI

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Background: Functional magnetic resonance imaging (fMRI) scanning of subjects at rest has become a major imaging paradigm, termed “functional connectivity” or “resting state” MRI. In these scans, subjects lie quietly, often staring at a crosshair, for 5-15 or more minutes, performing no particular instructed task. Data are typically analyzed for temporal covariance between the fMRI signals in different parts of the brain. Correlations in task-free fMRI signals are thought to reflect the functional relatedness of the tissues producing those signals. There is hope that diagnostic and prognostic information for neuropsychiatric conditions will be found in these “resting state” signals: resting state analyses have fractionated patients with depression into endophenotypes, with certain subtypes responding preferentially to transcranial magnetic stimulation. Large neuropsychiatric studies are now underway that use resting state fMRI scans as cornerstones of the datasets, for example the ABCD study scanning 10,000 children to find markers of developmental trajectories or the NIH Human Connectome Project and its progeny such as the Early Psychosis Project.

A potent modulator of cerebral blood flow, and thus the signal detected by fMRI, is the concentration of carbon dioxide in the blood (measured as the partial pressure [pCO2]). When subjects breathe deeply or quickly (i.e., hyperpnea), they exhale more CO2, pCO2 drops, and cerebral blood flow decreases; conversely, if breathing is shallow or slow (or stopped) (i.e., hypopnea or apnea), less CO2 is released, pCO2 rises in the bloodstream, and cerebral blood flow increases. The effect size can be large: both brief breath holds and the natural alternation between hyperpnea and hypopnea in periodic breathing can cause fluctuations in cerebral blood flow on the order of 50%, changes that should be sufficient to produce large, multiple-percent changes in fMRI signal. Little is known about the breathing characteristics of “healthy” young adults lying at rest in an MRI scanner, the kind of subject that forms the backbone of the functional connectivity literature. To address this gap in knowledge, we recently began to examine in detail the characteristics of respiration in a large, publicly available fMRI dataset.

Methods: The “900-subject” release of the Human Connectome Project (HCP) Young Adult data was obtained. Subjects were “healthy” in a broad sense: study criteria excluded subjects with diabetes, hypertension, or neurological or psychiatric disorders. Each subject underwent four 14.4 minute fMRI scans while staring quietly at a crosshair.

For all subjects, for each of the resting state fMRI runs, physiology data was obtained and visually examined, since traces are often partially or fully corrupted. 440 of 900 subjects had four 14.4-minute resting state fMRI scans while staring quietly at a crosshair. For all subjects, for each of the resting state fMRI runs, physiology data was obtained and visually examined, since traces are often partially or fully corrupted. 440 of 900 subjects had four 14.4-minute resting state fMRI scans while staring quietly at a crosshair. For each of their 1,780 scans, fMRI signals and paired physiological records were visually examined as “gray plots”, in which respiratory traces were plotted along with gray-scale heatmaps of all in-brain signals. Patterns observed by the lead author were independently re-identified by additional blinded authors.

Results: Beyond normal breathing, we came to recognize the existence of two common patterns of respiration (Fig. 1). One pattern was known to us, which we term “single deep breaths”. This pattern occurs in most subjects during the scanning period. The other pattern was not known to us and has no proper name in healthy, awake subjects. We term it a “burst”. It occurs in a large fraction of subjects, and is typically a set of serial taperings of respiratory depth, with each taper lasting dozens of seconds, and sets of tapers spanning minutes at a time. These two patterns have distinct time courses in fMRI signals, distinct influences on functional connectivity, distinct cardiovascular correlates, and distinct propensities to manifest in each sex such that deep breaths have no sex bias but males exhibit bursts substantially more than females (Figs. 2, 3). Because bursts are more common in males, and because bursts cause shared respiratory modulations of fMRI signals, one would expect increased mean voxelwise correlations in males. Males do have significantly higher mean functional connectivity than females (Fig. 4). Mean fMRI signal covariance scaled strongly with burst and deep breath prevalence both within and across subjects, with a stronger effect of bursts. Fits did not differ across sexes.

Conclusions: We describe two respiratory patterns commonly seen in young adults lying in MRI scanners, both with marked influence on fMRI signals and signal covariance. It is no surprise that most HCP subjects exhibit deep breaths, for all people sigh, and yawns are to be expected as subjects stare at a screen and become bored and perhaps drowsy. The occurrence of the burst pattern, and its prevalence and sex bias, was of great interest to us. Review of the medical literature suggests that the “burst” pattern shares a mechanistic basis with “periodic breathing”, which has a well-documented sex bias linked to sex hormone balance, as well as important associations with age and with neuropsychiatric health.

Keywords: Resting State Functional Connectivity, Respiration, Sex Difference

Disclosure: Nothing to disclose.

T63

Decoding Working Memory Load Using Machine Learning and Task-Based Functional Connectivity

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Background: Working memory (WM) is a key building block of human cognition and its dysfunction is associated with cognitive impairment in neuropsychiatric disorders. While precise mechanisms of WM processing are unclear, short-term maintenance of information is thought to be supported via interactions between brain networks involved in memory representations and attention processes. Functional connectivity is a powerful tool that has been used to characterize brain networks at numerous brain states. Machine learning (ML) has the unique advantage to assess covariance in connectivity patterns and detect subtle changes. In this study, we took advantage of both of these robust tools to determine the characteristic connectivity features that separate high and low WM load conditions in healthy young adults.

Methods: The study cohort included 182 healthy participants (93 F, age 24.96 ± 3.60), who underwent fMRI during working memory performance. Participants performed the Sternberg Item Recognition Paradigm (SIRP) while undergoing functional MRI. During this task, subjects memorized a set of 1, 3, 5, or 7 consonants. After a brief delay period, consecutive probes
(individual letters) were presented. At each probe, participants indicated whether the probe was a target (part of the memorized set) or foil. Functional images underwent standard and connectivity-specific preprocessing. Time courses were extracted from 611 seed regions covering the entire cortex to compute a 611x611 correlation matrix for each subject. Functional connectivity was computed during retrieval periods for each load (e.g., 1, 3, 5, or 7) and a task correlation matrix (611x611) was generated. Correlation matrices generated for each load were analyzed using a linear Support Vector Machines (SVM) algorithm (fitcsvm) in Matlab. We performed binary classification for all possible task condition pairs, which we validated using 5-fold cross validation. In order to determine which network features contributed most to low vs. high WM load classification (1T vs. 7T), we used Neighborhood Component Analysis (NCA) via fscnca in Matlab, which provided us with feature weights (as a measure of feature importance) for 1T-7T classification. We then computed classification accuracy iteratively using increasing number of "top" features. The accuracy was maximum at top 718 features. We then obtained the distribution of brain networks among these 718 features, assessing within-network and between-network features separately. Finally, we determined whether the connectivity of top features predicts behavioral performance (measured by response time) and load-dependent activation. False Discovery Rate (FDR) was used (Qval < 0.05) to correct for multiple comparisons.

**Results:** SVM successfully decoded different task conditions resulting in classification accuracies above chance level for each classification (see Table 1 below). Task conditions that substantially differed in difficulty depicted higher classification accuracy than the ones with similar levels of difficulty.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Mean accuracy</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1T-3T</td>
<td>72%</td>
<td>67%-77%</td>
</tr>
<tr>
<td>1T-5T</td>
<td>79%</td>
<td>76%-84%</td>
</tr>
<tr>
<td>1T-7T</td>
<td>87%</td>
<td>81%-91%</td>
</tr>
<tr>
<td>3T-5T</td>
<td>70%</td>
<td>69%-72%</td>
</tr>
<tr>
<td>3T-7T</td>
<td>81%</td>
<td>70%-87%</td>
</tr>
<tr>
<td>5T-7T</td>
<td>70%</td>
<td>67%-76%</td>
</tr>
</tbody>
</table>

(Abbreviations: 1T, 3T, 5T, 7T: Working memory load representing 1-, 3-, 5-, and 7-letter conditions.)

For 1T-7T classification, NCA determined feature weights for the most informative connectivity features. Distribution of top 718 connections across networks showed that, among within-network features, connections within-visual and within-ventral attention network (accounting for 40% and 32% of all within-network connections respectively) contributed most to the classification, whereas connections between visual and somatomotor as well as between visual and frontoparietal networks were the top contributors to the 1T-7T classification (accounting for 17% and 14% of all between-network connections respectively). Functional connectivity between dorsal and ventral attention networks (right superior parietal lobule and left supramarginal gyrus respectively) during high WM load was positively correlated with processing speed (punc = 0.000088). While connectivity within the frontoparietal control network at high WM load was positively correlated with load-dependent activation in left dorsolateral prefrontal cortex (punc = 0.00028), coupling between visual and somatomotor networks at high WM load predicted activation in right supplementary motor area (punc = 0.000024).

**Conclusions:** We leveraged machine learning to decode WM load from connectivity matrices, determined the most informative features separating high and low WM load, and identified the functional connectivity features that predict task performance and task-related activation. Our findings highlight the likely roles of visual-frontoparietal network connectivity in WM maintenance, visual-somatomotor connectivity in subvocal rehearsal, and frontoparietal-default connectivity in directing attention based on changes in WM load. Our results also provide an opportunity to investigate these features in neuropsychiatric disorders, in which working memory is impaired.

**Keywords:** Working Memory, Functional MRI (fMRI), Task-Based Functional Connectivity, Machine Learning, Cognitive Impairments

**Disclosure:** Nothing to disclose.

**T64**

**Neuroimaging of Transcranial Magnetic Stimulation for Suicidality**

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**Background:** Suicide is a public health emergency. Patients with depression and anxiety are at particularly high risk, especially those who do not respond to standard treatments. Repetitive transcranial magnetic stimulation (TMS) is an effective and non-invasive option for patients with treatment-resistant depression and anxiety. Moreover, the collective findings of several recent clinical trials indicate that TMS may be a promising treatment for suicidality, and that reductions in suicidality may be independent from those of other clinical symptoms. That in turn suggests that changes in specific neural mechanisms might uniquely mediate lessened suicidality. Here, we conducted a secondary analysis comparing resting-state functional magnetic resonance imaging (fMRI) before and after a course of TMS for comorbid posttraumatic stress disorder (PTSD) and depression, and evaluated whether there were specific functional connectivity changes associated with improvement in suicide-related symptoms. We also conducted an exploratory diffusion tractography analysis to identify associated anatomical pathways.

**Methods:** Neuroimaging data were collected from patients with comorbid PTSD and depression (N = 25, female = 13, age = 52 ±10) before and after an 8-week daily course of 5Hz TMS of the left dorsolateral prefrontal cortex (DLPFC). Diffusion imaging data were available in a subset of participants (n = 17). Depression and PTSD symptoms were measured at baseline and conclusion of TMS with the Inventory of Depressive Symptomatology-Self Report (IDS-SR) and PTSD Checklist for DSM-5 (PCL-5), respectively. Suicidality was derived from the IDS-SR item #18.

Our seed-to-voxel functional connectivity analysis focused on five seed regions, one at the left DLPFC stimulation site, and for each hemisphere, a dorsal and ventral striatum seed. We selected these striatal seeds because of the association of fronto-striatal functional connectivity with both suicidal severity and antidepressant response to TMS. Functional connectivity maps were entered into group models testing the between-subject effect of change in suicide scores and the between-conditions effect of session (post- vs. pre-treatment), after covariance for baseline suicidality and treatment-related changes in IDS-SR and PCL-5 scores. All results were corrected for multiple comparisons (voxel-level threshold, p < 0.001; cluster-level threshold, false discovery rate (FDR) corrected p < 0.05).

Diffusion images were reconstructed using a q-space diffeomorphic reconstruction algorithm that simultaneously computes the density of oriented diffusion spins and maps these estimates into Montreal Neurological Institute template space. We then conducted a group-level connectometry analysis in which we tested the association between functional connectivity coefficients of suicidality change and diffusion in white matter fibers tracked from significant clusters identified by our seed-to-voxel analyses. Five thousand randomized permutations were performed to estimate the false discovery rate with significance set at p-FDR < 0.05.
Results: Decreases in functional connectivity between dorsal striatum and frontopolar cortex were correlated with reduced suicide symptom severity after TMS (p-FDR < 0.001). This relationship was significant after covariance for overall change in symptoms of depression and PTSD. Connectometry analysis indicated that frontopolar cortex white matter connectivity in the left cortico-striatal pathway, left corticothalamic pathway, left prefrontal u-fibers, left inferior fronto-occipital fasciculus, and corpus callosum were associated with functional correlates of suicide symptom change after TMS (p-FDR < 0.05).

Conclusions: This is one of the first studies to investigate mechanisms underlying suicidality change after TMS. Our results suggest that reduction of fronto-striatal connectivity using TMS may be a promising treatment for suicidality. Notably, this fronto-striatal relationship was specific to suicidality and relatively independent of changes in overall depression. Additionally, our exploratory structural results provide novel targets for TMS optimization (including frontal pole). Future research can build on this multi-modal approach to advance individualized stimulation approaches in high-risk patients.

Keywords: Suicide, Transcranial Magnetic Stimulation, Neuroimaging, Depression, Suicidality

Disclosure: Nothing to disclose.

T65

Diurnal Patterns as Evidenced by Over Eleven Million Smartphone Keystrokes During Daily Usage: An IOS BiAffect Study

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Background: Our group has previously demonstrated that keyboard dynamics metadata, unobtrusively collected on Android smartphones, are associated with cognition and mood outcomes in bipolar disorder (Stange et al., 2018; Zulueta et al., 2018). Here, we report the initial results from a much larger ongoing open-science iOS study on mood and cognition using keyboard dynamics. Through an academia-industry collaborative partnership with Sage Bionetworks, our iOS BiAffect project leverages Apple’s ResearchKit framework to enroll and study eligible U.S. adults directly via their personal iPhones. The core technology behind BiAffect is a custom virtual keyboard that replaces the native iOS keyboard and thus allows for the collection of keyboard dynamics and typing kinematics metadata (i.e., not what is typed but how one types). The aims of this study were to establish how typing dynamics may change over the course of the day (i.e., diurnal patterns) and in relation to user demographics (gender and age), while controlling for the way each user interacts with their phone (e.g., one vs. two-handed typing, the number of autocorrects and backspaces [as a proxy of typing errors], and the total number of keypresses for that session).

Methods: We report analyses of our current iOS sample that have successfully undergone data quality control, cleaning and all preprocessing steps, a dataset comprising most active 365 users who collectively contributed more than 16 million keypresses and >24 hours of typing. Of those, 249 participants reported their age and gender. The active users span ages between 18 and 82 with a mean age of 37.7 (71% females 27% males and 2% non-binary).

To briefly outline our preprocessing pipeline, all inter-key delay times (i.e., the time between two consecutive keypresses) are calculated within a typing session (i.e., when the keyboard is deactivated or there is a pause between typing for more than 8 secs). Next, each inter-key delay is tagged by the specific keypress categories of the two keypresses involved (here we present only the categories that include character to character inter-key delays). To distinguish between one- vs. two-handed typing (i.e., typing mode), inter-key delay is linearly regressed to the distance between the center of the two touch events-of-interest on a per session basis. The slope of this linear regression and its corresponding p-value are used to classify one-handed (positive slope, p < 0.05) and two-handed (negative slope, p > 0.05) sessions. This method was validated via test data collected on internal testing phones. The slope and median inter-key delay for each test session were also classified via a Gaussian mixture model with a 99% accuracy.

As long inter-key delay times encode events other than word-level typing behaviors (e.g., pauses or being distracted in the middle of typing), to better infer pure typing speed we examined 11,446,443 character-to-character keypresses in ~115k sessions with inter-key delays between 0.1 and 2 seconds to capture general cognitive-motor processes in daily smartphone communication. Then, we conducted hierarchical growth-curve mixed-effects models relating typing speed to other variables of interest.

For our 2-level mixed-effects model, we set the dependent variable to be the median inter-key delay on the session-level. Random effects of the models included the user as the cluster (ICC = 0.81) and allowed each user to have their own slopes of the time of day (both linear and quadratic) and different intercepts for their typing mode. Fixed effects were tested hierarchically adding the time of day, age of the user, typing mode, gender, number of backspaces, autocorrects and characters per session. Model improvement was assessed via deviance testing.

Results: Results supported a second-order polynomial effect of diurnal patterns (first order, b = 1.10, t = 4.83, p < 0.0001; second-order, b = 2.66, t = 13.40, p < 0.0001). People typed more slowly in the middle of the night (midnight to 6am) as compared to during midday (noon to 6pm). There was a positive linear effect for age (b = 0.070, t = 14.60, p < 0.0001), such that older people typed slower. While there was no significant gender effect (F = 0.385, p = 0.819), the number of autocorrections (b = −0.008, t = −34.69, p < 0.0001) and backspaces (b = −0.0004, t = −2.20, p < 0.03) both resulted in shorter inter-key delays (i.e., faster typing) within the sessions.

There was an interaction between diurnal patterns and age, such that older people exhibited a more pronounced slowing in the typing speed at the end of the day/early hours of the morning (first order, b = 0.48, t = 2.06, p < 0.04; second order, b = 0.55, t = −2.72, p < 0.007). Furthermore, as we observed an interaction between age and the number of autocorrections (b = −0.001, t = −5.79, p < 0.0001). This is likely because faster typing leads to more typos/errors, which then trigger more backspaces/autocorrects and that might be amplified in older users.

Conclusions: Our main findings established 1) the utility of collecting keyboard dynamics in the wild to examine the association between performance and aging in the context of diurnal patterns, and 2) supports the feasibility of BiAffect in successfully recruiting participants using a crowd-sourced open-science research paradigm. Future analyses will further explore the relationship between keyboard dynamics and mood symptoms, diagnoses, as well as major domains of neurocognitive functioning as measured using BiAffect.

Keywords: Digital Phenotyping, Mobile Technology, Circadian Rhythm

Disclosure: Keywise Inc., Stock / Equity, nOCD, Advisory Board
Persistent Post-Operative Cognitive Dysfunction in Aging: Mechanisms Explored


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Background: Post-operative cognitive dysfunction (POCD) is a constellation of debilitating cognitive symptoms that disproportionately afflict older individuals immediately after surgery. These symptoms, lasting days to months, range from confusion to difficulties with executive functions, and inability to form long-term episodic memories, to dementia. Persistent symptoms are associated with greater risk of developing Alzheimer’s Disease, yet underlying mechanisms driving this risk are not known. Our previous findings showed that exploratory abdominal surgery (laparotomy) impaired hippocampal-dependent memory in aged, but not young adult rats, and that this effect was driven by the pro-inflammatory cytokine interleukin-1 beta. These impairments lasted 4 days following surgery, but did not persist beyond that time point. Here, we present a novel model of POCD made persistent in aged rats with a regimen of post-surgical morphine treatment.

Methods: Young adult (3 mos) and old (24 mos) male F344xBN rats (n = 6-10) underwent sham or laparotomy surgery under isoflurane anesthesia, and were treated with saline or morphine (2mg/kg) twice a day for 7 days after surgery. Two, four, or eight weeks later, rats were trained in a contextual fear conditioning paradigm and memory for what was learned was tested 4 days later. At the appropriate time points, rats were saline perfused and hippocampi were dissected and collected for PCR analysis of mRNA expression of various genes (e.g., IL-1 beta, IL-6, Tnf alpha, Nitrp3, Hmgb1, Tlr2, Tlr4, Synaptophysin, and Psd95). In a separate experiment, rats received a single (intra cisterna magna) injection of the IL-1 receptor antagonist, IL-1RA (1 mg/kg) immediately prior to surgery to determine the role of IL-1 in these impairments.

Results: Hippocampal-dependent memory was impaired at 2, 4, and 8 weeks post-surgery (versus just 4 days without morphine) in laparotomized aged rats that received morphine treatment. Young adult rats did not exhibit any impairments at any time point. The memory impairments were associated with elevated gene expression of various inflammatory markers and danger signals only in aged, laparotomized, morphine-treated rats. Memory deficits were completely prevented with the central administration of IL1RA, suggesting that IL-1 signaling is critical to initiating the cascade of mechanisms leading to dysfunction. Using a stereoisomer of morphine that binds the toll-like receptor 4, but not the mu opioid receptor, we found similar memory impairments in aged rats, suggesting that morphine-induced persistent POCD may be mediated via TLR4 activation. In addition, pre- and post-synaptic markers, synaptophysin and PSD95, showed aberrant expression in the aged morphine-treated hippocampus, suggestive of synaptic dysfunction. Data were analyzed using two- and three-way ANOVAs with both Statview and Prism software.

Conclusions: Together, these findings indicate that opioid treatment following surgery, in the already vulnerable aged population initiates an exaggerated inflammatory cascade that may trigger, possibly via TLR4 activation, synaptic plasticity dysfunction to cause very long-lasting memory deficits.

Keywords: Surgery, Morphine, Cognitive Decline, Ageing

Disclosure: Nothing to disclose.
defined as “synapse-enriched”. Gene ontology (GO) analysis of “synapse-revealed that “synapse-enriched” genes are predicted to have specialized neuronal functions that are synaptic in nature. We used differential expression analysis to determine which transcripts were expressed at significantly higher levels (FDR < 0.05) in synaptic samples relative to somatic samples, revealing 542 synaptic DEGs. 311 of the C. elegans synaptic DEGs have predicted mammalian orthologs. Of those mammalian orthologs, 269 have been previously validated as axonal or synaptic. GO analysis of mammalian orthologs of synaptic DEGs revealed an enrichment of translational regulators and mRNA binding proteins, including a number of orthologs of Pumilio1/2 (pufs). High-sensitivity in situ hybridization confirmed that puf mRNAs colocalize with presynaptic markers. RNAi-mediated knockdown of synaptic pufs reveals that they regulate memory formation, with puf-3/5 necessary for memory formation (p < 0.01).

Conclusions: By identifying the presynaptic transcriptome, we have demonstrated that presynaptic transcripts contribute to associative behaviors. Because many of these transcripts we identified have conserved functions in mammals, these findings set the framework for future studies for understanding the role that these presynaptic proteins play in plasticity, behavior and repair.

Keywords: Memory and Learning, Presynaptic mRNA, Transcriptomics
Disclosure: Nothing to disclose.

T68
Cumulative Life Stress Exposure, Lifetime Estrogen Exposure, and Cognition and Mental Health in Older Women

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Background: Studies have demonstrated that chronically high cortisol levels lead to deficits in cognition. Women may have a buffer against the negative effects of cortisol as estrogen has been shown to be effective at modulating the stress response. The aim of this study was to examine the effects of lifetime stress exposure and estrogen levels on cognition and mental health in older women.

Methods: Forty-four women with a mean age of 71 (SD = 6.9) from Chittenden County, VT completed stress and hormone questionnaires and performed two memory tasks. The Stress and Adversity Inventory for Adults (Slavich & Shields, 2018) was used to assess participants; lifetime exposure to acute and chronic stressors. The Older Adult Self Report (Achenbach et al., 2004) examined seven psychiatric syndromes including anxious/depressed, thought problems, worries, and memory/cognition problems. The Indices of Estrogen Exposure Questionnaire (Lord et al., 2009) examined lifetime hormone exposure. Cognition was assessed with the Buschke Selective Reminding test (SRT; Buschke & Fuld, 1974) that measured episodic memory, and the Letter Number Sequencing (Weschler, 1997) task that measured working memory.

Results: Lifetime estrogen exposure did not modulate the relationship between cumulative life stress exposure and cognition in the older women in our sample. Yet, this study showed a relationship between several indices of lifetime stress exposure and participants’ mental health. Total stressor severity in particular was strongly correlated with thought problem scores (p < 0.001) and critical psychiatric scores (borderline clinical or clinical) (p = 0.005) on the Older Adult Self Report.

Conclusions: The findings of this study suggested that estrogen did not modulate the relationship between stress and cognition in our sample of older women. However, the results showed relationships between mental health and cumulative lifetime stress exposure. Future research should examine objective measures of estrogen if possible. Although this study demonstrated specific relationships between lifetime stress exposure and mental health, these processes were not in turn associated with cognition. Therefore, additional studies are needed to investigate how lifetime stress exposure and psychiatric health affect cognition in later life.

Keywords: Acute and Chronic Stress, Estrogen, Aging
Disclosure: Nothing to disclose.

T69
mTOR Suppression Plays a Protective Role in Age-Related Cognitive Decline Through Potential Regulation of the Aging Neuroimmune System

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Background: In the face of a global surge in the aging population, research into the biological modulators of aging is pivotal to not only increase quality of life for those progressing through healthy aging but also for those afflicted by age-related disorders. Regarding brain function, normal aging is associated with alterations in structure, neuroinflammation and a concomitant decline in cognitive function. A leading target of therapeutic development for extending the lifespan is the mechanistic target of rapamycin (mTOR), which has been genetically and mechanistically linked with a number of neurodevelopmental, neuropsychiatric and age-related neurological disorders. Inhibition of mTOR via the immunosuppressant drug rapamycin prolongs longevity in multiple organisms, as well as alleviating cognitive decline in rodent models of aging. However, these pharmacological studies are hindered by multiple limitations, and the underlying mechanisms of mTOR inhibition have been left unstudied. Here we studied the developmental transcriptional regulation of mTOR expression in the prefrontal cortex (PFC) of mice across the entire postnatal lifespan, and assessed the impact of genetic haplinsufficiency of mTOR on spatial memory function as well as cortical neuroimmune status in adulthood and aging.

Methods: Using quantitative RNA profiling we assessed the trajectory of mTOR expression in the PFC of C57BL/6 male mice from postnatal day 1 to 2 years of age. Genetically engineered heterozygous male mice with a confirmed 50% reduction in mTOR expression (mTOR HET) were compared to their wildtype (WT) littermates at 3, 12, 18 and 24 months of age in a spatial memory task, and their PFC tissue simultaneously assessed for levels of multiple cytokines and chemokines using the Proteome Profiler Mouse Cytokine Array.

Results: In the typically developing postnatal murine PFC, expression of mTOR significantly increased with aging (n = 95, p < 0.001). WT and mTOR HET mice presented equal performance in the spatial memory task at 3 months of age; however, WT mice displayed a gradual decrease in task performance across aging and by 24 months of age were unable to perform the task and were significantly impaired compared to 3 month performance levels (P < 0.01). Conversely, mTOR HET mice performed the spatial memory task equally across all ages assessed, and displayed a significantly superior memory level at 24 months of age compared to WT littermates (p < 0.05) (n = 8-15 genotype/age). Finally,
mTOR HET mice show significantly less age-related cortical changes in chemokine and cytokine levels than WT littermates, including multiple analytes that have been previously associated with cognitive dysfunction.

**Conclusions:** Our data demonstrate for the first time that murine cortical levels of mTOR are temporally regulated across aging, and that cognitive decline co-occurring with this increasing expression trajectory can be mitigated by genetic suppression of mTOR levels. Moreover, using this novel genetic model we provide initial evidence for the interplay between mTOR, aging and the neuroimmune system as a potential molecular mechanism for the regulation of age-related cognitive decline. Our findings present significant advances in the understanding of normal aging, but also for multiple age-related neurological disorders in which disrupted mTOR function has been implicated.

**Keywords:** mTOR, Neuroimmune Mechanisms, Ageing, Memory, Cognition

**Disclosure:** Nothing to disclose.

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**T70**

**One-Year Clinical Outcomes Following Theta Burst Stimulation for PTSD**

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**Background:** We previously demonstrated that a novel form of transcranial magnetic stimulation, called intermittent theta burst stimulation (iTBS) improved PTSD symptoms alongside improvements in depression and social and occupational function. In that study, participants received two weeks of blinded stimulation, followed by two weeks of unblinded stimulation, for a total exposure to active iTBS of up to four weeks. We observed that those who had received greater exposure to active stimulation demonstrated superior clinical outcomes at one month after stimulation. This raised the important question of whether this dose-response effect impacted longer-term patient outcomes. The present study tested the hypothesis that greater cumulative exposure to active iTBS would be associated with superior outcomes at 1-year.

**Methods:** Using VA electronic records and with procedures approved by the Providence VA IRB, we reviewed naturalistic outcomes up to one year from study endpoint. Included in the study were participants who completed double-blind iTBS. Primary outcome was defined as clinical relapse, operationally defined as any serious adverse event (e.g., suicide, psychiatrics hospitalizations), or need for TMS retreatment in the year following participation in the original randomized controlled trial. Kaplan-Meier survival curves were generated for hypothesis testing. Odds ratios for clinical outcomes were generated, and the relationship between stimulation and medication use were also evaluated. Based on findings reported below, we revisited baseline functional neuroimaging to explore potential predictors of longer-term outcomes; imaging results were corrected for multiple comparisons and cross validated.

**Results:** Forty-six (92%) of the parent study’s intent-to-treat participants were included in the present study. Mean age was 51.0 ± 12.3 years and seven (15.2%) were female. Groups were balanced in terms of sample size, age, and sex (all ps > 0.1). Overall, 22 (47.8%) patients demonstrated clinical relapse by one year. When comparing groups, Kaplan-Meier survival curves demonstrated superior outcomes at one year in the four-week group (log-rank ChiSq = 5.871, df = 1, p = 0.015). This was based upon several factors; in the four-week group, only eight (33.3%) relapsed within one year (mean days to relapse = 2960 ± 22.1), compared to 14 (63.6%; mean days to relapse = 1820 ± 31.9). The odds of clinical relapse were significantly greater in patients that received two weeks of active iTBS compared to those who received four weeks (OR = 3.50, 95% confidence interval 1.04-11.79). When used, retreatment with standard TMS was generally effective. Medication use and changes thereof did not differ between groups. Exploratory imaging analyses indicated that default mode network connectivity was predictive of non-relapse (all corrected p < 0.05 and cross validated).

**Conclusions:** Four weeks of active stimulation was associated with statistically significant and clinically meaningful superiority at one year. In the four-week group, clinical relapse rates in our study were generally comparable to those observed in studies of standard TMS for depression. These findings indicate an important dose-dependence of iTBS on durability of clinical efficacy in PTSD. Default mode network connectivity was associated with longer-term outcomes, in a comparable fashion to prior observations.

Further investigation is needed to determine the optimal course of iTBS, other factors that contribute to durability of response, and the efficacy of this form of stimulation for other disorders.

**Keywords:** Theta Burst Transcranial Magnetic Stimulation, Post Traumatic Stress Disorder, Transcranial Magnetic Stimulation

**Disclosure:** Neosync (equipment loan), Grant, Neuronetics (unpaid), Advisory Board

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**T71**

**Using the Neuroscience of Fear Extinction for Anxiety Reduction: Study Design, Aims, and Preliminary Data**

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**Background:** Social anxiety disorder affects 12% of Americans, resulting in significant distress and disability. Although exposure therapy is one of the most effective treatments available, as many as 25% of patients do not respond and we do not know why. Extinction learning is thought to be the mechanism of exposure therapy, and the neuroscience of extinction learning has advanced significantly since exposure therapy was developed; however, there has been little application towards improved clinical outcomes. The Using the Neuroscience of Fear Extinction for Anxiety Reduction (UNFEAR) study aims to bridge this gap by directly linking exposure therapy response to the neurobiology of extinction learning. Here we present the design, aims, and some preliminary data from the UNFEAR study (NCT# 03465137).

**Methods:** The UNFEAR study aims to recruit 80 adults of both sexes ages 18–50 with primary social anxiety disorder and not receiving treatment (N = 15 currently, N = 20 anticipated by the time of presentation). Participants attend three assessment visits prior to randomization to either 10 sessions of exposure therapy or a waitlist. In the first assessment visit, participants complete self-report questionnaires and a structured diagnostic interview. In the second assessment visit, participants undergo a fear conditioning and extinction protocol. Participants first view a neutral abstract image repeatedly paired with a loud aversive noise (Conditioned Stimulus or CS+), 50% reinforcement rate, and another image (CS−) that is never paired (fear acquisition phase). Following this, participants view the same images without aversive consequences during functional magnetic resonance imaging (extinction learning phase). In the third assessment visit, participants view the same images without aversive consequences
one week later (extinction recall phase). Here we present skin conductance responses during the fear acquisition, extinction learning, and extinction recall phases for participants with clean data in all 3 phases (N = 7 so far). Data were down-sampled to 100Hz, phasic skin conductance was extracted using Continuous Decomposition Analysis implemented in Ledalab, and event-related skin conductance responding was quantified as the summed amplitude of phasic skin conductance between one and four seconds following each stimulus (amplitudes less than 0.05 μS were treated as zero). Overall study aims are to (1) identify neurobiological features of extinction learning that predict successful extinction recall, (2) identify pre-therapy associations between symptom severity and extinction learning, and (3) build a mechanistic predictive model of exposure therapy response.

Results: The initial 15 participants (5 female) had primary social anxiety disorder with baseline Liebowitz Social Anxiety Scale scores consistent with moderate to significant social anxiety severity (M = 80.8, SD = 20.9) and baseline Patient Health Questionnaire scores consistent with minimal to mild depression symptoms (M = 5.0, SD = 4.7). One participant met diagnostic criteria for current major depressive disorder and another for remitted major depressive disorder. Two additional participants met diagnostic criteria for anxiety comorbidity (one panic disorder with agoraphobia, one generalized anxiety disorder). Participants with clean skin conductance data (N = 7) showed a clear fear acquisition effect, with greater skin conductance responses to CS+ (M = 0.30 μS, SEM = 0.08 μS) than CS- (M = 0.13 μS, SEM = 0.07 μS) in the first four trials of each type. This effect was extinguished by the mid-point of extinction learning (CS+: M = 0.02 μS, SEM = 0.01 μS; CS-: M = 0.01 μS, SEM = 0.01 μS). At the one-week extinction recall test, skin conductance levels were elevated for the first four trials of both the CS+ (M = 0.26 μS) and CS- (M = 0.28 μS) with high variability particularly for CS+ responding (CS+: SEM = 0.12 μS, CS-: SEM = 0.25 μS).

Conclusions: Initial UNFEAR study participants show clear fear acquisition, and greater variability of extinction recall than extinction learning effects on skin conductance responding. This may have been due to a context effect, because only the extinction learning phase took place in the MRI scanner. Future analyses with the full study cohort can therefore examine the generalization of fear extinction learning to the acquisition context during the recall test and relate this generalization to the impact of in-session exposure therapy on participants’ day-to-day anxiety.

Keywords: Adult Anxiety, Fear Extinction, Exposure Therapy

Disclosure: Nothing to disclose.

T72

Differential Gray Matter Correlates and Machine Learning Prediction of Abuse and Affective Psychopathology in Adolescent Girls

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Background: Childhood abuse represents one of the most potent risk factors for the development of psychopathology during childhood, accounting for 30–60% of the risk for onset. Adolescent girls are particularly vulnerable to the effects of abuse, and more broadly show a 2- to 3-fold greater risk of developing affective disorders such as anxiety, depression, and PTSD. While previous studies have separately associated reductions in gray matter volume (GMV) with childhood abuse and affective disorders in youth, it is unclear whether abuse and affective psychopathology show distinct versus common structural neural substrates. Additionally, to our knowledge, no studies have tested which structural abnormalities are predictive of abuse and psychopathology at the individual level in adolescent girls.

Methods: In a pooled multisite, multi-investigator sample, 246 females (average age 14.1 years) were recruited into studies of interpersonal violence-related PTSD, depression, or anxiety. Youth completed assessments for psychopathology, childhood maltreatment history (CTQ abuse score), and underwent high resolution T1 structural MRI. First, we characterized how differences in GMV associated with childhood abuse exposure depend on the presence or absence of psychopathology using voxel-based morphometry (VBM) with whole brain correction, adjusting for age, intracranial volume, site, and scanner. Next, we implemented a novel feature-learning approach combining five common machine learning (ML) algorithms on whole brain parcelled GMV in order to determine which regional brain structures were most informative for accurate, individual-level predictions of abuse severity and psychopathology status. Algorithms included the general linear model trained with stochastic gradient descent, random forest, multilayer perceptron, support vector machine, and gradient boosting machine. Hyperparameter optimization was performed with 10-fold cross validation. Mann-Whitney U comparisons were used to determine GMV features showing prediction influence significantly greater than chance. Conjunction analysis was used to further limit false positives, requiring significant features to be present in 3 or more of the 5 algorithms.

Results: Participant breakdown using dichotomous abuse classification included the following: Healthy no abuse (n = 99), Healthy with abuse (n = 50), Psychopathology no abuse (n = 12), Psychopathology with abuse (n = 85). Biomarker identification was highly consistent across VBM and ML analyses. When characterizing childhood abuse continuously, occipital pole GMV was positively associated with abuse severity in VBM analyses and was the most informative in predicting both dichotomous and continuous severity of abuse. Algorithms achieved a continuous prediction of abuse severity within 1.3 points and a maximum f1 score in discrete predictions 21% greater than chance. When characterizing psychopathology, VBM analyses revealed reductions in temporal and parietal cortex and cerebellum GMV in girls with psychopathology. These regions were also informative in predicting the presence of psychopathology, with maximum f1 score 22% greater than chance. Finally, interactive effects in VBM showed that GMV in the ventromedial and dorsolateral prefrontal cortex was positively associated with abuse severity only in youth with psychopathology. Four-group ML predictions with discrete abuse exposure (+/- -) by psychopathology (+/- -) reached a maximum f1 score 22% greater than chance and were most informed by the occipital pole and ventrolateral PFC.

Conclusions: Both voxelwise general linear models and machine learning models suggest distinct structural correlates of childhood abuse and affective psychopathology in adolescent girls with a high degree of overlap between methodological. These findings suggest that trauma exposure may affect different brain circuits than psychopathology, potentially leading to unique biotypes of psychopathology in girls with and without abuse exposure. Furthermore, voxelwise general linear and machine learning models point to differential effects of abuse on gray matter in girls with and without psychopathology particularly in prefrontal regions. Additional study is warranted in larger samples with greater numbers of non-abused psychiatric cases to test the generalizability of these findings.

Keywords: Adolescent, Child Abuse and Neglect, Affective Disorders, Gray Matter Volumes, Machine Learning

Disclosure: Nothing to disclose.
Untangling the Stressful Relationship Between Sex, Stress, Anxiety, and Neuropathic Pain: Results From Animals and Patients With Diabetic Neuropathy. What are the Psycho-pharmacological Implications?

Abstract not included.

The Use of Social Media in Recruiting Participants for Mental Health Research Purposes: A Systematic Review and Recommendations


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Background: Social media holds exciting promise for advancing mental health research recruitment. These mechanisms, however, raise new methodological and ethical considerations. A systematic review was conducted to characterize the current use of social media in recruiting participants for mental health research.

Methods: A literature review was performed using MEDLINE, EMBASE, and PsychINFO. Only non-duplicative manuscripts written in the English language and published between 1/1/2004-3/31/2019 were selected for further screening. Data extracted included study design, inclusion criteria, and advertising strategies and costs.

Results: A total of 176 unique studies used social media for mental health clinical research participant recruitment, either exclusively (43.2%) or to supplement traditional recruitment methods were reviewed. The majority of studies were cross-sectional (67.6%) in design and recruited primarily adults (only 5.7% recruited children/adolescents). Facebook was overwhelmingly the recruitment platform of choice (92.6%), with the use of paid advertisements being the predominant strategy (5.7%). Recruited primarily adults (only 68.3% of studies, social media recruitment performed as well as, or better than, traditional recruitment methods. The mean cost per recruited study participant was $24.12 (SD $28.74). In 55.6% of studies that made comparison with traditional recruitment methods, social media recruitment was more cost-effective than some or all of the other methods.

Conclusions: Social media appears to be a cost-effective recruitment tool for mental health research recruitment across a variety of study types. However, the platform raises methodological and privacy concerns not covered in current research guidelines and regulations. Using federal government policies and the reviewed literature, we synthesized a patient-oriented set of best practices.

Keywords: Social Media, Social Media Use, Clinical Trials, Recruitment

Disclosure: Epiodyne, Consultant, Allergan, Consultant, Blackthorn, Consultant, Rugen, Consultant

A Phase 2a Randomized, Double-Blind, Placebo-Controlled Study Investigating the Efficacy, Safety, and Tolerability of the Fatty Acid Amide Hydrolase (FAAH) Inhibitor JNJ-42165279 in Subjects With Social Anxiety Disorder

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Background: JNJ-42165279 is a potent, selective, and orally bioavailable inhibitor of the enzyme fatty acid amide hydrolase (FAAH). FAAH is the enzyme primarily responsible for the degradation of the endocannabinoid N-arachidonoylthanolamine, or anandamide (AEA). The endocannabinoid system is thought to play a role in the regulation of fear and anxiety responses. We conducted a proof of concept study to assess the efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of treatment with JNJ-42165279 in subjects with social anxiety disorder (SAD).

Methods: This was a multicenter, double-blind, placebo-controlled, randomized, parallel-group study to assess the efficacy, safety, and tolerability of JNJ-42165279 during 12 weeks of treatment in subjects with SAD. Subjects were enrolled at sites in the United States, Canada, and Australia, randomly assigned in a 1:1 ratio to receive either 25 mg JNJ-42165279 per day or placebo (PBO). No other treatments were allowed. The study consisted of 3 phases: screening for up to 28 days, 12-week double blind treatment, and a follow-up examination between 7 and 28 days following the last dose of study drug. The primary efficacy endpoint was improvement in social anxiety symptoms, as measured by the change in the Liebowitz Social Anxiety Scale (LSAS) total score from baseline to the 12-week endpoint. The secondary endpoints included changes from baseline to the endpoint for: LSAS subscales, LSAS ≥30% and ≥50% Improvement from baseline on total score, Hamilton Anxiety scale (SIGH-A) total score, Hamilton Depression scale (HDRS17) total score, and the Clinical Global Impression – Improvement (CGI-I) score. Exploratory endpoints included changes in Sheehan Disability Scale (SDS), Generalized Anxiety Disorders 7 item scale (GAD-7), Snaitl-Hamilton Pleasure Scale (SHAPS), and the Medical Outcomes Study Sleep-revised (MOS Sleep R). Pharmacodynamic evaluations included plasma concentrations of FAAs. PK parameters were estimated using population PK modeling. Safety and tolerability were monitored at regular intervals. Blood samples were collected for pharmacogenomics and to evaluate the PK (plasma) and PD effects of JNJ-42165279.

This was a proof-of-concept study. The threshold for signal detection was a 1-sided test with an alpha <0.20.

Results: At the completion of enrollment, a total of 149 subjects (JNJ-42165279 N = 74, PBO N = 75) were included in the analysis. Average (SD) age was 37.8 (13.1) years; 65% were male. 10% had comorbid MDD and 16% had comorbid GAD. The mean (SD) baseline LSAS total score for JNJ-42165279 was 102.3 (16.4) and for PBO was 105.2 (16.3) (severe).

The mean change in LSAS total score (SD) from baseline to Week 12 was numerically greater for JNJ-42165279: −29.4 (27.5) compared to PBO: −22.4 (23.6) for PBO. Based on a mixed model for repeated measurements the least-square mean difference (SE) between JNJ-42165279 and PBO was −3.8 (−4.72) did not meet the protocol threshold for signal detection (p = 0.21, one-sided).

An analysis that censored subjects with no detectable drug
concentrations (11 out of 59 censored) revealed a larger change from baseline difference (−30.2(28.2) for JNJ-42165279, −22.4 (23.6) for PBO; least-square mean difference (SE) −4.5 (5.03) (p = 0.19), which did not meet the protocol threshold for signal detection (p < 0.20, one-sided). The percentage of subjects who had a >30% improvement from baseline in the LSAS total score after 12 weeks of treatment was significantly higher for JNJ-42165279 (42.4%) compared to PBO (23.6%) (p = 0.04).

The mean (SD) SIGH-A total score at baseline was 10.1 (7.44) for JNJ-42165279 and 10.6 (7.47) for PBO. At week 12, the LS mean (SE) treatment difference between JNJ-42165279 and PBO groups in change from baseline of SIGH A total score was −0.9 (1.01) (p = 0.19) which met the threshold for signal detection. The percentage of subjects with a CGI-I score of very much improved or much improved was significantly higher at the end of 12 weeks for JNJ-42165279 (44.1%) than for PBO (23.6%) (p = 0.02). The MOS Sleep-R Problems Index showed greater improvement for JNJ-42165279 compared to PBO.

The drug was well tolerated, and no notable neurological adverse events of interest or findings occurred in either treatment group. No deaths occurred, and the most adverse events were mild to moderate in severity. Two serious adverse events occurred: a subject admitted for alcohol use disorder and a subject with a known allergy to eggs who experienced an anaphylactic reaction after ingesting a bakery product that had been incorrectly labeled. Mean changes from baseline in hematology, serum chemistry, and urinalysis, ECG, and vital signs were minimal and were similarly distributed in the PBO and JNJ-42165279 treatment groups, with none considered clinically significant.

Post-hoc regression analysis of PK and biomarkers demonstrated a strong correlation between trough drug concentrations and plasma AEA levels.

Conclusions: Considering the totality of responses on the LSAS, CGI, and HAM-A, JNJ-42165279 appears to have a small to modest anxiolytic effect in patients with severe SAD. This was substantially less and later than the effect reported for SSRIs and venlafaxine.

The strong relationship between plasma AEA levels and trough concentrations of JNJ-42165279 suggest that escape from full FAAH inhibition occurred in subjects with lower trough concentrations. This may warrant exploration of higher doses of JNJ-42165279 in future trials.

Keywords: FAAH Inhibitor, Social Anxiety, CNS Clinical Trials, Anandamide

Disclosure: Janssen Pharmaceutica NV, Employee

T76

Intrinsic Brain Connectivity Moderators of Psychotherapy Response and Changes in PTSD: A Combined Connectomic, Network Level, and Seeded Connectivity Approach

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Background: Trauma-focused psychotherapy is a first-line treatment for post-traumatic stress disorder (PTSD). Treatment mechanisms are poorly understood, and heterogeneity in treatment response is vast. Brain phenotyping is a promising method to assess treatment suitability and identify treatment mechanisms, but an understanding of the neural processes underlying therapeutic benefit and facilitating favorable treatment response is nascent. Here, we assay intrinsic patterns of brain connectivity across multiple units of analysis (whole brain connectome, network-level interactions, and seeded connectivity of PTSD-relevant limbic and prefrontal structures) to determine the intrinsic connectivity features most useful for identifying treatment mechanisms and outcome-predictive effects, both on the group and individual patient level.

Methods: Individuals with PTSD were randomized to immediate treatment with prolonged exposure therapy (N = 36) or a treatment waitlist (N = 30). Individuals underwent clinical and resting state functional magnetic resonance imaging assessment prior to randomization and 1 month following cessation of treatment/waitlist. Treatment-moderating connectivity and treatment-related changes were assessed utilizing linear mixed models with multiple brain units of analysis: a 200 parcel whole brain connectome; resting network-level interactions; and seeded subregional connectivity in the amygdala, insula, hippocampus, and frontopolar cortex. Multivariate machine learning with cross-validation was also utilized to determine the connectivity features best able to predict treatment response on the individual level.

Results: While limbic and frontopolar seeded connectivity showed patterns of treatment-specific change distributed across the brain (FDR p < 0.05), connectome and network-level metrics did not demonstrate significant treatment-related effects. In contrast, only a network-level metric (lower baseline visual to default mode network connectivity) moderated the group-level effect of treatment on PTSD symptoms (FDR p = 0.027). Machine learning yielded a robust, cross-validated predictive model of individual treatment response when predictive features were restricted to connections within the ventral attention network (correlation between model-predicted and observed symptom changes: r = 0.77, p < 0.001), and this outperformed a model based on clinical and demographic features alone.

Conclusions: Intrinsic brain connectivity demonstrates potential as a future clinical PTSD biometric. Specifically, network-level connectivity metrics may be most useful for predicting PTSD treatment outcome, while seeded connectivity may best aid in therapeutic mechanism interrogation.

Keywords: PTSD, Psychotherapy, Resting-State Functional Connectivity, Resting-State fMRI, Clinical Trial

Disclosure: Nothing to disclose.

T77

Synchronized Transcranial Magnetic Stimulation for Post-traumatic Stress Disorder and Comorbid Major Depression

Abstract not included.

T78

Polygenic Risk Score Associations With Nonpharmacologic Interventions for PTSD

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Background: Mindfulness Based Stress Reduction (MBSR), Transcendental Meditation (TM), and Present-Centered Group Therapy (PCGT), are effective non-pharmacologic treatments for 28-49% of veterans with PTSD. Many studies have examined genetic associations with PTSD disease risk, but few have examined genetic relationships with treatment response. We examined
whether polygenic risk scores (PRS) for PTSD, Major Depressive Disorder (MDD), Generalized Anxiety Disorder (GAD), and Attention Deficit Hyperactivity Disorder (ADHD) were associated with treatment response to these interventions.

Methods: Veterans on stable pharmacotherapy regimens with PTSD receiving Meditation [(MBSR (n = 50), TM (n = 18)], or PCGT (n = 52) were enrolled from two separate studies. Participants (n = 101) were all of Caucasian ancestry, averaged 60±10 years of age, n = 84 (83%) male, with n = 40 (39.6%) identified as having exposure to early life trauma (e.g. abuse, neglect). The PTSD Checklist (PCL) was administered to assess improvements after 9 weeks with 32.7% responding (≥10 pt PCL improvement). Participants were genotyped using the Illumina PsychArray followed by imputation using the 1000Genomes reference panel. We explored PRS for PTSD, GAD, MDD, ADHD disease risk in association analyses. MDD and GAD were selected due to common comorbidity with PTSD, and ADHD was selected for overlap in some clinical features (e.g. hyperarousal and inattention) that may influence response to these interventions. Polygenic risk scores were constructed using p-values and effect sizes from the most recent Psychiatric Genomics Consortium GWAS summary data. The SNPs used in the analysis were selected using a two phased p-value-informed clumping approach in PLINK. We first removed SNPs with linkage disequilibrium (LD) r2 values > 0.5 within a 200-kb window. Then we used a more stringent cutoff r2 = 0.2 to select more independent SNPs within a 5000-kb window. After LD clumping, 143480 SNPs from PTSD, 115667 SNPs from Anxiety, 138369 SNPs from MDD and 135188 SNPs from ADHD were used. For each participant, separate PRS for PTSD, MDD, GAD, and ADHD were generated using SNPs with p-values less than 10 different p-values thresholds (p = 0.5, 0.1, 0.05, 0.01, 10-3, 10-4, 10-5, 10-6, 10-7, 5*10-8). Logistic regression models were fitted to detect the association of week 9 responder and PRS by adjusting for PCL baseline scores and gender. PRS for the different disorders were examined individually, then in multivariate models, and then all four PRS were combined together using the first principal component of a genetic correlation matrix. We fitted models with/without adjusting for history of childhood trauma, and then separately in only participants receiving a meditation (MBSR or TM) to compare the results.

Results: Individual disease risk analyses identified a significant relationship between MDD and treatment response to the three non-pharmacologic treatment interventions (OR 1.20, p = 0.02). The four disorders considered together based on the first principal component of the genetic overlap was significantly associated with treatment response (1000 permutation-adjusted ChiSq p-value=0.04). Including early life trauma in the models or considering specific treatment type did not improve the models. Baseline depression symptoms did not predict treatment response.

Conclusions: PTSD symptoms are difficult to treat, in part due to heterogeneity in disease etiology and psychiatric comorbidities. The nonpharmacologic interventions examined here (MBSR, TM, and PCGT) represent patient-centered stress reduction approaches that improved symptoms in veterans who were receiving stable pharmacologic treatments, but who were still symptomatic. Increased genetic risk for MDD was associated with an increased likelihood of response to these interventions. Furthermore, the combined genetic risks for four relevant disorders based on the first principal component of their overlap was related to response. In this combined genetic risk approach, for every one unit percent increase in this composite genetic measure of disease risk, there was a 6% increase in the odds of response. These results suggest that these interventions may be particularly beneficial for patients with increased genetic loading for PTSD and related disorders, and that identifying strategies to quantify these genetic factors may be useful in future studies to identify who may benefit most from these nonpharmacologic interventions.

Keywords: PTSD, Polygenic Risk Score, Mindfulness Meditation
Disclosure: Nothing to disclose.

T79
Single Nuclear Sequencing of the Non-Human Primate Orbitofrontal Cortex Reveals Diversity of Cortical Interneuron Types
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Background: While anxiety disorders (AD’s) are among the most common and devastating mental illnesses, the biological basis for these disorders is not well understood. In this regard, and due to their recent evolutionary divergence from humans, nonhuman primates (NHPs) can be used as a translational model to understand the cellular and molecular processes that are relevant to human anxiety. Our work in young rhesus monkeys has characterized the neural circuit that underlies early-life pathological anxiety, also called anxious temperament (AT). This and other work points to the orbitofrontal cortex (OFC) as a regulator of subcortical structures involved in responding to threat. Because the OFC is composed of many different cell types, an important next step is to understand the roles and contributions of specific OFC cell types in mediating anxiety responses in rhesus monkeys. Single nuclear sequencing (snRNA-seq) allows for the characterization of distinct transcriptional profiles of cells in a region, facilitating an unbiased analysis of distinct cell types. In this study, we focused on inhibitory interneurons of the OFC, as they regulate the function of excitatory cells and have been extensively linked to the circuit level dysfunction underlying many psychiatric disorders.

Methods: Fresh frozen tissue from Walker’s area 13 was collected from n = 2 female adult rhesus monkeys (Macaca mulatta), using the boundaries specified in Paxinos et al., 2009. Tissue was dissociated following previously published protocols. Nuclei were isolated using the Chromium Single Cell 3’ technology from 10X Genomics and were sequenced on the Illumina NextSeq, following manufacturer guidelines. Reads were aligned to the Genome Reference Consortium Human Build 38 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.39) and quantified using cellranger. Data filtering, processing and analysis were performed in Scanpy (https://github.com/thelislab/scanpy). Standard processing pipelines were followed, including selection of variable genes, dimensionality reduction using principle components analysis (PCA), embedding using uniform manifold approximation and projections (UMAP) and clustering using the Louvain method of community detection.

Results: After quality control, 15,768 nuclei remained for analysis. We observed a median of 5,874 genes per nucleus and 13,035 reads per nucleus. Clustering algorithms revealed many clusters which were enriched for markers of excitatory cells (SLC17A7), inhibitory cells (GAD1, GAD2), oligodendrocyte precursor cells (OLIG1, OLIG2), oligodendrocytes (OLIG), astrocytes (GFAP), and other cell types. Of these clusters, several were enriched for genes associated with GABAergic signaling, markers traditionally associated with inhibitory interneuron populations. Based on hierarchical clustering, these inhibitory cells could be further divided by markers associated with distinct developmental origins, with several clusters enriched for ADARB2 (p < 0.001, Benjamini-Hotchberg corrected), a marker of developmental origin in the caudal ganglionic eminence (CGE), and other clusters enriched for LHX6 (p < 0.001, Benjamini-Hotchberg corrected), for the medial ganglionic eminence (MGE). Results were largely

consistent with extant literature on the developmental origin of different characterized interneuron populations; for example, somatostatin (SST) interneurons, which are known to originate primarily in the MGE, formed a distinct cluster that was also enriched for LHx6. Interneurons that were enriched for the calcium binding protein, calbindin (CALB1), on the other hand, co-expressed markers indicative of origins in the CGE. This population of cells was also characterized by co-expression of corticotropin releasing hormone (CRH), an anxiogenic neuropeptide involved in mediating stress responses. Interestingly, expression of CRH binding protein (CRHBP), a protein that binds to CRH and affects its ability to bind to CRH receptors, was expressed in a distinct population of cells that also expressed SST, suggesting a role for post-synaptic regulation of CRH signaling.

Conclusions: snRNA-seq allows for an unbiased characterization of the cell types present in the brain. This method is particularly valuable to understand the cell types and their heterogeneity in specific brain regions that have been linked to psychiatric disorders. The analyses herein confirm the presence of known GABAergic cell types (e.g. SST, CALB1, CRH) and deepen our understanding of both developmental origins and co-expression patterns. Building on this work, studies will be performed on tissue from our brain bank that is constituted of 72 young rhesus monkey brains. These monkeys were fully phenotyped using anxiety-related behavioral measures and multimodal neuroimaging. Future experiments with this brain bank will provide a deeper understanding of the cells present in the OFC and other anxiety-related brain regions, with the aim of providing insights into specific cell types driving the circuit level dysfunctions underlying psychopathology.

Keywords: Single-Cell RNA Sequencing, Orbitofrontal Cortex (OFC), Rhesus

Disclosure: Nothing to disclose.

T80

Genome-Wide Profiling of Amygdala CRH Neuron Translation Following Fear and Fear Extinction Learning

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Background: Fear and fear extinction learning are biophysical processes that precipitate molecular changes in neuronal circuits. CRH neurons within the central amygdala have been consistently implicated in playing a central role in fear and fear extinction learning processes. Given the recent finding that the CRH Receptor (Crhr1) meets genome-wide significance in PTSD symptoms (Gelernter et al., 2019), further understanding the role of these cell populations has enormous significance for understanding the biology of threat- and fear-related disorders such as PTSD. Cataloguing the dynamic translational changes in this population following fear or fear extinction learning will inform our understanding of the amygdala CRH neuronal population’s role in and response to fear processes.

Methods: To examine actively translating mRNA transcripts following fear or fear extinction learning we generated a CRH-TRAP mouse by crossing a Crh-Cre line with a Cre-dependent L10a-GFP line. Mice were habituated to apparatus twice for 10 minutes, fear conditioned (5CS/US, 30s CS at 6000Hz, 90s ITI, .65mA US) or exposed to tone alone (5CS, 30s CS at 6000Hz, 90s ITI), and fear extinguished (30 CS, 30s CS at 6000Hz, 60s ITI) on consecutive days. Following tone exposure, fear conditioning or fear extinction one each of male and female cohorts of mice was sacrificed after a delay of 2 hours.

TRAP was performed in accordance with method published by Heintz et al. Tissue was homogenized and mRNA’s isolated from GFP-tagged ribosomes (TRAP). RNA quantity and quality were assessed using Bioanalyzer Pico Chip (Agilent). Libraries were prepared using SMARTer HV kit (Clontech) and NexteraXT DNAkit. Further analyses by WGCNA identify multiple significant gene networks activated or inhibited by fear extinction learning. Predixcan analysis was used to identify multiple genes across disease modalities that are significantly altered with fear and extinction learning. Mobilizing upstream regulator analyses we used to identify hub genes related to differential pattern of gene expression within the CRH neuron population TRAP dataset.

Results: Mice learned and extinguished fear as expected compared to control animals (F(5, 190) = 45.53, p < 0.0001). Expression pattern of ribosome tagged eGFP closely recapitulates that observed in native CRH expression. Differential expression analysis between tone-alone and fear extinction groups identifies differentially expressed genes surviving FDR in males. Examination of the top EXT DEGs reveals genes associated with neuronal activity or neuronal plasticity such as Juntb, Fos, Camk2n1, Bcl2l2, Xiap, Ddn, Cdk10 and Ppp1r1a (all p’s < 0.001), regulated in directions consistent with decreased neuronal activity or LTD. Additionally, genes such as CRH, Dusp1, Fkbp4, Fkbp5, Crh,Pja1, and Usp22 are regulated in directions consistent decreased glucocorticoid receptor signaling. Analyzing the genetic regulation of the genes significantly altered with fear extinction learning, we identified significant overlap with genes mediating neuropsychiatric risk.

WGCNA analysis identified 19 networks (modules) containing 3565 co-expressed genes. Further, gene co-expression network analysis identified several significant gene networks activated or inhibited by fear extinction learning. Mobilizing upstream regulator analysis of the gene and gene network changes we identified the cAMP responsive element binding (CREB) protein inhibited following fear extinction. Over-expression of CREB in CRH neurons in vivo concordantly modulated fear extinction expression and consolidation (RM ANOVA F(1, 13) = 8.244, p = 0.013).

Conclusions: We used TRAP-seq to isolate CRH neuron specific polysome associated RNAs following tone exposure, fear conditioning or fear extinction. Bioinformatic analysis of translational changes in CRH neurons following fear extinction identified differentially regulated genes and gene pathways involved in this learning process. Because CRH neurons are recruited during learning of weak threats and active responses, we hypothesized that stress from transport and tone exposures was likely sufficient to activate this neuron population, washing out differences between tone-exposure and fear conditioning groups. This hypothesis was tested in a separate cohort of mice demonstrating that stress related genes CRH, Sgk1, and Id3 are regulated in response to both tone exposure and fear conditioning. Thus, it is likely that the transcriptional signature of associative fear learning was obscured by stress related translational changes. Our data demonstrate that gene changes as a result of fear extinction indicate the movement from an activated stress translational program to a deactivate stress translational program.

Genes are co-expressed forming functional gene networks. The majority of Extinction DEGs were co-expressed in 5 networks, including CREB regulation. CREB was chosen for cell-type specific manipulation given was it affecting a single network, the one containing CRH and has been shown to be necessary for stress related increases in CRH transcription, modulation of CREB was chosen. Congruent with predictions, over-expression of CREB in CRH neurons enhanced fear expression and blunted fear extinction. In summary, TRAP provides a powerful approach to

T81
Severe Sexual Abuse Reduces Model-Based Reinforcement Learning Updates Within the Left Frontoparietal Network
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Background: Trauma and PTSD are characterized by aberrant fear and safety learning. Two learning processes that have been delineated using computational modeling are model-free and model-based reinforcement learning, the former of which is disrupted among individuals with a history of assaultive trauma and may contribute to habitual fear responding. Currently, it is unclear whether model-based reinforcement learning, which is prospective and goal-directed, is also impaired among individuals who have experienced trauma. The present study sought to identify the impact of assaultive trauma history on model-based reinforcement learning among adolescent females and to identify the neural correlates of this impact. It was hypothesized that a history of assaultive trauma, regardless of PTSD status, would predict impaired model-based reinforcement learning. Furthermore, based on previous research indicating that model-based reinforcement learning is implemented within cognitive-control regions, such as lateral prefrontal cortex, and the intraparietal sulcus, it was hypothesized that trauma would predict reduced activity within frontal and parietal regions and frontoparietal networks during model-based reinforcement learning updates.

Methods: Sixty-two adolescent females between the ages of 11 and 17 with (n = 30) and without (n = 30) histories of directly experienced physical or sexual assault were recruited to participate in the present study. Adolescents with an assaultive trauma history were recruited such that approximately half of the assaulted participants had a PTSD diagnosis (n = 16). Participants completed a three-arm bandit reinforcement learning task during fMRI. Based on previous work, a risk-sensitive anticorrelated Rescorla-Wagner (RW) model and a latent-state model were selected to capture model-free and model-based reinforcement learning, respectively. Effects of trauma and PTSD on model-derived parameters (e.g., overall model fit) were examined, as well as the impact of changes in latent-state beliefs on neural activity via voxelwise and network-level (i.e., independent component analysis [ICA]) analyses as a function of trauma and PTSD.

Results: Based on Akaike information criteria, the latent-state model provided a better fit for a greater proportion of participants than the Rescorla-Wagner model (n = 22 vs. n = 38, χ²(1) = 4.27, p = 0.040). Although neither trauma nor PTSD broadly predicted reinforcement strategy use, greater sexual abuse severity predicted less use of model-based versus model-free reinforcement learning (t(55) = 3.21, p = 0.003). With regard to neuroimaging, no significant effects of changes in latent-state beliefs emerged for the voxelwise analyses. However, ICA indicated that less use of the model-based versus model-free reinforcement learning strategy predicted less left frontoparietal network (FPN) activity during updates of latent-state beliefs (t(49) = −2.17, p = 0.035). This pattern also emerged as a function of sexual abuse, with greater sexual abuse severity predicting less left FPN activity during updates of latent-state beliefs (t(49) = −2.30, p = 0.026). Follow-up analyses revealed that the impact of sexual assault on FPN activity was specific to severe sexual abuse, as adolescents with a history of no and low-to-moderate levels of sexual abuse exhibited greater left FPN activity than those with a history of severe sexual abuse (t(48) = 2.17, p = 0.011 and t(48) = 2.28, p = 0.027, respectively).

Conclusions: Results indicate that model-based reinforcement learning is impaired among female adolescents with a history of sexual abuse. Severe sexual abuse was associated with greater use of trial-and-error learning rather than learning that involves building a complex cognitive map of the environment. Severe sexual trauma was also associated with less left FPN activity during changes in model-based latent-state updates. Regions within the FPN have been shown to be particularly vulnerable to the effects of early stress due to a protracted maturational time course, and early sexual abuse may be particularly pernicious, disrupting normative development of these regions. Given the significant impact that trauma, especially sexual trauma, has on mental health, it is plausible that aberrant model-based reinforcement learning is an important route through which impairment emerges.

Keywords: Trauma, PTSD, Computational Modeling, Cognitive and Affective Neuroscience

Disclosure: Nothing to disclose.

T82
A Peripheral Epigenetic Signature of Fearlessness in Rhesus Monkeys
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Background: An increasing body of evidence suggests an important role for epigenetic mechanisms mediating the effect of environmental factors on the risk for psychiatric disorders. In parallel, stable epigenetic marks in peripheral tissues may be particularly useful biomarkers for diagnostic approaches, treatment, and prevention. Human studies to date typically focus on DNA methylation profiling in peripheral tissues; however, multiple confounding factors make the identification of disease-relevant DNA methylation signatures of psychiatric phenotypes challenging. In contrast, well-controlled animal models may provide stronger evidence for peripheral epigenetic signatures but often lack translational value for human disorders. Here, we focus on a non-human primate model of fear and fearlessness in rhesus monkeys (M. mulatta) from the Caribbean Primate Research Center at Cayo Santiago, Puerto Rico. Using a translational platform to profile DNA methylation in rhesus and human, we provide evidence for fear-specific DNA methylation profiles in these monkeys, potentially influenced by exposure to a natural disaster in the form of Hurricane Georges (Category IV) in 1998.

Methods: A free-ranging population of rhesus monkeys exposed to the natural environment inhabits Cayo Santiago. In order to test fear/fearlessness in a subset of animals, each monkey was shown a grape, which was then dropped into a two-sided feeding bin with opaque lids. The monkey approached the bin, opened the lid and retrieved the grape. This was done twice. In...
the third trial, the bin was rotated to the opposite side, which contained a rubber snake hanging from the inside of the lid. Opening the lid to retrieve the grape exposed the monkey to the snake stimulus. Fear responses were assessed with a ratio comparing the time spent holding the lid open when the snake was present/when the snake was absent. Lid holding ratios ≥ 1 indicated fearlessness. Monkeys were only tested once with the snake stimulus. Due to the close genetic relationship between human and rhesus monkey we were able to use the Illumina EPIC methylation array platform to profile DNA methylation on a genome-wide level in the monkeys. This was achieved by first mapping the array probes to the in-silico bisulfite converted macaque genome and only retaining the successfully and uniquely mapped probes. Statistical modeling was performed in R using generally species-independent scripts derived from established methylation array analysis pipelines. Validation of differential methylation was performed using pyrosequencing. Available DNA methylation data from the Grady Trauma Project was used for the comparison of non-human primate and human data.

**Results:** Of 171 monkeys tested, 81% were fearful to the snake, dropping the lid quickly, whereas 19% were fearless and able to retrieve the grape. There was a bimodal distribution of lid holding ratios, 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 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. Of the 866,150 probes on the EPIC array, 50.3% successfully and uniquely mapped to the macaque genome, and 299,409 probes remained after strict data processing. Using a genome-wide screening approach, we identified 163 differentially methylated positions (DMPs) throughout the rhesus genome that were significantly associated with fearlessness (pFDR < 0.05) in monkeys born before the hurricane indicating that fear to a snake stimulus may indeed have a biological correlate in peripheral tissues. The top DMP (cg10639643) was located in ADCY9 (chr20:2936346; log2FC = 0.31; pFDR = 1.14E-05). ADCY9 encodes adenylate cyclase type 9, a ubiquitous adenyl cyclase that responds to beta-adrenergic stimulation. In contrast, no such associations were observed in monkeys born after the hurricane. Using the site-specific results, the presence of differentially methylated regions was investigated by combining nearby p-values, leading to the identification of 46 differentially methylated regions (DMRs) associated with fearlessness in the older animals. The most significant DMR was located in DDHA2 (chr4:32597546-32598449; pSidak = 2.68E-09), encoding N(G,N)(G)-dimethylarginine dimethylaminohydrolase 2, which plays a role in nitric oxide production. We are currently studying the overlap between the fear methylation signature in rhesus monkeys compared to fear phenotypes and fear related disorders in human populations.

**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. Human studies on epigenetic profiling so far have not been able to reliably identify fear-related peripheral biomarkers. Here we show evidence for fear/fearlessness associated DNA methylation profiles in a highly translational non-human primate model. We also provide potential evidence for the impact of Hurricane Georges on the development of a bimodal distribution of fearful and fearless monkeys that is strongly associated with the observed epigenetic changes. We speculate that the observed phenotype and the accompanying epigenetic changes in this non-human primate model are a result of a natural disaster with direct relevance for trauma-related outcomes in humans.

**Keywords:** Rhesus, Fear, Epigenetic

**Disclosure:** Nothing to disclose.

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**T83 PTSD- and IPT-Related Differences in Intrinsic Connectivity in a Pilot Sample of Female Adolescent Survivors of Sexual Assault With PTSD**

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**Background:** Age, gender and trauma-type exposure each contribute to the phenomenology and risk of posttraumatic stress disorder (PTSD; Breslau, 2009; Kerig, 2014; Kessler et al., 2017; Kilpatrick et al., 2003). Adolescent girls are at increased risk for trauma exposure, particularly sexual assault, as well as for PTSD (Kerig, 2017; McLaughlin et al., 2013). However, little is known about the neural correlates of PTSD and PTSD treatment in adolescent girls.

In recognition of the developmental considerations involved in treating PTSD during adolescence, evidence-based treatments have been adapted for use with adolescents (Gilboa-Schechter et al., 2010; Rosner et al., 2019). IPT, originally developed to treat depression, has been successfully adapted to PTSD (Markowitz et al., 2015) and current work is testing interpersonal psychotherapy for adolescent PTSD in a group format (IPT-APG).

IPT-APG may be particularly well-suited to treat adolescents with PTSD following sexual assault, as group treatment for PTSD has been theorized to reduce feelings of isolation and has been shown to be an effective treatment for women with PTSD (Krupnick et al., 2008; Zlotnick et al., 1997). Furthermore, IPT for PTSD has been found to be particularly effective for sexually traumatized patients, possibly due to its focus on interpersonal relationships (Markowitz, Neria, Lovell, Van Meter, & Petkova, 2017).

The current pilot study examines the efficacy of IPT-APG and PTSD- and treatment-related changes in resting state functional connectivity (RSFC) in adolescent girls with PTSD following sexual assault. Regions of interest include limbic areas, previously associated with PTSD in a number of studies (Aghajani et al., 2016; Bluhm et al., 2009; Cisler, Steele, Smitherman, Lenow, & Kilts, 2013; Malivoire, Girard, Patel, & Monson, 2018). Since IPT-APG focuses on changing inter-personal interactions, other regions of interest include nodes of the default mode network (DMN), which are important for social cognition (Laird et al., 2011; Schilbach, Eickhoff, Raska-Jagiela, Fink, & Vogele, 2008).

**Methods:** Six female adolescents with PTSD resulting from sexual assault and 5 same-aged female healthy controls participated in a pilot study at the Federal University of São Paulo (UNIFESP) in São Paulo, Brazil. PTSD symptoms were assessed using the CAPS. For patients, IPT-APG was adapted for adolescents in the following ways: 1) a group format was used to provide social support and examples of other adolescents girls who experienced similar traumas; 2) a session was added to provide psychoeducation about PTSD and treatment for the patient and her parents or guardians; and 3) a specific focus was placed on role transitions, which are developmentally appropriate in adolescence, and highly relevant following a trauma. IPT-APG was provided for 12 weeks, in 90-minute weekly sessions.

Resting fMRI scans of patients and health controls were collected at baseline and then again for patients only following IPT-APG treatment. RSFC was analyzed using SPM 12 and the...
CONNNtoolbox. Differences in RSFC between patients and controls were examined at baseline. Within-subject changes in symptomatology and changes in RSFC were correlated in patients before and following completion of IPT-APG. Results were found using a whole-brain voxel-wise threshold of p < 0.001, with a cluster-size FDR corrected threshold at p < 0.05, two-sided.

Results: All patients who completed treatment (5 out of 6) no longer met criteria for PTSD. Replicating previous studies, before treatment PTSD patients had decreased connectivity between the right hippocampus and the posterior cingulate cortex (PCC), a key node of the DMN (peak MNI: −22, −58, 12, cluster size: 89 voxels). Results from within-subject analyses examining the relationship between pre- and post-treatment PTSD symptoms and RSFC found that improvement in re-experiencing symptoms (CAPS-B) were associated with increased connectivity between limbic seeds and DMN regions (left amygdala – right lateral parietal cortex (LP), peak MNI: 40, −54, 16, cluster size: 33 voxels), and between DMN seeds and limbic regions (left LP and right hippocampus: peak MNI: 22, −26, −31, cluster size: 31 voxels; right LP and right hippocampus: peak MNI: 24, −28, −12, cluster size: 46 voxels).

Conclusions: These pilot results suggest that IPT-APG warrants further study as a novel treatment for adolescent PTSD. We replicated previous findings that PTSD is associated with decreased connectivity between limbic and DMN regions. Symptom improvement was associated with changes in RSFC between limbic and DMN nodes, consistent with IPT-APG’s focus on making positive changes in social interactions, and the DMN’s association with social cognition. Together, the decreased connectivity between limbic and DMN regions in PTSD patients before treatment, and the increased limbic-DMN connectivity related to improvements in re-experiencing symptoms suggest that IPT-APG may help restore a more normative pattern of RSFC. However, replication from a larger sample is required to better understand treatment-related neural changes in adolescents with PTSD.


Keywords: Adolescent PTSD, Resting State Functional Connectivity, Treatment-Related Changes in Resting State Functional Connectivity

Disclosure: Nothing to disclose.

T84

Examining Anxiety-Related Differences in Attentional Efficiency and Associated Neural Correlates in Adolescents

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Background: According to Attentional Control Theory (Eysenck & Derakshan, 2011), the ability to flexibly utilize and adapt attention processes in the face of threatening stimuli is impaired in individuals with anxiety. While there is support for this theory, there is also research suggesting that threatening stimuli can facilitate attentional processes. Extending this work in pediatric anxiety, the current study uses a Visual Search paradigm, to examine anxiety-related differences in the impact of emotional stimuli on attentional control processes (efficiency and effectiveness) and associated neural correlates.

Methods: Seventy-two adolescents (8-18 years old, M = 14.23, SD = 2.31) completed a Visual Search task during fMRI. Of the recruited participants, 38 (23 females) met DSM-5 criteria for at least one anxiety disorder. All participants completed the Screen for Child Anxiety Related Disorders (SCARED) within months of the imaging visit. SCARED total scores (averaged across child and parent reports) were used to examine the impact of individual differences in anxiety severity on task performance and brain responses.

In the Visual Search paradigm, participants were tasked with finding a slanted black bar (target) in a series of 1, 5, or 30 upright and slanted black and white bars (2000-ms). Once they located the target, they were instructed to press a button indicating which direction it was pointing. Each trial started with the presentation of an emotional distractor (300-ms), angry face, happy face, or scrambled image. Accuracy (i.e., effectiveness, percentage of correct trials) and search time (i.e., efficiency, RT) were analyzed as behavioral measures.

Neuroimaging analyses focused on neural responses during the search process (when the participants were presented with the search screen). Participants completed 3, 8-minute runs of the task. All behavioral and neuroimaging data was analyzed using a mixed-effects model wherein SCARED scores were entered as the continuous, between-subject variable and Emotion (angry, happy, scrambled) and Search Size (1, 5, 30) as repeated, within-subject variables. All analyses covaried for age. Neuroimaging analyses also covaried for RT and scanner. To demonstrate the impact of threatening faces on attentional control, Attention Bias Indices (ABI; Dodd et al., 2017) were created for each participant by subtracting RT/neural response on angry trials from RT/neural responses on happy trials.

Results: Full interactions (SCARED X Emotion X Search Size) for accuracy and RT were not significant. However, a significant SCARED X Emotion interaction emerged for RT. In particular, participants with higher SCARED scores took longer to find the target on trials preceded by happy and scrambled images. Further, ABI were positively correlated with SCARED scores (r = 0.32, p < 0.001) suggesting that angry faces facilitate attentional processes to a greater extent in participants with more severe anxiety but serve as a distractor in individuals with low/no anxiety.

Whole brain analyses (p > 0.005, k > 65) revealed a significant SCARED X Emotion interaction in the superior parietal lobule (SP, k = 106) and the precuneus (k = 70). In line with the behavioral findings, follow-up tests revealed more engagement of these regions in participants with higher SCARED scores on happy and scrambled trails regardless of Search Size. Individuals with low/no anxiety showed the opposite pattern. Further, ABI in the SP and precuneus were positively correlated with SCARED scores (r = 0.54, p < 0.001 and r = 0.50, p < 0.001, respectively) suggesting that these regions may help compensate for general attention deficits (when not facilitated by negative stimuli).

Conclusions: These findings highlight anxiety-related differences in the impact of threatening stimuli on attentional efficiency, but not effectiveness. Results showed that anxiety severity was associated with less attentional efficiency during positive and neutral trials. Of particular interest is the fact that negative stimuli facilitate search in adolescents with higher anxiety. And, this effect was linked with activation of the SP and precuneus. These findings support previous literature demonstrating attentional biases towards negative stimuli in anxious individuals. Finally, the present findings suggest that anxious youth may not demonstrate attentional efficiency deficits in the face of threatening stimuli.

Keywords: Adolescence, Anxiety, Human Neuroimaging, Attention

Disclosure: Nothing to disclose.
The Utility of Brain Age as a Biomarker for Accelerated Ageing in PTSD

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Background: Posttraumatic stress disorder (PTSD), a debilitating disorder characterized by prolonged fear and autonomic responses following a traumatic stressor, has been linked with accelerated aging. Individuals with PTSD exhibit more advanced age as measured by alterations in leukocyte telomere length, DNA methylation, cellular senescence of p16INK4a, and autonomic reactivity compared to individuals without a PTSD diagnosis. Given the deleterious effects of aging on the brain, identifying neural biomarkers may help stratify PTSD risk, effects of disease on the brain, and aid in the development of a personalized medicine approach to treating PTSD. Machine-learning has been applied to metrics derived from grey matter parcellations of T1-weighted structural MRI to predict brain age. This estimate of brain age can then be compared with chronological age to calculate a difference score, referred to as predicted age difference (PAD). Personalized predictions have been optimized by training algorithms using chronological age in a large sample of healthy controls and then tested to predict brain age in patient cohorts. Brain age that is greater than chronological age has been linked with poorer motor and lung function, lower intelligence, higher allostatic load, and increased mortality. The present study examined the potential effectiveness of predicted brain age as a biomarker of accelerated aging in 12 sites participating in ENIGMA-PGC PTSD working group. We hypothesized that both severity and diagnosis of PTSD would relate to higher brain age as assessed by PAD.

Methods: We analyzed 889 subjects that included 59% male (n = 525) and 41% female (n = 364) adults between the ages of 18–61 years (mean age = 36, SD = 9.9) that were aggregated from 12 ENIGMA-PTSD sites. All participants completed a high resolution T1-weighted structural magnetic resonance imaging (MRI) optimized for tissue contrast, as well as clinical assessment of PTSD diagnosis and severity using the Clinician Administered PTSD Scale for DSM-IV. Estimates of BrainAge from all sites were calculated centrally at Duke University by applying the trained algorithm implemented in Matlab to T1-weighted image. Linear modeling was used to examine relationships between chronological age and predicted BrainAge. To limit the impact of age on the relationship between PTSD and BrainAge, we explored the relationship between PTSD severity and diagnosis with the residual effects of the chronological age and BrainAge model (referred to as BrainAgeR). Linear mixed effects models were used to examine relationships between PTSD and BrainAge, PAD, and BrainAgeR, with site as a random effect. To control for multiple comparisons, a Bonferroni correction was applied for 6 comparisons (BrainAge, PAD, and BrainAgeR for PTSD severity and diagnosis). Results that met a critical p-threshold of <0.008 were used to determine significance.

Results: A total of 520 subjects met criteria for current PTSD (mean PTSD severity = 50, SD = 31.6). Age positively related to BrainAge (β = 0.53, SE = 0.03, t = 19.29, p < 0.001) and negatively related to PAD (β = −0.47, SE = 0.03, t = −17.31, p < 0.001). Importantly, BrainAgeR was not related to Age (β = −0.02, SE = 0.03, t = −0.56, p = 0.579), indicating that the linear effects of age were no longer impacting this estimate. Results testing our main hypothesis showed that neither PTSD severity nor PTSD diagnosis related to BrainAge (PTSD severity β = −0.003, SE = 0.01, t = −0.33, p = 0.740; PTSD diagnosis β = −0.41, SE = 0.66, t = −0.61, p = 0.541); PAD (PTSD severity β = −0.01, SE = 0.01, t = −0.53, p = 0.596; PTSD diagnosis β = −0.18, SE = 0.64, t = −0.017, p = 0.781) or BrainAgeR estimates (PTSD severity β = −0.004, SE = 0.01, t = −0.49, p = 0.624; PTSD diagnosis β = −0.28, SE = 0.56, t = −0.51 p = 0.609).

Conclusions: Contrary to our hypothesis, neither PTSD severity nor PTSD diagnosis related to measures of brain age, which may be a marker of brain health and possibly a proxy for accelerated aging. Although PTSD has been consistently linked with other phenotypes of accelerated aging, our results do not support evidence of accelerated brain aging, perhaps because predicted brain age is not yet a sensitive indicator of chronological age or accelerated aging. Our results could be explained by multiple factors. The first is that dissimilar to genetic and epigenetic markers, brain aging is not accelerated in PTSD. An important methodologic consideration is that current MRI technology may not be sensitive enough to detect small changes in brain morphology associated with accelerated aging. Alternatively, and importantly, models for predicting brain age are still in their infancy and continue to evolve. As such, it is possible that the present model, which was trained on a large but fairly homogenous sample may not generalize to our more heterogeneous samples. Furthermore, our findings that age is inversely related with predicted brain age (PAD) suggest that PAD may not be an accurate indicator of changes in neural health across the entire life span. Future research is warranted to test other models of predicted brain age to determine its utility as a biomarker for accelerated aging.

Keywords: Accelerated Aging, Posttraumatic Stress Disorder, Brain Age, Structural MRI Consortium

Disclosure: Nothing to disclose.

Role of Insula to Amygdala Projection Neurons in Anxiety- and Valence-Related Behaviors

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Background: Despite high societal burden of anxiety disorders, efficient anxiolytics without side effects are unavailable. Most pharmacological treatments of anxiety disorders are targeting the serotonin system. Selective serotonin reuptake inhibitors (SSRIs) are used as the first-line pharmacological treatment of anxiety disorders and buspirone, a partial agonist of serotonin 1A receptor, is known to have anxiolytic properties. However, those anxiolytics affect serotonin neurotransmission in the whole central nervous system, including the neural circuits irrelevant to anxiety, likely leading to the debilitating side effects.

Human neuroimaging studies revealed abnormal function of the insular cortex (IC) and amygdala in patients with anxiety disorders. Recent anatomical studies in animal models reported divergent connections between IC subregions and amygdala nuclei, including projections of the anterior IC to the basolateral amygdala (BLA) and projections from the posterior IC to the central nucleus of amygdala (CeA). The neural circuits of anxiety are well studied in the amygdala, whereas the function of the IC neural substrates in anxiety remains unclear. Therefore, we investigated the role of the anterior and posterior IC neurons in anxiety-related behaviors, along with the expression of serotonin receptors in those circuits.
**Methods:** First, we defined the distribution of IC-amygdala projectors by performing anterograde and retrograde tracing. For anterograde tracing, we injected an anterograde adeno-associated viral vector (AAV9) carrying the gene coding for a green fluorescent protein (eYFP) under the control of the CaMKIIa promoter, and quantified the density of processes in the downstream regions. For retrograde tracing, we injected retrograde tracer (CTB-555 and CTB-647) into the BLA or CeA of C57B6 mice. We combined these experiments with immunohistochemistry against the serotonin 1A or 2A receptors.

Second, we performed ex vivo whole-cell patch-clamp recordings in the BLA and CeA and perform channelrhodopsin2 (ChR2) assisted circuit mapping (CRACM) to define the nature of the insula to amygdala connections.

Third, we recorded the activity of anterior and posterior glutamatergic IC projection neurons, by performing in vivo fiber photometry during well-established anxiety assays. We expressed the genetically encoded calcium sensor GCaMP6f in those neurons by injection an adeno-associated viral vector (AAV9) carrying the gene coding for GCaMP6f under the control of the CaMKIIa promoter.

Lastly, we performed optogenetic activation of IC-BLA and IC-CeA neurons during anxiety and valence assays, by expressing ChR2 in IC-BLA or IC-CeA neurons using a double viral strategy. We injected a retrograde vector expressing cre (CAV2-cre) in the downstream region (BLA or CeA) and injected a cre dependent AAV carrying the gene coding for ChR2 in the anterior or posterior IC, respectively.

**Results:** Using anterograde tracing we found the anterior and posterior IC neurons, preferentially project to the BLA and CeA respectively. Thanks to retrograde tracing we found an inverted antero-posterior gradients of IC-BLA and IC-CeA projector populations with the IC-BLA neurons having the highest density in the anterior IC. We identified that 80% of IC-BLA and IC-CeA projectors express the serotonin 1A or 2A receptors, while only one third of GABAergic interneurons express these receptors. Ontogenetically assisted circuit mapping allowed us to demonstrate a monosynaptic connection of the IC to BLA glutamatergic neurons and CeA GABAergic neurons.

Using fiber photometry, we found that the activity of anterior, but not posterior IC neurons is correlated with anxiety levels. Specifically, glutamatergic neurons of the anterior insula are more active in the center of the open field test (OFT) and the open arms of the elevated plus maze (EPM) compared to the border of the OFT and the closed arms of the EPM. Finally, we found that optogenetic activation of IC-CeA projectors induces real time place aversion (RTPA).

**Conclusions:** Altogether, our findings revealed a new role of the anterior insular cortex projection neurons in anxiety-related behaviors.

**Keywords:** In Vivo Calcium Imaging, Circuit Optogenetics, Anxiety Circuitry, Serotonin, Valence

**Disclosure:** Nothing to disclose.

**Background:** Social function is a critical, yet understudied, factor in posttraumatic stress disorder (PTSD). A previous investigation of ‘social navigation’ found that the hippocampus tracked the trajectories of fictional characters in a 2D social space and additionally related to self-reported social function (Tavares RM et al. 2015, Schafer M & Schiller D 2018). Given that the hippocampus is impaired in PTSD, there is a clear rationale to predict that this task will be sensitive to social dysfunction in individuals with PTSD.

**Methods:** In a preliminary sample of 15 World Trade Center (WTC) rescue and recovery workers highly exposed to the 9/11 WTC attacks and/or their aftermath, 5 with WTC-related PTSD and 10 without any lifetime psychiatric disorders despite high WTC-related exposure (i.e., ‘highly resilient’), we explored whether their ‘social navigation’ in a 2D social space is related to social dysfunction. In a narrative-based role-playing computer task, participants move to a new town and interact with characters to achieve social goals. On the basis of the participant’s decisions, the characters are modeled as coordinates in a 2D social space of social power and affiliation. From these coordinate values, a ‘social dynamics’ measure of social geometry –going back and forth along the social dimensions (flexibility) vs. showing a more rigid trajectory (inflexibility) with larger absolute values on the dimensions of power and affiliation– was compared between groups and correlated with self-reported social support.

**Results:** Post-task memory questionnaire scores indicated that the participants were able to attend to and remember the narrative, as the scores were all above chance (M = 63%). Analyses of the ‘social dynamics’ measure described above revealed preliminary differences between the PTSD group (M = 32.21, min = 29.17, max = 35.55, arbitrary units) and the highly resilient group (M = 28.59, min = 22.16, max = 37.88). Further, previously assessed healthy controls (n = 21; M = 28.38, min = 19.74, max = 36.68) were much more similar to resilient participants on this measure. Social dynamics scores showed negative correlations with the MOS Social Support Survey subscales, which were stronger among those with PTSD than resilient individuals (average r = −0.66 versus r = −0.31 respectively).

**Conclusions:** Individuals with PTSD showed significantly greater social inflexibility in a naturalistic social interaction task that was strongly correlated with real-world social dysfunction. The task conceptualizes a 2D social space and yields a geometric outcome measure of social dynamics. Preliminary findings in this context may reflect a kind of social rigidity and enhanced sensitivity to power dynamics and affiliative interactions consistent with social dysfunction, known to be associated with PTSD. While these results are preliminary and should obviously be taken with caution, they provide a basis for hypothesis-generation in this ongoing study, with a larger projected sample of 35 WTC workers with PTSD, 35 highly resilient WTC workers and 35 low WTC-exposed controls.

**Keywords:** Social Behavior, PTSD, Social Cognition, Cognitive and Affective Neuroscience

**Disclosure:** Pending patent (submitted), Patent (not licensed), Patent
Background: Mindfulness involves present-moment attention to bodily sensations, and has been incorporated into psychotherapies for psychiatric disorders. There is accumulating evidence of efficacy for depression relapse prevention, anxiety, and posttraumatic stress disorder (PTSD). Studies of long-term mindfulness meditators and meditation naïve healthy people have shown alterations in cross-network connectivity between DMN and attention networks. PTSD has also been associated with DMN-attention network dysregulation. We have previously demonstrated increased connectivity between DMN (posterior cingulate seed) and fronto-parietal network (FPN, bilateral dIPFC) following 12 weeks of mindfulness practice in PTSD patients (Mindfulness-based Exposure therapy, MBET).

Methods: Male military veterans with PTSD (N = 32) deployed to Iraq or Afghanistan and healthy age-and-gender-matched community healthy controls (N = 20) underwent 3T fMRI paradigms to assess resting-state functional connectivity (rsFC) during unconstrained resting state (mind-wandering) and during a mindful interoception task. Seed-based analyses identified changes in functional connectivity associated with mindfulness-based interventions in both healthy and PTSD patients with nodes within DMN, frontoparietal (FPN), salience (SN), and dorsal and ventral attention networks (DAN, VAN). Whole-brain connectomes were estimated using joint independent components analysis (jICA) and examined shared and unique aspects of mindfulness task- and PTSD-associated connectivity patterns.

Results: Following an 8-week mindfulness intervention, healthy controls showed significantly increased functional connectivity between DMN (PCC seed) and FPN (dIPFC), similar to our previously published findings in PTSD patients. Both PTSD patients and healthy controls also showed substantial changes in cross-network connectivity during the mindfulness interoception task compared to unconstrained rest. The mindfulness task was associated with decreased connectivity within DMN, and increased connectivity (decreased anti-correlation) between anterior DMN and DAN, and FPCN and DAN in seed-based analyses, and in an independent component (IC) associated with these networks (p = 7.0 × 10^{-5}). PTSD patients had significantly decreased expression of this same connectivity IC during the mindfulness condition compared to healthy volunteers (p = 0.009), and level of change in this component from rest to mindfulness was correlated with PTSD avoidant symptoms (p = 0.007).

Conclusions: In this multivariate, whole-brain connectomic analysis, we demonstrate overlap between brain networks engaged during a mindfulness task and brain networks exhibiting baseline abnormalities in PTSD. In particular, both participants in a mindful interoceptive state and PTSD patients at rest exhibited increased connectivity between DMN, DAN, and FPCN networks, suggestive of increased interoceptive awareness and effortful regulation of attention. It is possible the therapeutic benefits of mindfulness in PTSD are produced by re-engagement of the same core neural circuits that are dysregulated in the disorder.

Keywords: Combat PTSD, Mindfulness Meditation, Fronto-parietal Network, Default Mode Network (DMN)

Disclosure: Nothing to disclose.

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Ventral Tegmental Area-Amygdala Circuit Mediates Approach-Avoidance Behaviors

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Background: Exploration of the environment while also preserving one’s self from threats are crucial behaviors to ensure survival and well-being, but they often result in a conflict between two innate drives: the drive to explore potential rewards and the drive to avoid potential harmful situations. Numerous brain circuits are involved in this approach-avoidance conflict. Amongst them, the mesocorticolimbic system—i.e. dopamine—plays a pivotal role in exploratory, motivational and reinforcing learning processes, while the amygdala (AMG) complex is known to be instrumental in avoidance and fear-learning mechanisms. However, little is known about how approach-avoidance behaviors are determined by the circuit activity of ventral tegmental area (VTA) dopamine neurons projecting to the AMG. In this study, we investigated VTA-to-AMG dopamine neuron’s activity on approach-avoidance behaviors, and further investigated how this circuit’s function is regulated by chronic stress, a major factor that impairs the innate approach-avoidance balance under disease conditions such as anxiety disorders.

Methods: To determine the VTA-to-AMG dopamine circuit’s function in approach-avoidance behaviors, we used a cell- and circuit-specific probing approach combining viral strategies with a transgenic male TH-BAC-Cre mouse line. The statistics sample value (n subject 8-15 per group) was analyzed depending on the sample size, normality, homoscedasticity and potential nested effects. All behavioral and electrophysiological datasets were subjected to Factorial ANOVA’s, followed with Bonferroni t-test corrected to maintain an experiment-wide alpha of p < 0.05. For data not following normal distribution as calcium imaging data, non-parametric analyses of correlation, means and variance was used. We characterized the VTA-to-AMG circuitry using retrograding tools, immunohistochemistry and IDISCO imaging. We then used fiber photometry recordings to probe VTA-to-AMG neuronal activity during the elevated plus maze (EPM) task. Further, mice were exposed to a repeated social defeat stress (RSDS) paradigm to analyze VTA-to-AMG circuit activity in animals displaying altered approach-avoidance behaviors. Finally, to define the causal link between VTA-to-AMG circuit activities with approach-avoidance behaviors, we used electrophysiological recordings to identify underlying pathological alterations and employed an optogenetic circuit-specific approach to manipulate VTA-to-AMG circuit activities and consecutive approach-avoidance behaviors.

Results: Our fiber photometry recordings from VTA-to-AMG projecting neurons showed that approach towards the EPM open arms is associated with increased VTA-to-AMG circuit activity in stress-naive mice (Spearman correlation r = 0.5, P < 0.05). Using the RSDS paradigm, we demonstrated that socially stressed animals undergo individual alterations in their approach-avoidance balance, displaying either or both social avoidance behaviors (One-way ANOVA P < 0.001) and a decreased time in EPM open arms and open-field center (One-way ANOVA P < 0.01). We then measured VTA-to-AMG projecting neuron activity during the expression of these phenotypes, which is correlated with decreased time in EPM open arms. Using in vitro electrophysiological recordings, we confirmed our fiber photometry results—decreased VTA-AMG dopamine neuron’s spontaneous activity is associated with a decreased time in EPM open arms (Linear regression, r = 0.5, P < 0.05). Utilizing optogenetic manipulations, we further showed that halorhodopsin-mediated decrease in VTA-to-AMG circuit activity decreased the time in EPM open arms (t test P < 0.01), and conversely that channelrhodopsin mediated activation of VTA-AMG circuit activity rescued the approach-avoidance imbalance observed following RSDS (t test P < 0.01).

Conclusions: Our results support VTA-to-AMG neuron’s activity mediates approach-avoidance behaviors. In response to repeated
exposure to social stress, the VTA-to-AMG circuit displays hypo-
dopaminergic activity. This hypo-dopaminergic state is causally
associated with RSDS-induced pathological approach-avoidance
behaviors. Overall, these data provide highly novel information
regarding the contribution of dopamine circuit-function specificity
in the expression of approach-avoidance behaviors. Moreover,
these findings may provide useful information in the identification
of new therapeutic targets for psychiatric disorders, in particular
for disorders related to approach-avoidance behaviors such as
anxiety disorders.

Keywords: Dopamine, Amygdala, Circuit-Function, Approach-
Avoidance

Disclosure: Nothing to disclose.

T90

Effects of Social Media Use on Self-Esteem, Depressive
Symptoms, and Physiological Markers of Stress in Depressed
and Healthy Adolescents

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Background: Social media use is an inherent dimension of
adolescent daily life and development. However, there are limited
data regarding the impact of social media use on neurodevelop-
ment and psychiatric outcomes. Research regarding specific
differences in social media use and effects among adolescent
with and without psychiatric disease is also lacking. The current
study sought to examine patterns of social media use and the
ensuing impact on self-esteem, depressive symptoms, and
biomarkers of stress among psychiatrically hospitalized adoles-
cents and healthy adolescent controls. It was hypothesized that
excessive social media use would correlate with depressive
symptom severity. It was further hypothesized that a brief period
of social media use would impact salivary biomarkers of stress and
clinical measures of self-esteem and depressive symptom severity.

Methods: This was a prospective, case-control study of
depressed adolescents aged 13–17 years hospitalized in a
psychiatric unit (n = 30) compared healthy control adolescents
matched for age, gender, and age of first social media use (n = 30).
After a period of abstinence from social media use (at least
24 hours) participants completed a series of clinical assessments
prior and subsequent to 20 minutes of social media use. Baseline
measures included the Mini-International Neuropsychiatric Inter-
view for Children and Adolescents (MINI-KID), Quick Inventory of
Depressive Symptomatology, 17 item, adolescent self-report
(QIDS-A17-SR), Rosenberg Self Esteem Scale (RSES), Bergen Social
Media Addiction Scale (BSMAS), and a Cyberbullying Scale. Post
social media use measures included the RSES and QIDS-A17-SR.
Saliva samples were obtained before and after social media use for
amyrase and cortisol levels quantified by enzyme-linked immu-
nosorbent assay (ELISA).

Results: At baseline the BSMAS scores were higher (p < 0.001)
in the depressed group (mean 17.57 [SD 5.48]) as compared to
the healthy controls (11.33 [SD 4.47]). Baseline, self-esteem scores
were lower (p < 0.001) in the depressed group (mean 22.17 [SD
4.84] vs. mean 34.27 [SD 4.38]). Among the entire sample (n = 60)
there was a positive correlation (r = 0.58, p < 0.001) between
BSMAS scores and depressive symptom severity rated on the
QIDS-A17-SR. Salivary alpha amylase (p < 0.001) and cortisol levels
(p < 0.05) increased after SMU among depressed participants but
not healthy controls. There were no significant differences in pre
and post social media use and self-esteem (Rosenberg Self Esteem
Scale) and depressive symptom severity (QIDS-A17-SR) measures.

Conclusions: To our knowledge, this is the first study of its kind
to examine biomarkers of stress and clinical measures after
prospective social media use in depressed and healthy adoles-
cents. There appear to be differences in social media use between
depressed and control adolescents. Depressed adolescents appear
to have more features of problematic social media use and are
more physiologically reactive to social media use. Further research
to delineate the specific characteristics of social media use in
depressed and healthy adolescents could assist with efforts to
understand the impact of social media as a risk factor for
psychiatric hospitalization, depression, and suicide. The present
findings provide further support for our assertion that social
media use should be assessed in standard psychiatric interviews.

Keywords: Adolescent Depression, Social Media Use, Acute and
Chronic Stress, Cortisol, Biomarkers

Disclosure: Neuronetics, Inc., Grant, Procter & Gamble Com-
pany, Consultant, Assurex, NeoySync

T91

Isolation Matters: Different Maternal Separation Protocols
Differentially Influence Pup Behavior and Perineuronal Net
Development in Male and Female Rats

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Background: Early life adversity (ELA) can increase susceptibility
to psychiatric disorders later in life, largely due to aberrant
development of regions controlling affective regulation, such as
the prefrontal cortex (PFC). Males and females experience sex-
specific consequences of early adversity, which may be due to sex-
dependent developmental trajectories or to sex-specific experi-
ences within the same early life environment. Animal models have
been helpful to clarify the causal relationships between ELA and
atypical phenotypes, however, they also bring new issues
including paradigm differences between laboratories. For exam-
ple, maternal separation (MS) is one ELA model that derails
development of behavior and brain circuitry, however the MS
paradigm sometimes involves isolation of pups, while sometimes
litters are kept together during the separation. In order to explain
reports of varied outcomes across laboratories, here we directly
compared the two types of MS. Additionally, cross-paradigm
predictors of later behavioral changes are needed to guide
strategies for identification and treatment of vulnerable indivi-
duals. Ultrasonic vocalizations (USVs) in pups act to evoke
maternal care such as searching and retrieval, and therefore play
an important role in mother-pup communication; we hypothe-
sized that early USV composition would predict later anxiety-like
and social behavior. We investigated 1) whether isolated MS and
litter MS confer different behavioral outcomes and PFC inter-
neuron development; and 2) how altered infant-mother commu-
nication following MS predicts later behavioral deficits in males
and females

Methods: Study 1) Pups either underwent MS for 4 hrs/day on
postnatal days (P)2-20 in isolation or with their entire litter, or
were control-reared. Pups were weaned from their mother at P21
and were first placed in a cage with home bedding for 5 min while
USVs were recorded using an Avisoft-Bioacoustics ultrasound
microphone positioned 10 cm above the cage. Spectrograms for
each recording were created using the DeepSqueak system for
Matlab, and 9 unique sonographic structures were identified.
Individual USVs were classified by type, and duration, peak


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ADHD (subcortical volume) and diffusion-weighted (fractional anisotropy) measures were correlated with later behavior. Following MS or control rearing, brains were perfused at weaning age (P20-22), adolescence (P40), or adulthood (P70). PFC sections were stained for Wisteria floribunda agglutinin (WFA) and anti-PV antibody to visualize WFA+ perineuronal nets and parvalbumin interneurons, respectively. Z-stacks were obtained using fluorescent microscopy in 4 serial sections of the PFC. ImageJ was used to count WFA+ PNNs, PV cells, and PNNs surrounding PV cells, and density was calculated.

Results: Both males and females exposed to isolation MS emitted fewer USV characterized as ‘upsweeps’ than controls upon weaning (p = 0.0025), while isolated MS decreased ‘short calls’ only in females (p = 0.0063). Litter MS had no effect on weaning USVs compared to controls. Isolation MS also delayed PNN formation around PV interneurons in females, but not males; PNN-ensheathment of PV cells was lower in isolation MS-exposed females at weaning (p = 0.034). In males (p = 0.033), but not females (p = 0.859), fewer upsweeps emitted during weaning predicted less time spent in the open area of the EZM in adolescence. Fewer upsweeps also predicted a longer latency to approach a conspecific in adolescent males (p = 0.043) but not females.

Conclusions: Short calls and upsweeps are effective at pup localization and therefore encourage retrieval; it is possible that aberrant development of PFC inhibition following isolation MS, but not MS with littermates is related to a reduced ability or motivation to solicit care. Social buffering from littermates may protect pups from some developmental effects of maternal deprivation. These results also demonstrate that male and female juveniles uniquely experience MS, which may provide insight into sex differences in the influence of adversity on development of brain circuitry.

Keywords: Rat Models, Ultrasonic Vocalization, Perineuronal Nets, Parvalbumin Neurons, Medial Prefrontal Cortex

Disclosure: Nothing to disclose.

Novel Brain-Behaviour Similarity Subgroups Across Neurodevelopmental Disorders

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Background: Neurodevelopmental disorders (NDDs) such as autism spectrum disorder (ASD), obsessive-compulsive disorder (OCD), and attention-deficit/hyperactivity disorder (ADHD) are each highly heterogeneous conditions that feature significant overlap in behaviours, genetic risk factors, cognitive and brain alterations. We used Similarity Network Fusion (SNF), a multi-data integrative clustering tool, to identify novel groups across NDDs featuring more homogeneous brain-behaviour profiles than those associated with categorical DSM diagnoses.

Methods: Measures from T1-weighted (cortical thickness, subcortical volume) and diffusion-weighted (fractional anisotropy) magnetic resonance imaging, and behavioural scores, were obtained for 182 children, aged 6-16 years with ASD (n = 91), ADHD (n = 52) or OCD (n = 39) from the Province of Ontario Neurodevelopmental Disorders (POND) Network. Data integration and spectral clustering were done using SNF. General adaptive functioning measures (not involved in cluster determination) were used to evaluate validity of the identified groups.

Results: Four groups with distinct brain-behaviour profiles that cut across clinical diagnoses were identified. Group formation and top contributing measures driving formation were shown to be stable with resampling. General adaptive functioning (F = 21.46, p < 0.0001, n2 = 0.28) was significantly different between groups, as were top contributing neurobiological features: right insula thickness (F = 47.76, p < 0.0001, n2 = 0.44) and right thalamic volume (F = 18.51, p < 0.0001, n2 = 0.24).

Conclusions: Our work provides preliminary indication that multi-modal data-integration methods such as SNF can identify novel participant subgroups that cut across conventional DSM categories in children with NDDs and feature more homogeneous brain-behaviour profiles than found with conventional diagnoses. Stability across other samples and testing of clinical validity of these groups is needed to determine whether they may have utility for diagnostic and treatment innovation.

Keywords: Neurodevelopmental Disorders, Clustering, MR Imaging

Disclosure: Nothing to disclose.

Interplay Among Trauma, Socioeconomic Status and Suicidal Ideation Throughout Adolescence

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Background: Early life environmental adversities are associated with teen suicidal ideation (SI), yet limited data exists on the relationship among types of environmental adversities (such as trauma exposure and low socioeconomic status (SES)) and their association with SI in different stages of adolescence. The Philadelphia Neurodevelopmental Cohort (PNC) is an investigation of clinical and neurobehavioral phenotypes in a diverse (56% Caucasian, 33% African American, 11% other) US youth community population assessed between 2009–2011. Participants were non-mental-help-seeking youth ascertained through the Children’s Hospital of Philadelphia (CHOP) general (non-mental) health system. Here we studied the interaction of early-life trauma exposure and SES in association with SI across adolescence in youths who were interviewed in the PNC (N = 7,054, age range 11-21, mean 15.8).

Methods: Clinical phenotyping was conducted using a K-SADS based interview that assessed lifetime exposure to potential traumatic stressful events (TSE) including situations in which the participant (1) experienced a natural disaster or (2) experienced a bad accident; (3) thought that s/he or someone close to him/her could be killed or hurt badly; (4) witnessed someone getting killed, badly beaten, or die; (5) saw a dead body; or if s/he ever was a victim of one of the following assaults: (6) attacked or badly beaten, (7) threatened with a weapon, or (8) sexually forced (including but not limited to rape). Individual-level SES score was calculated based on participants’ home addresses, that were geocoded and linked to US Census block-groups, and data from the Census’ American Community Survey was used to characterize block-groups (“neighborhoods”). Statistical modeling was conducted using binary logistic regression models with SI as the dependent variable and trauma exposure, SES, and their interaction behavior in a dyad, and weaning USV measures were correlated with later behavior. Following MS or control rearing, brains were perfused at weaning age (P20-22), adolescence (P40), or adulthood (P70). PFC sections were stained for Wisteria floribunda agglutinin (WFA) and anti-PV antibody to visualize WFA+ perineuronal nets and parvalbumin interneurons, respectively. Z-stacks were obtained using fluorescent microscopy in 4 serial sections of the PFC. ImageJ was used to count WFA+ PNNs, PV cells, and PNNs surrounding PV cells, and density was calculated.
interaction as independent variables. Models co-varied for age, gender, race and general psychopathology. Separate models evaluated interaction among environmental adversities and age.

Results: Trauma exposure (at least 1 TSE) was prevalent (n = 3,490, 49.5% of the sample), with almost 10% of the sample reporting lifetime history of SI (n = 671). Trauma was associated with SI (odds ratio (OR) = 1.5, 95%CI 1.4-1.6, p < 0.001, for each additional TSE), while SES was not associated with SI (p = 0.237). In youths with high trauma exposure (3+ TSEs), low SES youths reported less SI compared to high SES counterparts (Trauma X SES interaction, Wald = 14.3, p < 0.001, co-varying for age, gender and race). Evaluation of age effects showed that while trauma was associated with SI throughout adolescence (age 11-21), low SES was associated with SI only in early adolescence (under age 14, SES X age interaction, Wald = 7.7, p = 0.006, co-varying for gender, race and general psychopathology).

Conclusions: In a single large community youth sample, we show moderating effects of (1) SES on the association between trauma and SI; and (2) of age on the association between low SES and SI; and (2) of age on the association between low SES and SI. Results point to specificity in the relationship of SI with different types of environmental adversities in different adolescence epochs. Specifically, youths from high SES may be particularly at risk for SI if they have high trauma exposure, and coming from low SES may be associated with SI only in early adolescence. The cross-sectional study design limits causal inferences. Findings might inform risk stratification for youth SI.

Keywords: Suicide Risk Factors, Adverse Childhood Events, Environmental Risk Factors, Suicidal Ideation

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

T94

Youth Exposed to Maltreatment Show Age-Related Alterations in Hippocampal-Fronto-Amygdala Function During Extinction Recall

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Background: Exposure to childhood trauma is a major risk factor for psychiatric disorders. Delineating the neurodevelopmental mechanisms linking early-life trauma to psychopathology is critical for the early identification of risk and optimizing interventions to promote resilience following trauma. Youth exposed to maltreatment show alterations in fear conditioning and related hippocampal-frontoamygdala circuitry, yet much remains unknown about extinction and extinction recall, processes that are central to discriminating threat from safety following trauma.

Methods: Children and adolescents ages 8–17 years old (N = 161; 77 female, 84 male) with (n = 87) or without (n = 71) exposure to maltreatment (physical abuse, sexual abuse, domestic violence) completed a fear conditioning and extinction task which used blue and yellow bells as conditioned stimuli (CS+/CS-) and an aversive alarm noise as the unconditioned stimulus, across two separate days. At the first session participants completed acquisition and extinction during measurement of skin conductance response. Within one week, participants completed extinction recall and re-extinction during fMRI scanning. Analyses focused on the amygdala, hippocampus, and subgenual anterior cingulate cortex (sgACC) as a priori regions of interest defined using the Harvard Oxford probabilistic atlas. Given hypotheses about the impact of maltreatment on hippocampal and fronto-amygdala development, we tested whether maltreatment was associated with altered age-related patterns of activation during extinction recall and re-extinction.

Results: Youth with maltreatment exposure showed altered age-related patterns of activation in the amygdala (maltreatment x age interaction: F(1, 125) = 5.40, p = 0.022) and hippocampus (maltreatment x age interaction: F(1, 125) = 4.58, p = 0.034) during extinction recall. Specifically, whereas youth without a history of maltreatment showed stable activation with age, youth with maltreatment exposure showed age-related increases in amygdala and hippocampus activation to the CS- (vs. CS+) during recall. Findings also revealed an interaction between maltreatment, age, and sex (F(1,125) = 4.86, p = 0.029), such that females (but not males) with maltreatment exposure showed an altered age-related pattern of sgACC activation during re-extinction. Whereas females without a history of maltreatment showed an age-related decrease in sgACC activation to the CS+ (vs. CS-) during re-extinction, females with maltreatment exposure did not show an age-related change in sgACC activation.

Conclusions: Childhood maltreatment may alter the typical developmental course of fear extinction and related brain activation. In particular, youth exposed to maltreatment showed exaggerated amygdala and hippocampus activation to safety with age, suggesting a possible failure to discriminate between threat and safety in an age-expected manner at the neural level. Altered hippocampal and fronto-amygdala function may underlie difficulties learning or integrating environmental cues signaling safety that could increase risk for maltreatment-related psychopathology during development.

Keywords: Childhood Trauma, Maltreatment, Brain Development, Amygdala, Hippocampus

Disclosure: Nothing to disclose.

T95

Pattern of Cerebellar Lobular Volume Deficits in Adolescents and Adults With Fetal Alcohol Effects (FAE) and Fetal Alcohol Syndrome (FAS)

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Background: It is well-established that prenatal exposure to alcohol can cause distinct facial anomalies, behavioral and cognitive disturbance, and underdeveloped intracranial volume (ICV) of the brain. Among the brain regions this teratogen targets is the cerebellum. Despite its structural complexity, the cerebellum is commonly measured as an undifferentiated structure or as three regions of the midline vermis. The aims of this analysis were to use a recently developed method to parcelate and quantify selective regions of the midline vermis. The aims of this analysis were to use a recently developed method to parcellate and quantify selective lobules of the cerebellar hemispheres 1) to establish a pattern of volumetric sparing and deficit; 2) to determine whether regional volume deficits can be explained by ICV or whether they endure ICV-adjustment; and 3) to test for a graded severity effect from the less severe diagnosis of fetal alcohol effects (FAE) to the severe diagnosis of fetal alcohol syndrome (FAS). This analysis, which is based on legacy data, is unique, has not been published previously, and provides novel results on cerebellar morphology in adolescents and adults with FAE/FAS.

Methods: Native T1-weighted MRI legacy data (acquired on a 1.5T GE Sigma system between 1997–2000) from the Seattle FAS Follow-up Database included 60 controls, 58 individuals systematically diagnosed, using 1994 criteria, with FAE, and 61 with FAS. The groups were matched in age (mean ± SD years, FAS = 19.9 ± 5.4 for
controls; 20 ± 5.9 for FAE; 20.0 ± 5.8 for FAS) and sex (49% male). MRI data were analyzed with Ceres from volBrain [http://volbrain.upv.es], which yielded total ICV plus segmentation of total cerebellar tissue volume, gray matter cerebellar volumes, and a derived volume of white matter (total-gray matter = white matter) and parcellation of the gray matter volume into 12 lobules (I-II, III, IV, V, VI, Crus I, Crus II, VIIB, VIIA, VIIIb, IX, X).

Results: ICV showed a diagnostically graded effect with control > FAE and FAS (p < 0.0001); FAE > FAS (p = 0.02). Raw volumes unadjusted for ICV differences indicated marked deficits in FAE and FAS separately in all cerebellar regions relative to controls (p = 0.016 to 7.19-e) and graded volume deficits (FAE > FAS p ≤ 0.01) total gray matter, total white matter, and gray matter of lobules IV, V, VI, Crus II, and X. The unadjusted graded differences for lobular (but not total) volumes endured with residualized ICV correction based on the controls (p = 0.037 to 0.003). Volumes calculated as percent of one’s own ICV indicated that volume deficits in the total FAE+FA group endured in lobules II-L, III, V, Crus II, and X (p = 0.05 to 0.0014) but were attenuated in the diagnostic subgroups; nonetheless, the graded effect (FAE>FAS) persisted in lobules V (p = 0.0051) and X (p = 0.019), indicating that these lobules were even smaller than would be expected for an individual’s ICV.

Conclusions: In general, the groups exposed to alcohol during fetal development exhibited graded effects of cerebellar volume deficits, providing support for a spectrum based on severity and a diagnostic distinction of this legacy cohort of adolescents and adults. Compared with unaffected controls, both the FAE and the FAS groups had widespread cerebellar volume deficits, which extended to gray matter and white matter and to all 12 hemispheric lobules. The regional volume deficits in IV, V, VI, Crus II, and X gray matter, which included lobules associated with cognitive and emotional processing, were even greater than expected based on control group values for their abnormally small ICV. We speculate that the regional cerebellar FAE/FAS-related volume deficits may contribute to diagnostically-characteristic functional impairment involving emotional control, visuomotor coordination, and postural stability.

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Keywords: Cerebellum, Fetal Alcohol Spectrum Disorder, Age, MRI, Lobules

Disclosure: Nothing to disclose.

T96
Stress-Mediated Alterations in Maternal and Placental Extracellular Vesicles and Impact on Offspring Neurodevelopment

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Background: Parental lifetime exposures to perturbations such as stress, infection, malnutrition, and advanced age have been linked with an increased risk for offspring disease, especially neurodevelopmental disorders. In our well-established mouse model of early prenatal stress (EPS), maternal stress exposure during the first week of gestation impairs long-term developmental programming deficits in male, but not female, offspring, resulting in hypersensitivity to stress, cognitive impairments, and alterations in metabolic programming. The placenta, a fetally-derived tissue reflecting fetal sex chromosome complement, acts as an arbitrator between the mother and fetus, providing necessary factors for early fetal neurodevelopment. Previously, our lab found that gene sets important for endo- and exosomal cellular processes were downregulated in the male placenta in response to EPS, suggesting that EPS alters fetal extracellular vesicle (EV) signaling. EVs are small vesicles secreted locally and into the bloodstream by most tissues and function to transfer proteins, microRNAs, and other signaling factors between cells and tissues as a means of short- and long-distance communication. Preliminary data suggests that EPS alters key characteristics of fetal EVs in a sex-specific way, including EV size, small RNA content, and proteomic landscape. We hypothesize that these changes in EV characteristics contribute to sex differences in programmatic events in the developing brain. Here, we examine the dynamic interaction between both maternal and fetal EVs with the placenta as a potential mode of transmission of EPS to fetal brain development.

Methods: Pregnant female mice were exposed to early prenatal stress (EPS) from embryonic days (e) 0-7. To address the role of EV signaling via the placenta being altered by prenatal stress, pregnant females were sacrificed at e17.5 and EVs were isolated from trunk blood. EVs from control and EPS-exposed dams were injected into e17.5 naive dams. A cohort of naive dams received EVs labelled with near infrared dye and were sacrificed 24 hours following EV exposure. Using near infrared in vivo imaging of labelled EVs, we examined the effect of EPS on the trafficking of EVs in naive dams and fetuses. To examine the effect of EPS EV exposure on the placental transcriptome, a separate cohort was injected with non-dyed EVs from control and EPS dams at e17.5 and placental and fetal tissue was collected at e18.5 for RNA-seq. A cohort of naive dams was allowed to birth their litters following EV injection and offspring were followed through adulthood for growth and assessed as adults for hypothalamic-pituitary-adrenal (HPA) stress axis reactivity.

To address a role for EVs in the male-specific negative consequences of EPS, EVs were isolated from control and EPS-exposed neonates, labelled with near infrared dye, and were injected into age-matched naive neonates. A cohort of naive neonates was sacrificed at several time points within 24 hours following EV injection to investigate the disbursement of EVs throughout the body and into the brain via near infrared imaging. A cohort of naive neonates was followed through adulthood for growth and assessed as adults for hypothalamic-pituitary-adrenal (HPA) stress axis reactivity. Small RNA-Sequencing was performed on EVs isolated from control and EPS-exposed neonates to investigate potential alterations in EV cargo as a result of EPS exposure.

Results: EVs from control and EPS-exposed neonates were differentially trafficked into the brain of naive neonates in both EPS- and sex-specific ways. Preliminary analysis of labelled EVs suggests that EVs derived from EPS-exposed males promoted long-term changes in male body weight and HPA stress axis sensitivity, while female EPS EVs had no long-term programmatic effects on naive females. The hypothalamus of naive neonates injected with EVs derived from control and EPS-exposed neonates was subject to RNA-Seq, allowing us to link individual EV microRNAs with changes in mRNA expression in the developing brain.

Conclusions: Together, these studies support a role for EVs as circulating molecules that are sensitive to prenatal stress exposure. These results suggest that EVs may be an important mode of transmission from mother to fetus and that EPS-induced changes in offspring brain development may be due to altered EV cargo or targeting at the placenta. Together, these studies provide insight into the role of EVs in both maternal and fetal circulation, their interaction at the level of the placenta, and the impact of prenatal stress on important signaling dynamics between mother and fetus during gestation.

Keywords: Extracellular Vesicles, Prenatal Stress, Sex Differences

Disclosure: Nothing to disclose.
Perinatal Interference With the Serotonergic System Affects VTA Function in the Adult

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Background: Serotonin and dopamine are neurotransmitters associated with multiple psychiatric disorders. How they interact during development to affect subsequent behavior remains unknown. Knockout of the serotonin transporter or administration of selective-serotonin-reuptake inhibitors (SSRIs) during early-life lead to novelty-induced exploration deficits in adulthood.

Methods: Using a combination of optogenetics, behavioral testing and electrophysiology we tested the effects of perinatal exposure to fluoxetine (PN-FLX) on dopaminergic system’s function in the adult. Between 10 to 15 mice per group, male and female, were administered with saline or fluoxetine (10 mg/kg IP) from P2 to P11. Mice were tested after 8 weeks of age.

Results: Here we show that Raphe nucleus serotonin neurons activate ventral tegmental area (VTA) dopamine neurons via glutamate cotransmission and that this cotransmission is impaired in postnatally SSRI treated animals. Moreover, we show that the SSRI-induced hypolocomotion is mimicked by blocking serotonin neuron glutamate cotransmission. Optogenetic activation of dopamine neurons rescued this hypolocomotor phenotype.

Conclusions: Our data demonstrate that serotonin neurons modulate dopaminergic activity via glutamate cotransmission and that this pathway is developmentally malleable, with high serotonin levels during early life blunting this capacity, resulting in reduced novelty-induced exploration in adulthood.

Keywords: Serotonin, Dopamine, Early-Life, Development

Disclosure: Nothing to disclose.

Multimodal Assessment of Trauma Exposure on the Developing Brain

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Background: Childhood adversity has been shown to increase risk for psychiatric disorders across the lifespan. Most studies showing the effect of childhood trauma on the brain are retrospective and mainly focus on childhood abuse, whereas different types of trauma as well as timing of trauma could have important effects on the developing brain. The current MRI study assessed the effect of different types of trauma exposure and age on structure and function of the fear neurocircuitry in children.

Methods: 69 children ages 8-14 years were recruited through an ongoing study of an inner-city population with high trauma exposure. Clinical measures included the Traumatic Events Screening Inventory (TESI) to assess for lifetime exposure to traumatic events, and the Violence Exposure Scale for Children, Revised (VEX-R) to assess lifetime frequency of exposure to violence. Structural and functional MRI scans were collected. Freesurfer v6.0 was used to extract hippocampal and amygdala volumes (N = 63). An emotional Go/NoGo fMRI task was used to measure response inhibition in an emotional context. Region of interest analyses were performed for the bilateral hippocampus, amygdala and vmPFC (N = 47). Correlation analyses were conducted for TESI or VEX-R with brain measures. Secondary regression analyses were conducted using age group (<10 vs. ≥10 years) and location of violence (inside the home vs. community/school) as additional predictors. Analyses were corrected for intracranial volume (ICV; for structural measures), age and sex.

Results: The number of traumatic events (TESI) was negatively related to left hippocampal volume (r = -0.35, p = 0.006) and positively with left amygdala volume (r = 0.26, p = 0.046) correcting for ICV, age, and sex. No significant correlations between TESI and any functional measures were observed. Violence exposure was positively associated with inhibition-related activation (NoGo versus Go trials) in the hippocampus (r = 0.39, p = 0.009), amygdala (r = 0.34, p = 0.024), and vmPFC (r = 0.35, p = 0.017), correcting for age and sex. Secondary analyses showed an interaction between age group and violence exposure for hippocampal (F(3, 46) = 4.63, p = 0.007; interaction, t = -2.08, p = 0.043) and amygdala activation (F(3, 46) = 3.59, p = 0.021; interaction, t = -2.42, p = 0.020), such that significant correlations were only observed in younger children (hippocampus, r = 0.59, p = 0.037; amygdala, r = 0.75, p = 0.003). Additional analyses were conducted to investigate the effect of violence location (home versus community/school). The model including VEX-R frequency and violence location was significant for amygdala activation (F(2, 46) = 4.23, p = 0.021). In this model, violence frequency was a significant predictor (t = 2.03, p = 0.049) and location was only marginally significant (t = 1.89, p = 0.066). Exploratory analyses showed that children experiencing more violence at home versus at school or neighborhood demonstrated more amygdala activation (t(45) = 2.02, p = 0.049).

Conclusions: Here we show differential effects of type and timing of trauma on the brain’s fear neurocircuitry structure and function. Lifetime traumatic experience is associated with structural changes in the limbic system, whereas violence exposure is related to increased limbic and prefrontal activation. The effects of violence exposure on brain functioning are specifically observed in the younger children, underscoring the importance of a potential critical developmental period. Given the importance of fear neurocircuitry for psychiatric disorders, understanding the effects of different types of trauma exposure during development is essential for early detection of individuals at risk.

Keywords: Early Life Stress, Hippocampus, Amygdala, Inhibition, Neuroimaging

Disclosure: Nothing to disclose.

The Longitudinal Relationship Between White Matter Structural Integrity and Emotion Regulation in Neonates: A Preliminary Study

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Background: The rapid development of the human brain in the first years of life establishes important brain-behavior relationships that set the stage for future health outcomes. Most of this research has focused on normative neurodevelopmental processes; little is known about how early alterations in white matter microstructure
relate to clinically relevant behavioral outcomes. Emotional dysregulation is an early transdiagnostic risk factor for subsequent child behavioral and emotional problems. Thus, our goal was to examine white matter structural integrity within key limbic (i.e., uncinate fasciculus and cingulum) and association (i.e., forceps minor) bundles in 3-month-old neonates and determine their prospective relationships to emotion regulation behaviors at 9 months.

Methods: Mothers (age 19–24) and their 3-month-old infants (male and female) were recruited from the population-based, longitudinal Pittsburgh Girls Study (PGS). Pregnant or recently delivered participants were identified during annual PGS interviews. Following informed consent, mother-infant dyads completed research visits at the Children’s Hospital of Pittsburgh (CHP).

At 3 months, magnetic resonance imaging was performed on a 3T scanner (Siemens Skyra, 32-channel head coil) at CHP during natural, non-seated sleep. Diffusion-weighted imaging data was acquired using a ‘neurite orientation dispersion and density imaging’ (NODDI) multishell diffusion scheme. The b-values were 100, 250 and 750 s/mm² and the numbers of diffusion sampling directions were 8, 32 and 64. The in-plane resolution was 2 mm and the slice thickness was 2 mm. Following image acquisition, NODDI images were manually realigned, motion and eddy current corrected and reconstructed in native space using generalized Q-sampling imaging (GQI) in DSI Studio. Images with artifact, signal loss, or distortion were excluded from further analyses. Tractography was performed for each tract of interest (e.g., cingulum, uncinate fasciculus and forceps minor) within each infant. Quantitative anisotropy (QA) was extracted from each tract from uncinate fasciculus and forceps minor) within each infant.

Neonatal, White Matter Integrity, Cingulum, Uncinate Fasciculus, Emotional Regulation

Disclosures: Nothing to disclose.

T100

Are Social Attention Deficits Consistent Across Social Orienting Eye Tracking Paradigms in Autism Spectrum Disorder?

Abstract not included.

T101

Placental Programming in a Rodent Model of Maternal Immune Activation

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Background: Placental programming can be triggered by adverse experiences that take place across pregnancy, affecting the developmental trajectory of the fetus. In contrast, the influence of ‘positive’ gestational experiences remains unclear. In animal models, rearing in environmental enrichment (EE) has rescued impairments in brain and behavior following exposure to prenatal stressors. Using a maternal immune activation (MIA) model in rats, we test whether EE attenuates maternal, placental and/or fetal responses to an inflammatory challenge, thereby offering a mechanism by which fetal programming may be prevented.

Methods: Female Sprague Dawley rats (Charles River Laboratories, Wilmington, MA), were housed in one of two conditions: EE, comprised of large multi-level cages with ramps and access to toys, tubes, chew bones, and Nestlets® (Ancare, Bellmore, NY), or standard cages (SD) with only a tube, chew bone and Nestlets®. Following breeding, on gestational day 15, dams received an i.p. injection of 100 μg/kg of lipopolysaccharide (LPS; Escherichia coli serotype 026:B6; L-3755, Sigma, St. Louis, MO) or an equivalent volume of pyrogen-free saline. Two groups of dams (N = 56) were euthanized by ketamine/xylazine (40–80 mg/kg, i.p./5–10 mg/kg, i.p.) at either 3 h or 24 hr following the LPS challenge. Maternal plasma samples were collected and stored for later validation of MIA by corticosterone and interleukin (IL)-1β levels. Dams were perfused intracardially with a phosphate buffer solution and the uterine horn removed. Placentas, whole fetal brains, and bodies were saved for DNA and RNA isolation and sex identification. A third set of dams (N = 37), also treated with LPS or saline, were permitted to continue their pregnancy through until birth (postnatal day P11). Offspring were maintained in their respective housing conditions with their mothers until weaning. At that point, male and female offspring were placed
into clean cages, maintaining their same neonatal housing conditions until testing. Two (gestational treatment x housing) and three-way (sex x gestational treatment x housing) ANOVAs were conducted, as appropriate. If data were significantly skewed (Shapiro-Wilk), Kruskal-Wallis tests (non-parametric equivalent to ANOVA) were employed, followed by Mann-Whitney U tests. Pairwise comparisons were made using conservative adjusted alpha correction values.

Results: Maternal immune activation was confirmed by elevated plasma corticosterone (p = 0.0001) and IL-1β (p = 0.0001) 3 h after LPS challenge. A simultaneous decrease in the placental enzymes Hsd11b2 (p = 0.008) and Hsd11b2/Hsd11b1 (p = 0.024) suggested disturbances in glucocorticoid metabolism. While the maternal glucocorticoid implications of MIA were sustained, the placental effects were mitigated by EE. Moreover, there were placental and fetal brain changes in epigenetic marker gene expression (OGT, MECP2, DNMT1, DNMT3a) as a consequence of environmental experience and sex. Social discrimination ability was impaired in both male and female juvenile animals following MIA (p = 0.014). Life-long EE mitigated these impairments (p > 0.05), in addition to the sex specific MIA associated disruptions in central Fkbps (p = 0.002) and Omp1 (p = 0.009).

Conclusions: Overall, changes in placental functioning offer a potential mechanism by which the positive care of mothers can lead to a beneficial or protective outcome for the offspring. This underscores the importance of supporting maternal health throughout pregnancy and beyond.

Keywords: Maternal Immune Activation, Environmental Enrichment, Placenta

Disclosure: Nothing to disclose.

T102

Maternal Childhood Adversity and Infant White Matter Connectivity: A Pilot Study of Prenatal Maternal Cortisol Effects

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Background: Childhood adversity (conceptualized here as sexual, physical, emotional abuse and/or neglect) confers increased risk for a host of physical and mental health problems (Hughes et al. 2017). Increased risk for negative outcomes persists though adulthood and includes increased risk for all the common mental health disorders (e.g. mood, substance use disorders (Green et al. 2010), as well as chronic health problems (Norman et al. 2012). Atypical stress responses have been postulated as a potential mechanism explaining the long-term consequences of childhood adversity, as trauma exposure during important developmental periods may permanently dysregulate stress responses (Tarullo et al. 2006, Kuras et al. 2017). One such stress response is the hypothalamic–pituitary–adrenal (HPA) axis. Cortisol, when dysregulated, can contribute to allostatic load (McEwen 1998) and health deterioration. Because maternal cortisol can cross the placenta, and glucocorticoids have been consistently found to alter hippocampal structure and function (Bunea et al. 2017), it is possible that childhood adversity and associated cortisol dysregulation can have intergenerational effects by impacting fetal neurodevelopment. This pilot study of mothers with and without a history of childhood adversity and their neonates in São Paulo, Brazil aimed to examine if prenatal maternal cortisol impacts offspring white matter microstructure, particularly focusing on hippocampal connectivity.

Methods: This pilot study was conducted at the Universidade Federal de São Paulo (UNIFESP) and approved by the local IRB (2.496.978). Sixty-four pregnant women (26.9 ± 16.21 years) were recruited during their third trimester of pregnancy. Half of the mothers had experienced childhood adversity (+a group), half had not (-a group). Women completed a psychiatric assessment including the Childhood Trauma Questionnaire (Grassi-Oliveira et al. 2014) and MINI (Sheehan et al. 1998). Substance use was Maternal hair samples were collected (~10 mg of hair) from the posterior vertex as close to the scalp as possible. The most proximal 3 cm of hair was processed and dried samples were reconstituted in 0.4 ml assay buffer used in the enzyme immunosassay (D’Anna-Hernandez et al. 2011). Sleeping infants underwent MRI scans −1 to 2 weeks post birth at the UNIFESP on a Siemens 3T Skyra (32-channel coil). White matter structural connectivity (connectome) using diffusion-MRI was analyzed with Mirtx (Raffelt et al. 2017). Three connectivity indices were examined: streamline counts, length, and fractional anisotropy (FA). Analyses examined streamlines connecting the right and left hippocampus (Bastiani et al. 2019). White matter (WM) connectivity was examined in 28 infants (13 offspring of +a group and 15 from -a group scanned at 9.40 ± 8.30 days of age.

Results: The +a group had higher levels of hair cortisol (pg/mg) prenatally, F(1, 55) = 4.78, p = 0.03; (+a group 9.89 ± 6.23, -a group 5.95±7.20) Partial correlations controlling for infant sex, weight, and mother’s age were used to examine the relationship between prenatal maternal cortisol and infant WM connectivity. Cortisol levels predicted less length (r = −0.51, p = 0.02) and FA (r = −0.51, p = 0.02) between the left hippocampus and the right parietal lobe. Similarly, prenatal maternal cortisol predicted less length (r = −0.48, p = 0.02) and FA (r = −0.46, p = 0.03) between the left hippocampus and the right posterior cingulate gyrus. Cortisol also predicted less FA between the right hippocampus and the left frontal lobe (r = −0.47, p = 0.03). On the other hand, cortisol levels were predictive of higher streamline counts between the left hippocampus and parietal lobe (r = −0.43, p = 0.04) and amygdala (r = −0.49, p = 0.02). Findings remained significant when controlling for prenatal maternal mood. Additional analyses examined infant sex effects, yet none were detected.

Conclusions: In line with prior research, the present study documents that a history of childhood adversity is related to increased levels of hair cortisol in adulthood (Schreier et al. 2015). Prior research has related elevated prenatal maternal cortisol to infant cognitive development (Davis et al. 2010) and atypical stress responses (O’Connor et al. 2013) yet to date our study is the first to directly examine prenatal cortisol and infant white matter connectivity. Here we document that prenatal maternal cortisol is related to alterations in hippocampal white matter connectivity. A wealth of research documents that chronic stress and elevated glucocorticoid exposure impacts hippocampal morphology, volume, and connectivity and is related to hippocampus-dependent memory impairments and anxiety- and depression-symptoms and behaviors. Although this pilot study is limited by its sample size and lack of longitudinal follow-up, our findings suggest that elevated prenatal maternal cortisol may be an important pathway thought which childhood adversities have intergenerational consequences. Future research with larger samples should re-examine sex effects, as we may have been underpowered to detect differences. Future studies should also examine if the white matter differences detected in this study persist through development and are related to offspring cognitive and emotional development.

Keywords: Prenatal Programming, Cortisol, Adverse Childhood Experiences (ACE), Early Brain Development, White Matter Integrity
Characterizing Tract-Specific White Matter Trajectories and Stability of Individual Differences in Infant Rhesus Monkeys: Implications for Developmental Psychopathology

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Background: Understanding the factors that influence individual differences in postnatal brain development is important for providing insights into the earliest origins of neuropsychiatric and other neurodevelopmental disorders. While studies performed in human infants are informative, non-human primate (NHP) models can be used to investigate these factors in greater depth. The development of myelinated white matter (WM) in the brain is of particular interest due to its role in modulating signal transmission. It is especially important to consider the growth of WM tracts in the period immediately following birth as this may be a critical time for genetic, hormonal, and environmental factors to influence the development of WM. Previously, we demonstrated homologies between WM tracts in rhesus monkeys and humans, focusing on altered WM in relation to early life anxiety. To understand and characterize the earliest stages of postnatal WM development, we examined whole-brain and tract-specific patterns of myelination using a longitudinal study of rhesus macaques imaged 5 times from birth to one year of age.

Methods: Thirty-five rhesus macaques (11 males, 24 females) were imaged with T1-weighted MRI (MPnRAGE) and single-shell (b = 1,000 s/mm²) DTI in a 3T scanner, at 2, 7, 13, 26, and 52 weeks. The structural MPnRAGE images for each subject and timepoint were co-registered with a diffeomorphic, non-linear registration algorithm to produce a time-averaged population template. A diffusion tensor was estimated and prototypical DTI parameter maps (fractional anisotropy, FA; mean diffusivity, MD; radial diffusivity, RD) were calculated in native space and subsequently warped to our population template using diffeomorphic nonlinear registration. The warped ROIs were visually assessed for alignment and 15 white matter structures were selected. For each subject and timepoint, average values of FA, MD, and RD were extracted from all 15 ROIs and used to construct longitudinal trajectories. Non-linear regression (via SSE minimization) was implemented to fit these trajectories to a range of potential models. Information criterion parameters (AIC and BIC) were utilized to evaluate the goodness of fit for each proposed model. The DTI metrics in most of these ROIs conformed to a logarithmic growth model: FA (or MD or RD) = A ln(age) + B. In this model, the parameter A represents the extrapolated rate of change of FA (or MD or RD) at 1 week of age and the parameter B represents the value of FA (or MD or RD) at 1 week.

Results: Mathematical modeling of the average FA growth trajectories of the 15 tracts revealed that WM development is best represented by a logarithmic model. Specifically, average FA in all delineated tracts exhibits robust logarithmic growth with age (R² = 0.36-0.79, p < 0.001). Tract-specific continuous growth rate curves across the first year of life (produced from first-order derivative calculations of individual log functions for each tract) indicate that: 1) the first 10 weeks of postnatal life constitute very rapid myelination processes, and 2) this growth begins to plateau at approximately 25 weeks. In addition, individual differences in measures of WM microstructure, namely FA, are stable, such that FA at ~2 weeks of age is strongly predictive FA at ~1 year (R² = 0.32-0.72, p < 0.001). K-means longitudinal clustering (based on Calinski-Harabatz indices) of both the FA logarithmic fit parameters and raw longitudinal trajectory data suggest regional differences in myelination levels immediately post-birth and across the first year of life in NHPs that are indicative of asynchronous patterns of myelination levels seen in adult human and NHP studies (i.e. center > periphery, occipitoparietal regions > frontotemporal, and projection fibers > association and commissural). Specifically, we found that, at as early 2 weeks of age, the corpus callosum, internal capsule, superior fronto-occipital fasciculus, and corona radiata exhibit the greatest microstructural integrity (i.e. FA), followed by the external capsule, fornix, and anterior commissure, while the cerebellar peduncles, cingulum, and uncinate fasciculus are the least myelinated.

Conclusions: The results from this early-life longitudinal sample with multiple WM assessments across the first year of life constitute a systematic and in-depth characterization of the spatiotemporal dynamics of postnatal WM development in NHPs. Because each infant monkey was assessed 5 times, we were able to examine individual differences in growth rates as well as within-subject stability of FA. The results demonstrate rapid maturation of WM FA across the first year of life in NHPs, particularly in the first 10 weeks following birth. Importantly, individual differences in FA at 2 weeks of age predicted FA at 1 year, highlighting the need to understand the factors that influence white matter development at the earliest stages of life. Taken together, our findings present a comprehensive quantitative analysis of WM development in a primate species evolutionarily related to humans that is of value in modeling human psychopathology. These findings set the stage for using NHP models to explore brain-behavior relationships as they relate to the neurodevelopmental origins of mental illness.

Keywords: Neurodevelopment, Nonhuman Primate Models, Diffusion Tensor Imaging (DTI), Translational Neuroscience, Developmental Psychopathology

Disclosure: Nothing to disclose.

Maternal Pre-Pregnancy Body Mass Index is Associated With Fetal Growth, Neonatal Brain Connectivity, and Toddler Adaptive Skills

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Background: Higher maternal pre-pregnancy body mass index (BMI) is associated with obesity, poorer cognition and social abilities, and increased risk of psychiatric disorders in offspring. However, potential mediators of these outcomes, such as early brain development, are largely unknown. Furthermore, behavioral antecedents of the BMI-related psychiatric outcomes are poorly characterized. The current study investigates the association of maternal pre-pregnancy BMI with fetal growth, functional connectivity of the neonatal brain, and toddler adaptive skills.

Methods: One hundred and five 3rd trimester pregnant women, aged 14 to 19, were enrolled in the 2nd trimester and completed psychosocial and physical assessments. Their pre-pregnancy BMI and fetal growth indices via ultrasound were determined from chart review. They received routine prenatal care and had no major health problems. For 45 neonates, resting-state functional MRI were acquired, and standard preprocessing was performed. Intrinsic connectivity white matter tracts in the period immediately following birth as this may be a critical time for genetic, hormonal, and environmental factors to influence the development of WM. Previously, we demonstrated homologies between WM tracts in rhesus monkeys and humans, focusing on altered WM in relation to early life anxiety. To understand and characterize the earliest stages of postnatal WM development, we examined whole-brain and tract-specific patterns of myelination using a longitudinal study of rhesus macaques imaged 5 times from birth to one year of age.

Methods: Thirty-five rhesus macaques (11 males, 24 females) were imaged with T1-weighted MRI (MPnRAGE) and single-shell (b = 1,000 s/mm²) DTI in a 3T scanner, at 2, 7, 13, 26, and 52 weeks. The structural MPnRAGE images for each subject and timepoint were co-registered with a diffeomorphic, non-linear registration algorithm to produce a time-averaged population template. A diffusion tensor was estimated and prototypical DTI parameter maps (fractional anisotropy, FA; mean diffusivity, MD; radial diffusivity, RD) were calculated in native space and subsequently warped to our population template. A publicly available WM region-of-interest (ROI) atlas of young rhesus macaques was warped to our population template using diffeomorphic nonlinear registration. The warped ROIs were visually assessed for alignment and 15 white matter structures were selected. For each subject and timepoint, average values of FA, MD, and RD were extracted from all 15 ROIs and used to construct longitudinal trajectories. Non-linear regression (via SSE minimization) was implemented to fit these trajectories to a range of potential models. Information criterion parameters (AIC and BIC) were utilized to evaluate the goodness of fit for each proposed model. The DTI metrics in most of these ROIs conformed to a logarithmic growth model: FA (or MD or RD) = A ln(age) + B. In this model, the parameter A represents the extrapolated rate of change of FA (or MD or RD) at 1 week of age and the parameter B represents the value of FA (or MD or RD) at 1 week.

Results: Mathematical modeling of the average FA growth trajectories of the 15 tracts revealed that WM development is best represented by a logarithmic model. Specifically, average FA in all delineated tracts exhibits robust logarithmic growth with age (R² = 0.36-0.79, p < 0.001). Tract-specific continuous growth rate curves across the first year of life (produced from first-order derivative calculations of individual log functions for each tract) indicate that: 1) the first 10 weeks of postnatal life constitute very rapid myelination processes, and 2) this growth begins to plateau at approximately 25 weeks. In addition, individual differences in measures of WM microstructure, namely FA, are stable, such that FA at ~2 weeks of age is strongly predictive FA at ~1 year (R² = 0.32-0.72, p < 0.001). K-means longitudinal clustering (based on Calinski-Harabatz indices) of both the FA logarithmic fit parameters and raw longitudinal trajectory data suggest regional differences in myelination levels immediately post-birth and across the first year of life in NHPs that are indicative of asynchronous patterns of myelination levels seen in adult human and NHP studies (i.e. center > periphery, occipitoparietal regions > frontotemporal, and projection fibers > association and commissural). Specifically, we found that, at as early 2 weeks of age, the corpus callosum, internal capsule, superior fronto-occipital fasciculus, and corona radiata exhibit the greatest microstructural integrity (i.e. FA), followed by the external capsule, fornix, and anterior commissure, while the cerebellar peduncles, cingulum, and uncinate fasciculus are the least myelinated.

Conclusions: The results from this early-life longitudinal sample with multiple WM assessments across the first year of life constitute a systematic and in-depth characterization of the spatiotemporal dynamics of postnatal WM development in NHPs. Because each infant monkey was assessed 5 times, we were able to examine individual differences in growth rates as well as within-subject stability of FA. The results demonstrate rapid maturation of WM FA across the first year of life in NHPs, particularly in the first 10 weeks following birth. Importantly, individual differences in FA at 2 weeks of age predicted FA at 1 year, highlighting the need to understand the factors that influence white matter development at the earliest stages of life. Taken together, our findings present a comprehensive quantitative analysis of WM development in a primate species evolutionarily related to humans that is of value in modeling human psychopathology. These findings set the stage for using NHP models to explore brain-behavior relationships as they relate to the neurodevelopmental origins of mental illness.

Keywords: Neurodevelopment, Nonhuman Primate Models, Diffusion Tensor Imaging (DTI), Translational Neuroscience, Developmental Psychopathology

Disclosure: Nothing to disclose.
connectivity distribution (ICD) was performed to measure global connectivity on the voxel level. Controlling for infant sex and age at scan, linear regression was used to relate pre-pregnancy BMI to neonatal ICD. Post-hoc seed connectivity was performed to explore which specific connections most likely contributed to the ICD findings. The women completed the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III), Adaptive Skills scale when their toddlers were 14-months.

Results: All neonates were appropriate for gestational age (birthweight: 3206.9 ± 461.3 kg, gestational at birth: 39.3 ± 1.3 weeks) and were scanned at 42.5 ± 1.7 weeks postmenstrual age. The majority of participants were male (61.1%). Maternal BMI was correlated positively with the slope of estimated fetal weight, but not head circumference. A positive correlation between pre-pregnancy BMI and global neonatal connectivity in the left thalamus was observed. Using the thalamic region as a seed, higher pre-pregnancy BMI was associated with greater local thalamic connectivity and lower fronto-thalamic connectivity. Maternal BMI also correlated positively with BSID-III Self-Direction (r = 0.73, p < 0.0001), Communication (r = 0.49, p = 0.02), and Socialization (r = 0.62, p = 0.005) scales at 14 months. Estimated fetal growth was associated with a different index of social abilities (Leisure: r = 0.48, p = 0.01).

Conclusions: Similar to findings in adults in which BMI and the thalamus are associated, we observed this association across a generation – between maternal pre-pregnancy BMI and neonatal thalamic functional connectivity. The thalamus is involved in integration and regulation of sensorimotor processing. Maternal pre-pregnancy BMI also correlated positively with a fetal growth index and measures of social and adaptive development on the BSID-III. These results suggest that maternal BMI has multilevel influence on offspring development including physical, brain, and social development.

Keywords: Connectivity, Neonatal, Fetal Brain Development

Disclosure: Nothing to disclose.

T105

Obsessive-Compulsive Symptom Severity and Decision-Making Behavior: Using a Transdiagnostic Approach to Better Characterize Goal-Directed Control Deficits Across Neurodevelopmental Disorders

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Background: Neurodevelopmental Disorders (NDDs) co-occur frequently and share many phenotypic and neurocognitive characteristics, likely due to partially overlapping underlying pathophysiology. For example, individuals with autism spectrum disorder (ASD) show increased traits of obsessive-compulsive disorder (OCD), attention-deficit/hyperactivity disorder (ADHD), and Tic Disorders (TD). Prominent theories suggest that compulsive behaviors, characteristic of OCD, are driven by deficits in goal-directed control, which confers vulnerability for developing rigid habits. However, recent studies have shown that impaired goal-directed control accompanies several NDDs, including those without an obvious compulsive element. Given this lack of clinical specificity, a transdiagnostic dimensional approach may better characterize the clinical manifestations of goal-directed control deficits. Using a child-friendly version of a well-established, two-step Markov decision process task, we tested reinforcement-learning in youth to dissociate the contributions of goal-directed (‘model-based’) and habitual (‘model-free’) learning to their choice behavior. We also examined whether individual differences in OCD symptom severity were associated with a parameter estimate from a logistic regression analysis predicting choices in the learning task.

Methods: To date, 26 individuals with NDDs (9F; Age = 12.0 ± 2.5 years; IQ ≥ 70) and 7 Healthy Controls (3F; Age = 11.9 ± 2.9 years; IQ ≥ 70) have completed our study protocol. Participants in the NDD group must have had a prior diagnosis of ASD, OCD, and/or ADHD. Individuals with comorbid diagnoses were not excluded: ADHD (15.38%); ADHD, ASD (46.15%); ADHD, TD (3.85%); ADHD, ASD, OCD (3.85%); ADHD, ASD, TD (7.69%); ASD (19.23%); ASD, OCD (3.85%). Parents completed the Obsessive–Compulsive Inventory-Revised (OCI-R) while children and adolescents completed the reinforcement-learning task. In each trial of the two-step task, a first-stage choice between two options led to a second-stage choice which was reinforced with a reward. Each first-stage choice was predominantly associated (70% of trials) with one or the other of the second-stage states. We conducted logistic regression analyses using mixed-effects models; the model tested for effects of reward (coded as rewarded, unrewarded) and transition type (coded as common, rare) from the preceding trial on predicting each trial’s choice (coded as switch, stay) relative to the previous choice.

Results: Our data suggest that, similar to studies at population level, children and adolescents with and without NDDs recruit both goal-directed and habit-based strategies during the two-step reinforcement-learning task. Participants with NDDs showed a higher probability of staying (P(Stay)) following a rewarded trial regardless of transition type [common: F(1, 31) = 9.479, p = 0.004; rare: F(1, 31) = 5.768, p = 0.022].

As expected, parents of the NDD group reported significantly higher symptom severity on the OCI-R (Total Score) than parents of the HC group [F(1, 31) = 6.153, p = 0.019]. After covarying for sex and age, P(Stay) (choice behavior) following ‘common rewarded’ trials was a significant predictor of OCI-R Scores (R Square = 0.313, F(3, 32) = 4.406, p = 0.011); P(Stay) following ‘rare rewarded’ trials (R Square = 0.266, F(3, 32) = 3.496, p = 0.028) also predicted OCD symptom severity.

Conclusions: Despite high comorbidity rates and overlapping features among NDDs, studies assessing neurocognitive functioning often include only youth within one diagnostic category. Results suggest youth with NDDs may be more likely to show model-free choice behavior, and that this behavioral signature is related to increased OCD symptomatology. Using a transdiagnostic dimensional approach to examine patterns of neurocognitive functioning in a diagnostically heterogeneous sample is more consistent with real clinical situations. It has the potential to improve our ability to match youth to appropriate treatments and to inform development of more applicable treatments.

Keywords: OCD Phenotypes, Neurodevelopment, Decision Making, Transdiagnostic, Comorbidity

Disclosure: Roche and Autism Treatments, Advisory Board

T106

Links Between Poor Sleep and Lower Cognitive, Executive Function and Social-Emotional Skills in Urban, Low-Income Preschool Children

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Background: Adverse Childhood Experiences (ACES), poverty and low parent education have often been linked to delays in
cognitive and social-emotional development, but there has been much less attention to the impact of poor sleep on developmental delays. In a small 2018 pilot feasibility study with 23 children we found poor sleep was the only factor that was correlated with delays in cognitive and social-emotional development. Using mediation analysis, we are now examining the pathways through which ACEs, poverty, low parent education and parent-child interactions impact the development of cognitive, social-emotional and executive function skills in a larger sample of young children.

Methods: Ninety-four families, with children 3–5 years of age, accessing community agencies for food, housing, clothing and/or childcare assistance were evaluated with measures of demographics, ACEs, cognitive, executive function and social-emotional development (Ages & Stages Questionnaires (ASQ & ASQ-SE), NIH Toolbox tests (Dimensional Change Card Sort & Picture Vocabulary Test)), the BRIEF-P rating of executive function, sleep (Child Sleep Habits Questionnaire (CSHQ) & sleep log), and parent-child interactions coded in a 10 min video recordings of parents and children playing together. Videos were coded using the Simple Interactions coding system, adapted for quantification of behaviors. Principal Component Analysis (PCA) of family income, parent education, parent ACEs and child ACEs was carried out using SPSS version 24. Mediation analysis was also carried out using SPSS, model 4.

Results: Principal Component Analysis of family income, parent education level, parent ACEs and child ACEs found a primary component combining adversity measured by the four variables together which explained 63.6% of the variance (the first component is referred to as “Family Environmental Stresses”). Mediation analysis found both direct effects of Family Environmental Stresses on executive function and indirect effects with sleep as a mediator ($r^2 = 0.23, p < 0.001$). Separate mediation analysis found no direct effects of Family Environmental Stresses on communication skills, but there has significant mediation through sleep ($r^2 = 0.35, p = 0.005$). Mediation analysis of Family Environmental Stresses on social-emotional skills found significant direct effects but no significant mediation through sleep ($r^2 = 0.48, p = 0.0001$). For communication skills ($r^2 = 0.49, p = 0.001$), problem-solving skills ($r^2 = 0.40, p = 0.008$) and executive function skills ($r^2 = 0.46, p = 0.005$) there were significant correlations with Family Environmental Stresses in boys but not in girls. Importantly, strong parent-child interactions are significantly correlated with better sleep in children ($r^2 = 0.23, p = 0.035$).

Conclusions: We conclude that sleep plays a very important role in mediating the impact of family environmental stresses on children’s cognitive skills, including executive function skills, and that boys are more susceptible to the impact of family environmental stresses on cognitive development compared to girls. Importantly, sleep represents an important intervention target and strong parent-child interactions are associated with an improvement in children’s sleep.

Keywords: Cognitive Development, Executive Function, Sleep, Poverty

Disclosure: Working For Kids Building Skills LLC, Royalties

T107

Prenatal Exposure to Environmental Tobacco Smoke Alters the Structure and Function of Cognitive Control Circuits in Childhood

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Background: Active maternal smoking during pregnancy acts as a neurotoxicant, affecting fetal brain development and increasing the risk of a range of mental health problems including Attention Deficit Hyperactivity Disorder as well as deficits in attention and cognitive control. Environmental tobacco smoke (ETS) exposure (passive or second-hand exposure), also acts as a neurotoxicant. A growing body of literature documents parallel risks associated with prenatal exposure to active maternal smoking and ETS. To date, however, effects of prenatal ETS exposure on cognitive control processes and the structure and function of the brain circuits that support these processes remain understudied. We used structural and task-functional Magnetic Resonance Imaging (fMRI) to probe the effects of prenatal ETS exposure on frontostriatal portions of cortico-striatal-thalamo-cortical (CSTC) control circuits in 7 to 9 year-old children drawn from a prospective longitudinal birth cohort. The central hypothesis was that structural and functional abnormalities in frontostriatal portions of the CSTC circuits in children with prenatal ETS exposure, measured by maternal prenatal urinary cotinine levels, would contribute to impairments in cognitive control.

Methods: African American and Dominican children between 7 and 9 years old were recruited from an ongoing longitudinal birth cohort. Prenatal exposure to ETS was assessed through maternal urinary cotinine levels (limit of detection 0.05ng/mL) measured during the third trimester of pregnancy (mean gestational age: 34.7 weeks; standard deviation: 3.4). ETS exposure was determined based on maternal urinary cotinine values greater than 1.0 ng/ml. Forty-one children (17 exposed, 24 non-exposed) had complete structural data. Subcortical volumes (thalamus, caudate, putamen) were extracted from subcortical segmentation, and cortical thickness values were tested vertex-wise using Freesurfer 6.0. Structural tests included age, sex, and total intracranial volume and were false discovery rate (FDR) corrected. Thirty children (10 exposed, 19 non-exposed) had useable performance and functional activation data from the Simon Spatial Incompatibility task. Differences in reaction times for incongruent (I) versus congruent (C) trials measured the conflict effect. Because longer latency is associated with conflict, we used parametric modulation to assess associations of reaction time (RT) with activation within a pre-defined frontostriatal mask (SPM 12). Maps were thresholded at voxel-level significance $p < 0.001$ and FDR corrected cluster size threshold $q < 0.05$. Functional tests included age, sex, and mean motion as covariates and were family wise error (FWE) corrected.

Results: Significant group differences (exposed versus non-exposed) in subcortical volume were detected in left and right thalamus ($p < 0.02$). Exposed children had smaller thalamic volumes than non-exposed children ($538 \text{ mm}^3$ and $487 \text{ mm}^3$ adjusted mean reduction, respectively). Across all children, longer RTs associated with increased activation in anterior cingulate cortex (ACC) and left insula (all $p \text{ FWE} > 0.02$). Significant small-volume corrected group differences were detected in left inferior frontal gyrus (IFG; $p \text{ FWE} = 0.005$). Whereas non-exposed children showed no association between RTs and activation in left IFG, exposed children showed increased activation in left IFG with increased RTs. Across all children, the correlation between RT and activation in left IFG associated inversely with the conflict effect and with thalamic volume ($p < 0.04$). That is, greater RT/IFG activation associated with smaller thalamic volume and reduced conflict.

Conclusions: Relative to non-exposed children, those with prenatal ETS exposure showed reduced thalamic volumes and activation in left IFG during longer responses to Simon task stimuli. These effects were significantly associated with each
other suggesting prenatal ETS exposure may affect both the structure and function of control circuits. Whereas all children showed an association between increased RT and increased activation in ACC and left insula, only exposed children recruited left IFG during longer latencies. Such activation was associated with a smaller conflict effect, perhaps suggesting that IFG engagement enhances the ability of exposed children to resolve cognitive conflict.

Overall, our findings are consistent with those from studies of prenatal exposure to active maternal smoking documenting decreased brain volume and increased frontostrial activation during the resolution of cognitive conflict in exposed individuals. Such parallel results suggest prenatal exposure to nicotine and other compounds in ETS may be equally neurototoxic as active maternal smoking.

Keywords: Functional MRI (fMRI), Cognitive Control, Environmental Risk Factors, Nicotine Exposure

Disclosure: Nothing to disclose.
Background: Functional cortical connectivity from prefrontal cortex (PFC) to sensory cortical regions matures through adolescence to enable top-down cognitive processes such as attention. Integration of long-range inputs onto the prefrontal cortex with local circuitry is an essential step for a proper top-down control of sensory regions. However, molecular mechanism in control of this local/long-range input balance is poorly understood. Here, we examine the role of neuremodulation in circuit maturation of PFC top-down neurons projecting from anterior cingulate cortex to primary visual cortex (PFC->VIS) in mice.

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

Results: Rabies input mapping identified robust basal forebrain cholinergic inputs onto top-down PFC->VIS projection neurons established by adolescence. Electrophysiological recording reveals decreased nicotinic ACh response following adolescence in PFC->VIS projection neurons (p = 0.0032, N = 21-22 cells, t-test) as the projection neurons increase their expression of a nicotinic brake, Lynx1. Exploration of Lynx1-dependent changes in connectivity onto PFC->VIS projection neurons revealed that Lynx1 facilitates a selective reduction in heightened local connectivity onto top-down projections through the suppression of excessive dendritic spine formation that shifts the balance of local/long-range inputs in adulthood (p = 0.03, N = 6-7 mice, t-test). Bidirectional viral manipulations of Lynx1 expression within PFC->VIS projection neurons revealed that adolescent, and not adult, Lynx1 expression is necessary and sufficient to develop adult attentional performance on the 5CSRTT (p = 0.005 for knock-down, p = 0.008 for over-expression, N = 6-9 mice, 2 way ANOVA). Further exploration of gene pathways modified by Lynx1 expression revealed overlap with genes disrupted in autism spectrum disorder with significant overlap with Fragile X syndrome. Importantly, Lynx1-mediated modulation of nicotinic tone onto top-down neurons in FMR1KO mice resulted in improved visual attention (p = 0.04, N = 9 mice each, 2 way ANOVA) and improved local/long-range input balance into top-down projection neurons (p < 0.05, N = 5 mice each, t-test).

Conclusions: Our study reveals that adolescent cell-autonomous molecular control over nicotinic tone by Lynx1 is essential for prefrontal top-down projection neurons to shift the connectivity balance of local and long-range inputs to establish adult attentional control. These findings propose "local and long-range balance" as a key developmental milestone for cognitive development, and offer a novel conceptual framework for the better understanding of neurodevelopmental disorders and their therapeutic interventions.

Keywords: Top-Down Control, Prefrontal Cortex, Nicotinic Acetylcholine Receptors, Attention, Autism Spectrum Disorder and Related Syndromes

Disclosure: Nothing to disclose.

T110

Neural Concordance of Brain Response in Mother-Infant Pairs: Links to Mutual Positive Affect

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Background: Mother-infant interactions during the first three years of life likely shape infant brain development via synchronization of physiologic and neurologic systems. Prior studies have shown that mothers and infants synchronize physiology (e.g., heart rhythms, hormones) during periods of social bonding and affiliation (Feldman et al., 2011). However, little work has evaluated whether mother-infant dyads show synchronization of neural systems during positive interactions. The medial prefrontal cortex, particularly BA 10, has been demonstrated to be involved in social cognition, bonding and affiliation in both infants and adults, and to be activated by gentle touch (Kida & Shinohara, 2013). The inferior parietal lobe has been demonstrated to facilitate language and emotion perception, key components of dyadic social interaction. The use of near-infrared spectroscopy (NIRS), an optical imaging method that allows for in vivo assessment of cortical brain response, can allow for assessment of brain synchrony during naturalistic mother-infant interactions.

Methods: The current study used near-infrared spectroscopy (NIRS) to examine how mother-infant mutual positive affect would be related to coordination of brain activity in mother-infant pairs. Participants were 52 mother-infant pairs (Mage of infant = 25.87 months, range 10-43 months; 54% girls). Mothers and infants completed a structured play protocol (e.g., read a book together). During the play interaction, NIRS hyper-scanning was used to simultaneously measure both mother and infant brain activity via a TechEn Cw6. Mothers and infants were coded separately on positive affect (PA) in 5-second intervals by trained and reliable coders. PA was characterized as facial, vocal, and/or bodily expressions of happiness or excitement (e.g., laughter, smiling, clapping hands). Proportion of epochs in which both mothers and infants were simultaneously showing PA was computed ("PA synchrony"). Mixed effect models were run using NIRS Brain AnalyzIR toolbox (Santosa et al., 2018) to evaluate connectivity between mother and infant brain activity during play and how it was modulated by PA synchrony. Child age was entered as a fixed effect.

Results: During dyadic play, greater activity in mother medial and lateral BA 10 and inferior parietal regions was associated with greater activity in infant BA 10 and inferior parietal regions. Further, mother-infant PA synchrony moderated the mother-infant neural synchrony. Specifically, greater mother-infant PA synchrony was associated with a stronger association between mother inferior parietal activity and infant medial BA 10 and inferior parietal activity (r = 0.48, t = 4.57, q = 0.002; r = 0.53, t = 4.06, q = 0.005).

Conclusions: Findings illustrate that interactions between mothers and infants that are characterized by mutual warmth and positivity may be related to dyadic synchronization of neural systems. This finding is important as it demonstrates that, similar to other physiological systems (e.g., heart rhythms, hormones), infant neural systems develop via co-regulation during positive interactions with a caregiver.

Keywords: Positive Affect, NIRS, Synchrony

Disclosure: Nothing to disclose.
in the prefrontal cortex of male—but not female—rats. The mechanisms underlying this alteration in cortical myelin remains unknown. Studies have shown a significant increase in the total number of oligodendrocyte lineage glial cells, as well as proportion of these cells that myelinate axons, prior to adolescent development in the mouse brain. Alcohol may reduce myelin by targeting mature myelinating oligodendrocytes or interfering with proper oligodendrocyte maturation and subsequent survival of these cells.

Methods: In the present study, we used the drinking-in-the-dark model to test the effect of voluntary alcohol drinking during adolescence on oligodendrocytes in the prefrontal cortex in male and female mice. Mature myelinating oligodendrocytes were immunofluorescently labeled with a CC-1 antibody and quantified using confocal microscopy.

Results: Alcohol drinking reduced the number of myelinating oligodendrocytes in the prefrontal cortex of male mice only. Differential sensitivity to alcohol may be due to sex differences in proliferation and/or maturation of oligodendrocyte lineage cells. To address this, we are using transgenic reporter mice to determine how adolescent alcohol alters the kinetics of oligodendrocyte precursor cells differentiating into mature oligodendrocytes.

Conclusions: Overall, these studies will provide insight into the mechanism by which alcohol affects cortical myelination, increasing our understanding of the relationship between cognitive and emotional deficits and heavy drinking during adolescent development.

Keywords: Sex Differences, Alcohol, Myelin, Mice, Prefrontal Cortex

Disclosure: Nothing to disclose.

T112
Differential Impact of Alcohol Drinking on Oligodendrocyte Lineage Cells in Adolescent Male and Female Mice

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Background: Prefrontal cortical development extends from adolescence into adulthood, with significant increases in white matter volume and electrical signal transmission velocity. White matter is primarily comprised of axons wrapped by segments of lipid-dense myelin sheaths. We have found that voluntary binge-like alcohol drinking during adolescence decreased myelin density in the prefrontal cortex of male—but not female—rats. The mechanisms underlying this alteration in cortical myelin remains unknown. Studies have shown a significant increase in the total number of oligodendrocyte lineage glial cells, as well as proportion of these cells that myelinate axons, prior to adolescent development in the mouse brain. Alcohol may reduce myelin by targeting mature myelinating oligodendrocytes or interfering with proper oligodendrocyte maturation and subsequent survival of these cells.

Methods: In the present study, we used the drinking-in-the-dark model to test the effect of voluntary alcohol drinking during adolescence on oligodendrocytes in the prefrontal cortex in male and female mice. Mature myelinating oligodendrocytes were immunofluorescently labeled with a CC-1 antibody and quantified using confocal microscopy.

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Conclusions: Overall, these studies will provide insight into the mechanism by which alcohol affects cortical myelination, increasing our understanding of the relationship between cognitive and emotional deficits and heavy drinking during adolescent development.

Keywords: Sex Differences, Alcohol, Myelin, Mice, Prefrontal Cortex

Disclosure: Nothing to disclose.
measures and 70 individuals with TYP (mean age = 18.0 years; mean IQ = 109). They completed 4 28 trial runs of the rapid Preparing to Overcome Prepotency (rPOP) task in the scanner. In the rPOP, they were first presented with a fixation cross. This was followed by a cue signaling whether they should push a button on the same side (cued by a green square) as indicated by the probe, which was an arrow, or to the opposite side relative to where the arrow pointed (cued by a red square). After an inter-stimulus interval, the probe arrow was presented. Both inter-stimulus and inter-trial intervals were jittered (mean time = 4,500 ms). Data was acquired using a 3 Tesla Siemens Tim Trio with a 32 channel head coil. Data were preprocessed and analyzed using SPM12. Cue and probe phases of red and green trials were modeled separately in the GLM. Error and post-error trials were modeled separately from correct trials and excluded from contrasts. Participants with < 70% accuracy also were excluded. The GLM included translational and rotational movement and ART outlier regressors. ROI-to-ROI whole brain functional connectivity (FC) analyses of the salience, cognitive control, and default mode networks were implemented using PPI in the CONN functional connectivity toolbox (http://www.nitrc.org/projects/conn). All ROIs were derived from the Schaefer et al. (2017) Atlas. Associations between network functioning, task performance, ASD symptoms assessed with the ADOS-2 and attention problems assessed with the Connors Rating Scale were conducted using parametric and non-parametric correlations.

**Results:** Behavioral performance on the rPOP task as indexed by RT analyzed in a repeated measures ANOVA that controlled for IQ showed that there was a main effect of trial type (red/green) (F = 4.86, p < 0.05) no effect of diagnosis and a significant interaction (F = 4.27, p < 0.05), indicating that the ASD group performed more poorly on the task. Whole brain analyses showed few group differences in recruitment for the red-green contrasts in either the cue or the probe phases of the task. FC analyses showed that during the cue phase, the TYP versus the ASD group exhibited greater recruitment of prefrontal brain regions associated with the default mode, while the ASD group exhibited greater engagement of regions that were part of the salience network. Members of the ASD group that showed greater connectivity in regions found in those with TYP, performed better than other members of the ASD group. Members of the ASD group with greater salience network connectivity exhibited greater attention deficit and ASD symptom severity. During the probe phase of the task, the TYP versus the ASD group showed greater in prefrontal regions that were part of the cognitive control and default mode networks. Here too members of the ASD group who showed greater connectivity in these networks characteristic of TYP, performed better than other members of the ASD group. During the probe phase of the task, unlike those with ASD, younger children with TYP showed stronger connectivity between regions of the cognitive control and default mode networks. TYP also showed an association between prefrontal connectivity and IQ.

**Conclusions:** A growing body of work suggests that several of the neural networks involved in the implementation of cognitive control operate differently and in a less well integrated fashion in individuals with ASD compared to TYP. Results of the current study support this contention, and suggest that those with ASD who are able to recruit similar networks to TYP do better on the task. In addition, the ASD group shows greater preparatory recruitment of the salience network during the rPOP that is associated with attention deficits and ASD symptom severity. Future work will continue to examine this atypical recruitment of the salience network by those with ASD, other issues in comparative network dynamics, and their potential association with measures of adaptive functioning.

**Keywords:** Cortical Circuit Function, Autism, Adolescent Brain Cognitive Development Study, Attention Deficit Hyperactivity Disorder

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**Keywords:** Cortical Circuit Function, Autism, Adolescent Brain Cognitive Development Study, Attention Deficit Hyperactivity Disorder

**Disclosure:** Nothing to disclose.
connectivity between left OFC and left cerebellum (k = 133, p = 0.001), and the right precuneus and the bilateral middle cingulate cortex (k = 330, p = 0.0001). Increased PDI in that group was also associated with increased connectivity between the right OFC and the mid cingulate cortex (k = 240, p = 0.0001). In contrast, in the SA group, greater perceived pain disability was associated with decreased connectivity between the left OFC and the right angular gyrus (k = 104, p = 0.003), and both the left angular gyrus and left middle temporal gyrus (k = 86, p = 0.01). There was no statistically significant association with pain scores and the FC in the right OFC in the SA group.

**Conclusions:** To our knowledge, this is the first study to examine OFC connectivity as it relates to perceived pain disability in suicide ideators compared with suicide attempters. This cross-sectional study found that veterans who reported SI demonstrated reduced connectivity from the left OFC to the angular gyrus and middle temporal gyrus which was associated with increased perception of pain disability. The angular gyrus is a key parietal node of the default mode network and along with the middle temporal gyrus is crucial in semantic processing. The negative association for the PDI scores and connectivity between these regions in the SA group may underlie poor decision-making and impaired valuation of painful stimuli typically observed in attempters. This is consistent with reports that the inability to suppress or reappraise negative affect may increase suicide risk. In contrast, veterans who reported SI demonstrated increased connectivity from the OFC to the cerebellum, the mid cingulate cortex, and the precuneus that was associated with greater perceived pain disability. The positive association between the pain scores and connectivity between the precuneus and the left OFC in the SI group may be a result of the role of the precuneus in linking self-thoughts to negative emotions. Overall, these results suggest that resting state connectivity associated with pain disability may aid in identifying neurobiological signatures that are distinct in suicide ideators and attempters and indicate areas of potential clinical intervention.

**Keywords:** Suicide Risk Factors, Pain, Veterans, Orbitofrontal Cortex (OFC)

**Disclosure:** Nothing to disclose.

**T115**

**Dissecting the Dynorphinergic Circuitry Underlying Pain-Induced Negative Affective States**

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**Background:** Pain is a multifaceted experience composed of both sensory and emotional disturbances. The persistence of pain over time can lead to the formation of co-morbid psychiatric disorders such as anxiety, depression and negative affective states. Uncovering the circuitry underlying the emotional component of pain in its early stages represents a promising avenue to improve pain treatment, patient well-being and decrease the occurrence of co-morbid disorders. Previous studies have demonstrated that inflammatory pain induces changes in opioid systems in the central nervous, yet altering rewarding properties of external stimuli and inducing aversive states. In this line of evidence, we recently demonstrated that the dynorphin-kappa opioid system in the nucleus accumbens shell (NAcSh), a brain structure highly involved in reward and aversion processing, is actively recruited to drive pain-induced negative affective states. Interestingly, using rodent micro Positron Emission Tomography results we uncovered an overall recruitment of kappa opioid receptors (KORs) in the brain. While we uncovered that silencing NAcSh dynorphin-containing neurons can fully prevent the effects of pain on reward-driven motivation in rats, we hypothesize that their projections outside of this structure are also necessary to drive pain-induced negative affective states.

**Methods:** We used an inflammatory pain model (CFA in the hind paw of rats) to assess pain-induced changes in KOR function using GTPgammaS binding in the Lateral Hypothalamus. We also monitored the impact of pain on the excitability of LH neurons using ex vivo patch clamp recording. In addition, we used in vivo behavioral experiments to further dissect the involvement of KORs in LH to drive decrease in motivation for natural rewards. NorBNI (a long-term antagonist of the KOR) was administered bilaterally in the LH to investigate the necessity of KOR in pain-induced decrease in motivation for sucrose (using a progressive ratio schedule). Using a chemogenetic approach we were also able to silence specifically NACSh to LH projections to uncover their role in pain-induced negative affect. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee at Washington University in St. Louis.

**Results:** In this study, we demonstrate that inflammatory pain increases KOR receptor function in the Lateral Hypothalamus using GTPgammaS radiobinding assay 48 hours after the induction of pain. In order to assess the necessity of these receptors in the LH to drive pain-induced negative states, we bilaterally micro-injected norBNI (5ug per side) in rats. We uncover here that the KORs in the LH are necessary to drive the decrease in motivation for sucrose observed in inflammatory pain conditions. In addition, we show that neuronal excitability in the LH is increase in pain conditions as compared to control animals. While we previously demonstrated that pain decreases the firing threshold in NACSh dynorphin-containing neurons, we are currently assessing if silencing these projections selectively to the LH are sufficient to prevent the negative affective states induced by inflammatory pain. To do so, we bilaterally infused an HSV-dynorphin-hM4Di-mCherry in the NACSh and locally applied CNO in the LH. This allowed us to determine the role of NACSh to LH projections in driving pain-induced decrease in motivation.

**Conclusions:** We recently demonstrated that inflammatory pain recruits the kappa opioid system in the NACSh to drive negative affective states. Our current findings expand on these results and uncover for the first time a necessary role for KORs in the LH for the development of these co-morbid emotional disturbances. While the dynorphin-KOR system undoubtedly represents a promising avenue for pain pharmacotherapies, our study expands our understanding of the dynorphin-KOR circuitry underlying the emotional component of pain.

This work was supported by NIH grant R01 DA041781, DA042499, DA045463 to JAM. Authors declare no conflict of interest.

**Keywords:** Dynorphin, Pain, Negative Affect, Nucleus Accumbens, Kappa Opioid Receptor

**Disclosure:** Nothing to disclose.

**T116**

**5-HT1A Autoreceptor Binding and Cortisol Response to Stress in Alcohol and Substance use Disorders and Risk for Suicidal Behavior**

Nadine Melhem*, Jeffrey Miller, Lisa Pan, Francesca Zanderigo, Todd Ogden, Barbara Stanley, M. Elizabeth Sublette, David Brent, J. John Mann
Background: Impaired serotonin signaling has been implicated in the pathophysiology of neuropsychiatric disorders. High 5-HT1A autoreceptor binding in the brainstem raphe nuclei has been previously associated with major depressive disorder (MDD) and more lethal suicide attempts in people with MDD. Suicide attempters are also reported to have blunted hypothalamic-pituitary-adrenal (HPA) axis activity and cortisol response to stress compared to other high-risk individuals without a history of attempt. Alcohol and substance use disorders (ASD) are a major risk factor for suicidal behavior. Here, we examined the relationships of ASD with SHT1A autoreceptor binding and cortisol response to stress in individuals at high risk for suicidal behavior.

Methods: The sample consisted of 73 medication-free participants (70% females) at high risk for suicidal behavior either due to a prior history of suicide attempt (n = 21) or a family history of suicide attempt (n = 52); and a sample of controls with no prior personal or family history of an attempt (n = 25). Participants were recruited at two sites: the University of Pittsburgh and Columbia University. Participants underwent positron emission tomography (PET) imaging and 5-HT1A autoreceptor binding was quantified using [11-C]CUMI101. Participants were also administered the Trier Social Stress Task to determine cortisol response to stress and the Structured Clinical Interview for DSM-5. T-tests were used to compare groups (ASD vs. no-ASD) and regression analyses were conducted to control for covariates (e.g., age, sex, race, site) and clinical (e.g., suicide attempt, family history of attempt) covariates.

Results: Suicide attempt and family history of suicide attempt were not associated with SHT1A autoreceptor binding in any of the regions of interest (ROIs). However, a history of ASD was strongly associated with lower PET binding in anterior cingulate (1.42 ± 0.21 vs. 1.57 ± 0.26, p = 0.018, Effect size or ES = −0.64), dorsolateral prefrontal cortex (0.95 ± 0.17 vs. 1.08 ± 0.20, p = 0.009, Effect size or ES = −0.70), insula (1.65 ± 0.24 vs. 1.85 ± 0.25, p = 0.002, Effect size or ES = −0.82), medulla (1.1 ± 0.2 vs. 1.25 ± 0.23, p = 0.008, Effect size or ES = −0.69), and the orbitofrontal cortex (1.06 ± 0.16 vs. 1.22 ± 0.24, p = 0.004, Effect size or ES = −0.80). ASD was also associated with higher total cortisol output (β = 2.9, Standard Error (SE) = 1.05, p = 0.006, ES = −0.32) during the TSST even after controlling for personal and family history of suicide attempt.

Conclusions: Alcohol and substance use disorders were found to be strongly associated with lower serotonin autoreceptor binding and higher cortisol levels in response to a laboratory stressor. Lower binding may reflect an effect of substance use on serotonin neurons. These findings are consistent with previous findings of higher binding only being present in higher lethality suicide attempters; and higher resting cortisol levels have been previously associated with lower SHT1A binding in medication-free depressed patients and healthy controls. Our current sample was medication-free and hence discrepancies in findings may be attributed to differences in the severity and type of psychopathology and lethality of suicidal behavior between samples. Future studies are needed to investigate changes in biological mechanisms in the serotonergic and HPA axis with substance use in patients at risk for suicidal behavior.

Keywords: Serotonin 1a Receptor, PET Imaging Study, Suicidal Behavior, Alcohol and Substance Use Disorders, Cortisol Response to Stress

Disclosure: Excelsa Health, Board Member (Spouse), Fresenius Medical Care, Consultant, (Spouse), Select Specialty Hospital, Consultant (Spouse)

T117

Neural Correlates of Disengagement-Coping Behaviors and Their Relation to Social Discrimination

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Background: Social discrimination, a type of psychological stressor, is associated with increases in negative coping behaviors, which have been linked to greater maladjustment, depression, and anxiety. Yet, little is known about the neural underpinnings of discrimination-related coping responses. The current study was conducted to examine the neural correlates of negative coping behaviors, specifically, behavioral and mental disengagement. Of interest were fronto-limbic regions, which are central to affect regulation and stress response. Frontal-lobe regions contribute to executive functions (e.g., cognitive reappraisal) thought to protect against the negative psychological effects of social discrimination. Frontal-lobe regions are also implicated in top-down control of amygdala activity, which prior studies indicate is elevated in the context of social discrimination. Accordingly, we hypothesized that greater fronto-limbic thickness and stronger resting-state functional connectivity (rsfc) between the amygdala and frontal-lobe regions would be associated with lower levels of negative coping. We further hypothesized that fronto-limbic structure and function would mediate the relation between discrimination exposure and negative coping behaviors.

Methods: We included 51 adults (aged 23–64 years; 14% European American/White). Self-report questionnaires assessed levels of negative coping, i.e., behavioral and mental disengagement (the COPE), and perceived discrimination (Everyday Discrimination Scale). Structural and resting-state functional MRIs were obtained. FreeSurfer was used to analyze structural MRIs, providing measures of cortical thickness. Whole-brain analyses assessed the relation of cortical thickness to behavioral and mental disengagement. Whole-brain seed-based rsfc analyses were conducted in AFNI using the left amygdala the seed region, based on prior data demonstrating associations between left amygdala function and discrimination. Regression analyses assessed, conducted in AFNI, the relation of disengagement behaviors to left amygdala rsfc on a voxelwise basis; these analyses were restricted to regions of the executive control network (Shirer et al., 2012). Regression analyses, conducted using R 3.5, examined whether brain regions identified in the aforementioned analyses accounted for associations between discrimination exposure and disengagement behaviors (log-transformed).

Results: Behavioral and mental disengagement was negatively associated with dorsolateral prefrontal cortex (DLPFC) thickness (vertexwise p < 0.0001, cluster FWE corrected p < 0.001) and positively associated with amygdala-DLPFC rsfc (voxelwise p < 0.005, cluster FWE corrected p < 0.05). Discrimination exposure correlated with DLPFC thickness (r = −0.27, p = 0.05) and amygdala-DLPFC rsfc (r = 0.38, p = 0.02). Regression analyses revealed that the association between discrimination exposure and disengagement behaviors (beta = 0.023, p < 0.01) was partially accounted for by DLPFC thickness (beta = −1.08, p < 0.0001; change R2 = 0.36) and amygdala-DLPFC rsfc (beta = 1.26, p < 0.001; change R2 = 0.16). These effects were maintained even after controlling for age, estimated IQ, sex, and race.

Conclusions: Our findings provide the first evidence that negative coping behaviors (i.e., behavioral and mental disengage-
ment), which correlate with social discrimination, are related to fronto-limbic function. These data suggest that DLPFC-mediated processes may contribute to an individual’s ability to cope with experiences of social discrimination. Such findings help to elucidate potential targets of intervention and future investigation.

Keywords: DLPFC, Amygdala, Acute and Chronic Stress, Social Discrimination, Stress Coping
Disclosure: Nothing to disclose.

T118
Alcohol Misuse and Neural Responses to Food Cues in Adult Drinkers

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Background: Alcohol accounts for approximately 10% in total calorie intake for all drinkers and nearly 50% for alcoholics, representing a significant source of energy. Whereas casual drinking may temporarily enhance appetite, those with moderate to severe alcohol use have reported reduced food consumption and energy deficits both of which can result in homeostatic imbalance. As food and alcohol both engage the reward circuits, it has been proposed that alcohol may compete with food in incentive salience, leading to the displacement and devaluation of food as a reward. Nevertheless, little is known about whether and how problem drinking may alter the neural substrates implicated in the processing of food stimuli.

Methods: To examine the potential role of alcohol use in the alterations of brain responses to food stimuli, we acquired functional and structural magnetic resonance imaging data in 82 adult drinkers (33 females, aged 22–74 years) who performed a food craving task. All subjects gave written consent prior to participation in accordance to institute guidelines approved by the Yale Human Investigation Committee. During the task, subjects were presented with blocks of palatable food and non-food images and instructed to report their food craving. The Alcohol Use Disorders Identification Test (AUDIT) was employed to measure the extent of problem drinking in each subject. Using multiple regression and generalized psychophysiological interactions (gPPI) analyses, we assessed the relationship of individual differences in AUDIT scores with both brain activity and connectivity during the viewing of food relative to non-food images. In a voxel-based morphometry analysis, the gray matter volumes of brain regions with preferential activation to food as well as non-food images were also computed and related to drinking severity. The effect of age was accounted for in all analyses, and the results were evaluated at voxel p < 0.001 and cluster p < 0.05, FWE corrected threshold.

Results: A whole-brain multiple regression with AUDIT scores as the predictor showed that higher AUDIT score was significantly associated with reduced activity in the lateral orbitofrontal cortex during the viewing of food vs. non-food images. Food, relative to non-food images, elicited activations in multiple regions including the insula, middle frontal gyrus, superior parietal lobule, and occipital cortices. Connectivity strength among these regions during the viewing of food images exhibited a negative correlation with AUDIT scores. Finally, there was a significant negative relationship between AUDIT scores and the gray matter volume in the same regions. In contrast, the gray matter volume in those which activated to non-food images did not show a relationship with AUDIT scores.

Conclusions: We found evidence of attenuated food-related activity in the lateral orbitofrontal cortex in correlation with problem drinking. As the region is commonly implicated in the processing of food vs. non-food stimuli and likely involved in the reward aspect of gustatory function, the current finding suggests that alcohol misuse may impair food motivation. Connectivity and brain morphology analyses further demonstrate that various regions which preferentially responded to food stimuli, including those related to cognitive control (e.g., middle frontal gyrus), reward and interoceptive (e.g., insula), and visual (e.g., occipital cortex) processing, showed both functional and structural alterations in association with drinking severity. Taken together, we provide confirmatory evidence that alcohol misuse influences the neural processes involved in different aspects of food stimulus response, potentially contributing to the devaluation of food and energy deficits in heavy drinkers.

Keywords: Alcohol Drinking, Food Intake, Reward Devaluation, fMRI, Structural MRI
Disclosure: Nothing to disclose.

T119
Changing Self-Concept in Eating Disorders

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Background: Eating disorders are serious mental illness marked by altered perception of oneself and one’s body as well as altered eating behaviors. We have identified neural differences related to social perspective-taking and identified a cognitive marker of self-attribution, the externalizing bias, that is related to both clinical status (ill or recovered) and brain function (insula activation during self-evaluations). The externalizing bias measures one’s tendency to attribute positive versus negative scenarios to oneself.

Methods: We developed an outpatient intervention, the Self-Blame and Perspective-Taking Intervention (SBPI), targeting these two social perception concepts. The SBPI involved four two-hour groups meeting weekly for one month. Discussion of eating-disorder cognitions and behaviors was prohibited during the groups. Four cohorts (20 women with eating disorders) provided pre/post data including measures of interpersonal attribution, self-esteem, eating, depression, and anxiety. MANOVAs evaluated self-concept measures and clinical symptoms.

Results: At one-month post SBPI, there were significant improvements in self-concept including increases in both the externalizing bias (p = 0.02) and state self-esteem (p = 0.03). Clinical symptoms also improved, including eating disorder symptoms (EDE-Q, p = 0.002), depression (QIDS, p = 0.01), and anxiety (SIGH-A, p = 0.03).

Conclusions: A brief (8 total contact hours) intervention rapidly improved clinical symptoms in adult women with eating disorders. Targeting more general forms of self-concept, self-attribution and social perspective-taking, may have more clinical impact than focusing on traditional eating disorder symptoms such as body image or eating behaviors.

Keywords: Social Brain, Self-Perception, Psychosocial Treatment, Eating Disorders
Disclosure: Nothing to disclose.
T120

Normalization of Dysregulated Fatty Acids in Women With Anorexia Nervosa

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Background: Anorexia nervosa (AN) is a psychiatric disorder involving psychological, environmental, and biological factors. Individuals with AN avoid high-fat, high-calorie diets and have shown abnormal metabolism of fatty acids (FAs), which are essential for brain and cognitive/neuropsychiatric health. To clarify the relationship between FAs and AN, fasting and postprandial plasma FAs in AN and age-matched control women were examined in this food-challenge study.

Methods: The fasting and postprandial plasma FAs were analyzed via mass-spectrometry. Psychiatric phenotypes were assessed using Becker Anxiety Inventory and Becker Depression Inventory. Statistical analysis was done in Python and R (version 3.5.2).

Results: AN and controls exhibited different FA signatures both at fasting and postprandial timepoints. Fasting lauric acid, eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA), and fasting and postprandial alpha-linoleic acid (ALA) were higher in AN than in controls (lauric acid: 15081.6 ± 14970.2 vs. 8257.4 ± 4740.2 pmol/ml; ALA: fasting: 2217.7 ± 1587.6 vs. 1087.9 ± 821.2 pmol/ml; postprandial: 1830.9 ± 1115.6 vs. 1159.4 ± 664.7 pmol/ml. EPA: 33788.3 ± 17487.5 vs. 22860.6 ± 12642.4 pmol/ml; DPA: 32664.8 ± 16215.0 vs. 20969.0 ± 12350.0 pmol/ml; ALA: fasting: 2217.7 ± 1587.6 vs. 1087.9 ± 821.2 pmol/ml; postprandial: 1830.9 ± 1115.6 vs. 1159.4 ± 664.7 pmol/ml. FDR-adjusted p-values < 0.05). Food intake and AN status affected correlations specifically correlations between these FAs, BMI, depression, and anxiety. Desaturase SCD-18 showed a trend (p-value ~0.05) toward lower activity in AN (AN:0.08; control:0.12).

Conclusions: Altered FA signature, specifically correlations between elevated n-3 FAs and worsened symptoms illustrate metabolic underpinnings in AN. Future studies should investigate the mechanism by which FA dysregulation, specifically elevated n-3 FAs, affects the risk and outcomes of AN.

Keywords: Fatty Acids, Anorexia Nervosa, Metabolism

Disclosure: Nothing to disclose.

T121

Neural Basis of Meal Related Interoceptive Dysfunction in Anorexia Nervosa

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Background: Anorexia nervosa (AN) has the highest mortality rate of any psychiatric illness (Arcelus et al. 2011), yet the pathophysiology of this disorder remains poorly understood. Individuals with AN commonly exhibit avoidance of food intake and abnormal brain activity in regions implicated in interoception such as the insular cortex (Kaye et al. 2009), but it is unclear to what extent these abnormalities are driven by differences in the anticipation versus receipt of interoceptive signals. The current study probed the neurocircuitry of interoceptive processing in anorexia nervosa using bolus infusions of isoproterenol, a rapidly acting peripheral beta-adrenergic agonist similar to adrenaline, during the pre-meal time period.

Methods: We examined brain BOLD fMRI responses to the application of isoproterenol 2 micrograms and normal saline infusions in weight-restored AN (n = 19) and healthy comparison (HC; n = 22) females. Infusion order was randomized and double-blinded. Throughout each infusion scan participants rated the intensity of their perceived cardiorespiratory sensations by rotating a dial. To target pre-meal anticipatory processing, all participants were informed they would be asked to eat a 1000 Calorie meal upon scan completion. We evaluated changes in affective body signals (indexed via heart rate; HR), perceptual responses (indexed via dial ratings), and brain responses during the periods of anticipatory and peak HR change relative to a pre-infusion baseline period. For this initial neuroimaging analysis we conducted 1) a voxelwise whole brain analysis using a threshold of p < 0.001 uncorrected (recruitment target: n = 27 per group), 2) a region-of-interest (ROI) time series analysis of the insula using the Brainnetome cytoarchitectonic parcellation of anterior (agranular) and posterior (dysgranular/granular) insula (Fan et al. 2016), and 3) a recently developed computational signal processing technique called multivariate Functional Principal Components Analysis (mFPCA). mFPCA allows for estimating subject’s individual trajectories from intensely-sampled longitudinal data by empirically determining smooth principal modes of variation of trajectories around mean levels. Using spline basis functions, an individual’s response is modeled in a multivariate fashion via a matrix of spline coefficients to characterize the major modes of variation from subject to subject. The strength of association among variables was modeled on the smoothed response level, via correlations between principal component scores.

Results: Using the voxelwise analysis we found that the insular cortex showed the most selective responses to isoproterenol across the whole brain in both groups (p < 0.001 uncorrected), replicating our previous two studies (Hassanpour et al. 2016 and 2018). However, the AN group showed exaggerated neural responses in the anterior insula during the anticipatory time period immediately after infusion administration, prior to the onset of any HR changes. While both groups showed similar insula responses during the peak period of stimulation with isoproterenol, during the corresponding period for the saline condition the AN group showed blunted activity in the anterior insula relative to HC. The ROI time series analysis of the insula further illustrated this pattern: increased anterior insula activity during anticipation of isoproterenol in AN vs. HC, and reduced responses during the peak period for isoproterenol and saline. Using the mFPCA analysis we identified four principal components explaining the majority of variance in anterior and posterior insula activation. We evaluated the top two FPCA components from the anterior insula data via a linear mixed effects analysis of individual components. We observed a significant group by dose interaction for the first component on the anterior insula data (t = −3.44, p = 0.0007, Cohen’s d = 0.39) but not for the second FPCA (t = −1.65, p = 0.10, Cohen’s d = 0.11).

Conclusions: These findings 1) provide preliminary evidence supporting the idea that the pathophysiology of AN may be related to a neural circuit dysfunction whereby exaggerated anterior insular activity during anticipation distorts inferences about the internal state of the body, and 2) lay a foundation for evaluating interoceptive stress responses at the individual level by examining variability of insula reactivity to interoceptive probes in relation to AN illness characteristics.

References:


Keywords: Interoception, Eating Disorders, Functional Neuroimaging

Disclosure: Nothing to disclose.

T122

Intra-Cortical Myelination is Associated With 33 Impulsive and Compulsive Behaviors: A Latent Phenotyping Study of Disinhibition and Enriched Brain Gene Expression

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Background: Impulsive and compulsive symptoms are common, tend to co-occur, and collectively account for a substantive global disease burden. We previously found that approximately 70% of expressed variance in 33 impulsive and compulsive problems was accounted for by a single latent phenotype termed “disinhibition”. Neurobiological mechanisms underpinning this novel trans-diagnostic phenotype have yet to be elucidated.

Methods: To study the neurobiology of this latent phenotype, we utilised the Neuroscience in Psychiatry Network (NSPN), a cohort of young people in the United Kingdom, who provided questionnaire data and Magnetic Resonance Imaging (MRI) scans. Partial Least Squares (PLS) was used to identify brain regions in which intra-cortical myelination (measured using Magnetisation Transfer, MT) was significantly associated with the trans-diagnostic disinhibition phenotype. We then identified genes whose expression was enriched in these disinhibition-associated brain regions, using data from the Allen Human Brain Atlas.

Results: The sample comprised 126 participants, mean (standard deviation) age 22.8 (2.7) years, being 61.1% female. Disinhibition scores were significantly and positively associated with higher MT in the following regions bilaterally: inferior frontal cortex, middle and superior frontal cortex, posterior cingulate cortex, superior parietal cortex, paracentral gyrus, post-central gyrus, supramarginal gyrus, and precuneus. Genes involved in receptor signalling pathways were significantly over-expressed in these disinhibition-related cortical regions, including noradrenergic receptors (ADRA1B, ADRA2C), opioid receptors (OPRM1, OPRK1), dopaminergic receptors (DRD5) and serotonergic receptors (HTR1E).

Conclusions: This study integrates and extends beyond established disease models of impulsivity and compulsivity using a trans-diagnostic, dimensional approach. These findings indicate brain regions and biological processes implicated in a multitude of related, impairing mental disorders characterised by disinhibition. Such a latent phenotyping approach could be used in future to quantify effects of pharmacological and other treatments, with the aim of ameliorating a range of disorders, including in their early stages.

Keywords: Trans-Diagnostic, Phenotyping, Impulsivity, Compulsivity

Disclosure: Cambridge Cognition, Promentis, Isco Digital Health, Shire, Consultant, Elsevier, Honoraria, Wellcome Trust, Grant

T123

Event-Related Potential Neural Correlates of Aggressive Response Selection in Intermittent Explosive Disorder

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Background: Intermittent explosive disorder (IED) is a DSM disorder characterized by recurrent impulsive aggressive behavior. Research suggests that IED is characterized by abnormal functioning of the corticolimbic circuit. Specifically, IED has been associated with decreased orbitofrontal cortex (OFC) activity in response to angry faces relative to healthy research subjects, as well as heightened amygdala response to angry faces. Evidence for functional brain abnormalities in IED derive primarily from fMRI studies using static threatening stimuli. Accordingly, little is known about the temporally rapid functional neural correlates of aggressive retaliation to provocation in IED. The N2 component of the event-related potential (ERP) is associated with response selection and inhibitory processes. The current study investigates the functional significance of N2 during provoked aggression and compares healthy participants and participants with IED on task performance and the N2 ERP neural correlate of provoked aggression.

Methods: Adult men and women ages 21 to 54 (mean = 36, SD=10) were recruited from the community who: (1) currently met criteria for IED (N = 20; male=11, female=9); or (2) were healthy control subjects (N = 26; male=17, female=9). Participants were assessed for current and lifetime psychopathology using semi-structured diagnostic interviews. Participants completed an EEG session during which high-density EEG was recorded using 128 channels. During the session, participants completed a laboratory paradigm simulating a provocative aggressive interaction. Under the guise of competing in a reaction-time contest, participants were provoked by a (fictitious) opponent at varying levels of intensity across low and high provocation blocks. Provocations took the form of threats of electric shock to the fingertips, which were calibrated to participants’ tolerance thresholds. Participants could ‘retaliate’ aggressively (or respond non-aggressively) by selecting a shock intensity for the opponent. The loser of each competitive trial received the shock selected by the other player. Following artifact correction, the data were epoched relative to the retaliation responses. The N2 ERP component was analyzed at the frontocentral midline (FCz) electrode. Provocation and retaliation effects on N2 amplitude (i.e., trial-provocation, block-provocation, retaliation intensity, and available responses) were assessed using generalized linear modeling.

Results: Provocation intensity and intensity of retaliation were not significant predictors of N2 amplitude (ps > .9). Average provocation intensity within task blocks also did not impact N2 amplitude (p = .18). Number of available response choices (Wald Chi-square=9.127, df=1, p = .003) and the interaction between available responses and block (low versus high provocation; Wald Chi-square=4.617, df=1, p = .032) significantly predicted N2 amplitude. N2 amplitude was larger when participants had more options for retaliation (3 versus 2). N2 was smallest when response options were limited and included the most aggressive (and the
least utilized) response option, indicating that N2 observed during the task is sensitive to subjects’ subjective response space. Compared to other groups, female participants with IED retaliated the most intensely when provoked and showed corresponding higher amplitude of N2 (indicating the least constrained subjective response space) under high provocation.

Conclusions: In the current context, N2 amplitude appears to index subjective response space while retaliating to provocation. Rather than reflecting extent of inhibitory processing, N2 appears to index larger response space. Female participants with IED showed more aggressive responding to intense provocation than male participants with IED and healthy control subjects. Although IED is somewhat more prevalent in men, the current results suggest that women with IED may be more sensitive to intense provocation and show a pattern of neural activity associated with less constrained decision-making when responding to provocation.

Keywords: Irritability/Aggression, Intermittent Explosive Disorder, Threat Context, EEG, Event Related Potentials

Disclosure: Nothing to disclose.

T124
Safety and Efficacy of Esketamine Nasal Spray in a Depressed Patient Who was Being Treated With Tranylcypromine: A Case Report

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Background: Monoamine oxidase inhibitors are associated with a number of drug interactions, including antidepressants. Esketamine nasal spray is a newly FDA approved antidepressant which is unlikely to pose a drug interaction with MAOIs. However, MAOIs were not used in the clinical trials supporting the approval of esketamine nasal spray for the adjunctive treatment of individuals with treatment resistant major depressive disorder.

Methods: We describe a 61 year old woman with recurrent episodes of chronic major depressive disorder and multiple treatment failures to antidepressants of different classes as well as failure to respond to a course of ECT who was treated at our Center with esketamine nasal spray while taking tranylcypromine 60 mg daily. Initial mood and anxiety ratings were in the moderate range of severity. Because of concerns regarding safety, she was initially treated with half the usual starting dose (28 mg). This dose was increased to 56 mg for one session but lowered due to adverse effects, mainly dizziness.

Results: She responded well to treatment with esketamine nasal spray after the first treatment and mood and anxiety ratings were in the normal range after completion of the acute treatment phase (8 treatments over 4 weeks). Blood pressure tended to increase during treatment sessions but never reached levels of clinical concern. At baseline, blood pressure ranged from 91-108 mm Hg systolic and 56-70 mm Hg diastolic. Measurements at 40 minutes after dose administration ranged from 99-135 mm Hg systolic and 60-82 mm Hg diastolic. There were no indications of symptoms of a serotonin syndrome. Side effects were mild and included dissociation and sedation, which dissipated during the 2 hour post drug administration period. Her dose for the final 4 sessions was 42 mg.

Conclusions: Our patient was successfully treated with esketamine nasal spray and tranylcypromine for treatment resistant depression. There were no elevations of blood pressure outside the normal range and there was no indication of a serotonin syndrome. Our patient is likely the first to be treated with this combination, although there are reports in the literature of patients treated with MAOIs and IV ketamine or IV S-ketamine.

Keywords: Treatment Resistant Depression, Esketamine Nasal Spray, Tranylcypromine

Disclosure: Janssen, Honoraria

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Background: Ketamine exerts rapid and robust antidepressant effects that are thought to be mediated by activation of the mechanistic target of rapamycin complex 1 (mTORC1). To test this hypothesis, depressed patients were pretreated with rapamycin, an mTORC1 inhibitor, prior to receiving ketamine.

Methods: Twenty-three patients suffering a major depressive episode were randomized to oral rapamycin (6 mg) or placebo, each was followed 2 hours later by intravenous ketamine 0.5 mg/kg in a double-blind cross-over design with treatment days separated by at least 2 weeks. Depression severity was assessed using Montgomery–Åsberg Depression Rating Scale (MADRS). Antidepressant response was defined as a MADRS improvement of 50% or more.

Results: Over the two-week follow-up, we found a significant treatment by time interaction (F(8,245) = 2.02, p = 0.04), reflecting prolonged antidepressant effects post rapamycin+ketamine treatment. At 2 weeks, we found higher response (41%) and remission rates (29%) following rapamycin+ketamine compared to placebo+ketamine (13%, p = 0.04, and 7%, p = 0.003 respectively). However, rapamycin pretreatment did not alter the acute effects of ketamine.

Conclusions: Rapamycin pretreatment failed in blocking the antidepressant effects of ketamine. Unexpectedly, pretreatment with rapamycin prolonged the antidepressant effects of ketamine. This observation raises questions about the role of systemic vs. local blockade of mTORC1 in the antidepressant effects of ketamine, demonstrates that rapamycin may extend the benefits of ketamine, and thereby potentially sheds light on mechanisms that limit the duration of ketamine effects. Registered at clinicaltrials.gov (NCT02487485).

Keywords: Depression, Ketamine, Rapamycin, Rapid-Acting Antidepressant

Disclosure: FSV7, Advisory Board, Lundbeck, Advisory Board, Janssen, Honoraria

T126
A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of TS-121, a Novel Vasopressin V1b Receptor Antagonist, as an Adjunctive Treatment for Patients With Major Depressive Disorder

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Background: There are several lines of evidence indicating the dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis in patients with major depressive disorder (MDD). Arginine vasopressin (AVP), in cooperation with corticotropin-releasing factor (CRF), primarily regulates HPA axis activity through its receptor subtype V1b receptor, and hyperactivity of AVP-V1b receptor system has been noted in patients with MDD. Therefore, blockade of V1b receptor is an attractive approach for the treatment of patients with MDD, in particular for those with impaired HPA axis function. This hypothesis has been underpinned by the animal studies where several V1b receptor antagonists exhibits antidepressant-like and anti-stress-like effects.

THY1773, the active ingredient of TS-121, is a potent and selective V1b receptor antagonist. Pre-clinical studies indicate that THY1773 exhibits antidepressant-like effects in animal models, including the one in which HPA axis function is severely impaired, at doses which occupy the pituitary V1b receptor and thereby block desmopressin/CRF-increased adrenocorticotropin.

A randomized, double-blinded, placebo-controlled Phase 2 trial was designed to evaluate the safety and efficacy of TS-121 in patients with MDD who had an inadequate response to current antidepressant treatment.

Methods: The multicenter study enrolled 51 patients who had a total score of $\geq 18$ on the 17 item Hamilton Depression Rating Scale (HAM-D) at baseline. Patients remained on their current antidepressant therapy with a fixed dose throughout the course of the study. Following a 2 week placebo lead-in period, eligible patients were randomized to receive TS-121 10 mg, TS-121 50 mg or placebo once daily for 6 weeks in a 1:1:1 ratio, followed by a 2 week follow up period. Doses (10 and 50 mg) of TS-121 were determined based on the pituitary V1b receptor occupancy estimated by positron emission tomography (PET) using a selective V1b receptor PET ligand in healthy subjects. The reduction of depressive symptoms compared to placebo, assessed by Montgomery-Åsberg Depression Rating Scale (MADRS) score change from baseline at Week 6, was the primary endpoint. Secondary endpoints included changes from baseline at Week 6 for Clinical Global Impressions-Severity of illness score (CGI-S), Hamilton Anxiety Rating Scale (HAM-A) and Symptoms of Depression Questionnaire (SDQ), percentage of MADRS responders and percentage of CGI-I improvers. Safety and tolerability were assessed by standard safety parameters. Biomarkers to predict response to TS-121 with retrospective stratification of patients via baseline values of HPA axis and inflammatory markers were evaluated.

Results: Of a total of 51 subjects randomized, 43 subjects completed the trial (12 subjects in the TS-121 10 mg group, 15 subjects in the TS-121 50 mg group, and 16 subjects in the placebo group). The changes from baseline in MADRS score at week 6 (Least Square Mean [95% Confidence interval]) were: TS-121 10 mg (-9.0 [-13.9, -4.1]), TS-121 50 mg (-9.0 [-13.4, -4.5]), and placebo (-6.4 [-10.7, -2.2]). TS-121 groups showed greater reductions in the mean MADRS score from baseline compared to placebo, although these reductions did not achieve statistical significance. Moreover, TS-121 groups revealed similar trends of greater improvements compared to placebo group across secondary endpoints (CGI-S, HAM-A, SDQ), indicating an overall improvement in MDD symptoms. Notably, exploratory biomarkers analyses indicated that higher baseline cortisol levels (urinary cortisol and hair cortisol) were associated with a greater separation between TS-121 groups (both doses) and the placebo group in the primary endpoint, which support the concept that V1b receptor antagonists may be more efficacious in individuals who exhibit abnormalities in HPA axis function. There were no death and no severe adverse events reported in the trial. Two patients discontinued the trial due to non-serious adverse events; one in TS-121 10 mg group and the other in the placebo group. Four treatment-emergent adverse events (lenticular opacities, urinary tract infection, medication error, and headache) were reported for two patients in the total TS-121 group, which showed no dose-related trends.

Conclusions: Overall, greater improvement in depression symptoms were observed for subjects treated with TS-121 compared to those treated with placebo, and the trend was more apparent in subjects with higher urinary and hair cortisol levels. These findings combined with good tolerability warrant further investigation of TS-121 in patients with MDD.

Keywords: Antidepressant, V1b Antagonist, HPA, Major Depressive Disorder, Arginine Vasopressin

Disclosure: Taisho Pharmaceutical Co., Ltd.
exploratory endpoint using the patient-reported 36-item Short Form Survey, acute version 2 (SF-36). HAM-D and SF-36 results were assessed using mixed-effects models for repeated measures. Established minimally important differences (MIDs) for SF-36 domain and summary scores were applied to determine clinical relevance. Safety and tolerability were assessed by adverse event (AE) reporting, standard clinical assessments, and the Columbia-Suicide Severity Rating Scale.

**Results:** At the Day 15 primary endpoint, SAGE-217 demonstrated a statistically significant decrease in least-squares (LS) mean HAM-D total score compared with placebo (-17.8 vs. -13.6, p = 0.0028). Statistical separation occurred as early as Day 3 (p = 0.0252) and was sustained through Day 45 (p = 0.0027). The SAGE-217 group showed greater numerical improvements versus the placebo group in all SF-36 domain and summary scores at Days 15 and 45, with statistically significant improvements in 5/8 domains (Physical Functioning, Role Physical, Bodily Pain, Social Function, and Mental Health) and the Mental Component Summary score at Day 45 (p < 0.05 for all). At Day 15, 7/8 domains scores (all except General Health) for the SAGE-217 group achieved MIDs versus the placebo group. At Day 45, in addition to sustaining MIDs versus placebo for the 7 domain scores, the SAGE-217 group achieved a MID in the Mental Component Summary score. Somnolence, headache, dizziness, upper respiratory tract infection, diarrhea, and sedation were the most common (≥5%) AEs in the SAGE-217 group. There was no signal for increased suicide ideation.

**Conclusions:** SAGE-217 administration was associated with rapid (by Day 3), statistically significant, and sustained (through Day 45) reductions in depressive symptoms in women with PPD in this double-blind, randomized, placebo-controlled Phase 3 trial. This improvement in clinician-rated depression was followed by statistically significant improvements across multiple domains of patient-reported HRQoL at Day 45. These results support the further study of SAGE-217 in PPD.

**Keywords:** SAGE-217, Postpartum Depression, GABA, Health-Related Quality of Life

**Disclosure:** Sage Therapeutics, Inc., Consultant

## T129

**Increase in Adult Neurogenesis Maintains Dentate Gyrus Volume and Induces Stress Resilience**

Abstract not included.

## T130

**A New Care Model to Treat Bipolar Disorder Using a Six-Week Cycle Organized in an Integrated Practice Unit: Preliminary Results of 100 Individuals Into the First Cycle**


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**Background:** Bipolar disorder is associated with a high disease burden and cost with up to 2.4% of the population experiencing bipolar spectrum disorders. It occurs consistently across sex, race, ethnicity, geography, and socioeconomic class. Bipolar disorder is associated with the highest rate of suicide, occurring in up to 15% of individuals. Individuals with bipolar disorder frequently experience a relapsing and remitting course of illness. It impacts mood, cognition and psychosocial function across a wide range of behaviors and psychosocial function. Taken together, bipolar disorder requires a systematic and integrated treatment approach that incorporates multiple modalities, from psychopharmacological to psychosocial, that are challenging to address in general psychiatric practice. To address this complexity, we implemented an integrated practice unit specialized to treat bipolar disorder in the form of a six-week cycle where individuals meet weekly with a therapist and psychiatrist in tandem. We conducted a retrospective chart review of the first 100 cases receiving care at this outpatient unit.
Methods: The integrated practice unit developed at the Mulva Clinic for the Neurosciences, in partnership with Integral Care, uses a predetermined care pathway organized around the individual with bipolar disorder using a multi-disciplinary team, including psychiatrist, therapist, pharmacist, and peer support specialist. The care pathway is modeled on a six-week cycle with systematic integration of evidence-based pharmacological and psychosocial treatments. The term “cycle” is deliberately used within the team and person receiving care to create a limited time frame that is measurable and repeatable (i.e., one may enter into subsequent cycles if no improvement is achieved). Each week, the person meets with a psychiatrist for up 30 minutes and with a therapist for up to 60 minutes. These two visits are provided in the same room, one after the other to reinforce the person-centered nature of the care delivery. Prior to entering a cycle, each person participates in a comprehensive diagnostic evaluation that includes a structured clinical interview. In the first visit of the cycle, the team develops a personalized psychosocial treatment plan based on the six most problematic areas identified by the patient out of a menu of twenty-four common problems typically seen in bipolar disorder. Each problem is managed using predetermined treatments that are evidence-based. Moreover, any medication change is based on the latest guidelines. Finally, each individual also completes patient reported outcomes (PROs) as part of the standard of care at every visit that delineates the person’s clinical composition and helps to guide treatment decisions. Consistent with an integrated practice unit principles, these PROs are dynamic and subject to change based on perceived value to the patient and clinician (i.e., if measured outcomes are not sensitive/informative, they will be dropped in a subsequent integrated practice unit generation). Here, we completed a retrospective chart review of a set (function level, depression and mania symptoms, quality of life level) of patient reported outcomes using repeated measures anova with week number as within-subject factor (6 levels, one for each week); episode type (i.e. depressive vs hypo/manic episode) and gender as covariate. Individuals in remission were excluded from the analysis (n = 2). Missing data was replaced using the mean of two nearby points. Statistical differences at p <= 0.05. IRB approved study.

Results: The majority of the sample was characterized by individuals with bipolar disorder type 1 (n = 79, 69% female, mean age 32.2 years old, depressive episode 81%, manic episode 14%, hypomanic episode 3% and on remit (n = 3%); followed by individuals with bipolar disorder type 2 (n = 17, mean age 32.4 years old, depressive episode 76%, hypomanic episode 24%). The remaining 4 individuals had either unspecified (n = 1) or other specified bipolar disorder and related disorder (n = 3). Repeated measures anova indicated a main effect with respect to week number (i.e. improvement with longer duration in the program) and manic symptoms (F(3.5, 316) = 6.7, p < 0.001), and function level (F(4.1, 375) = 4.1, p = 0.003) but not for depression symptoms (F(4, 377) = 1.1), nor to quality of life level (F(2.5, 228) = 1.3). As expected, between-subject effects were significant for manic symptoms (i.e. worse in hypo/manic episode, F(1, 91) = 3.9, p = 0.05) and depression symptoms (i.e. worse in depressive episode, F(1, 91) = 6, p = 0.015) but not for quality of life level (F(1, 91) = 0.1). A trend towards worse functioning level was present in individuals during a depressive episode (F(1, 90) = 3.2, p = 0.08). Week number and episode type interactions were present with respect to depression symptoms (F(4, 377) = 3, p = 0.02), and manic symptoms (F(3.5, 316) = 6, p < 0.001), but not for quality of life level (F(2.5, 228) = 1.4), nor to function level (F(4.2, 375) = 1.3).

Conclusions: The first generation of our bipolar disorder integrated practice unit modeled as a six-week cycle program improved individuals function level regardless of episode type. Manic symptoms also improved but more so in individuals during hypo/manic episodes, as one would expect. Depression symptoms and quality of life improvements were not statistically significant, regardless of episode type. It is reasonable to speculate that depressed individuals are more likely to improve during a second cycle of the program.

Keywords: Bipolar Disorder, Care Coordination, Patient Reported Outcomes

Disclosure: Nothing to disclose.

T131

Novel Loci Identified for Affective Disorders via Whole Genome Sequencing in Extended Pedigrees

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Background: Our goal is to identify genes that increase risk for affective and psychotic disorders. Although these highly heritable diseases are associated with substantial morbidity and mortality, their etiologies remain poorly understood. Identifying genes that contribute to their risk should provide critical information leading to the development of novel diagnostic and therapeutic strategies. We describe novel and unpublished findings from an eight-site international consortium designed to identify rare causal variants for affective and psychotic illnesses using extended multiplex pedigrees.

Methods: Multigenerational families (N = 4371; 55% female; mean age = 43.13 years, SD = 16.76, range = 10–97) included at least three individuals affected by an affective or psychotic disorder. Diagnostic phenotypes were harmonized across sites. For sequence analysis, we focused on the identification of rare variants (MAF <= 0.01) that have a large absolute effect size, despite being present in only a small number of related affected individuals. Samples were sequenced to a minimum of 30x average coverage using Illumina HiSeq 2000 systems and the sequencing analysis pipeline utilizes the Isaac Genome Alignment and Isaac Variant Caller to detect SNPs and small indels. Measured genotype association analyses were conducted using SOLAR. Empirical kinship matrices were used in place of the standard pedigree-driven kinship matrix. Discrete phenotypes were analysed as inverse normalized pseudo-quantitative traits. Age and sex plus their interactions (age^1,1,2,3,sex) were included as covariates into the model.

Results: 469 individuals were diagnosed with psychosis, 182 with bipolar disorder I (BDI) and 956 with a major depressive disorder. We identified novel associations for BDI (r = 56.68, p = 5.13 x 10^{-14}, 19q13.2) in the PRX gene and (r = 47.00, p = 7.10 x 10^{-12}, 2q21.2) adjacent to the IL-1 cluster. Suggestively significant variants were identified for MDD (r = 26.40, p = 2.77 x 10^{-9}, 16p13.13) in the TTNDC1 gene, and psychosis (r = 25.41, p = 4.64 x 10^{-7}, 17q24.3) and also potential pleiotropic effects with cognitive ability (mean rhog between risk for psychosis and general cognitive ability = 0.41, se = 0.22).

Conclusions: This study shows the utility of pedigree-based designs for genetic study of psychiatric disorders as a complement to more typical case-control designs and highlights a number of genes implicated in immune-response in the etiology of affective disorders. This approach represents an implicit enrichment strategy for identifying the rarest (e.g., private or pedigree-specific) variants, as Mendelian transmissions from parents to
offspring maximize the chance that multiple copies of rare variants exist in the pedigree.

**Keywords:** Human Genetics, Extended Pedigrees, Whole Genome Sequencing, Affective Disorders, Psychosis

**Disclosure:** Nothing to disclose.

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### T132

**The Muscarinic M1 Receptor Regulates Motivated Behavior via Excitation of Nucleus Accumbens D1 Medium Spiny Neurons and Facilitation of Dopamine Release**

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**Background:** Motivational symptoms are debilitating features of several neuropsychiatric disorders and are highly correlated to long-term treatment outcomes. Recent studies have highlighted the regulatory role of nucleus accumbens (NAc) dopamine (DA) release and D1 medium spiny neurons (MSNs) in motivated behavior. Increased NAc DA release and D1-MSNs are pro-motivational and a disruption in either has been correlated with anhedonic-like phenotypes, suggesting that agents that increase DA release or increase excitability of D1-MSNs may be efficacious for the treatment of motivational dysfunctions. Gq/11-coupled muscarinic acetylcholine (mACh) M1 receptors are highly expressed in the NAc and have been reported to regulate the transition states of striatal MSNs and NAc DA transmission; however, the exact role of M1 on NAc D1-MSNs or DA release has not yet been investigated. The current studies will greatly advance our understanding of motivational circuitry and elucidate the therapeutic potential of M1 as a novel target for the treatment of motivational dysfunctions.

**Methods:** We first assessed M1 activation on NAc DA release via in vivo fast-scan cyclic voltammetry following administration of highly selective tool compounds for M1. Using whole-cell electrophysiology, we assessed the effect of M1 activation in the modulation of excitability and burst activity of NAc D1-MSNs by monitoring changes in membrane potential and the number of spike discharges in response to near threshold depolarizations. Next, we examined real-time activity of M1 activation on NAc D1-MSNs in vivo, by recording calcium (Ca2+) transients in D1-cre mice following administration of saline-vehicle, the M1 agonist VU0364572 and positive allosteric modulator (PAM) VU045395. To normalize the data, the control channel was fitted to and then subtracted from the raw trace, giving the ΔF/F. For peak detection, data were high-pass filtered and transformed to a Z-score. To confirm the effects on motivated behavior, global M1 knockout (M1−/−) and mice lacking the M1 receptor in D1-MSNs (D1-M1−/−) were trained in a traditional progressive ratio (PR) concurrent choice task based.

**Results:** Bath application of VU0364572 and VU045395 increased D1-MSN excitability and DA release in a concentration dependent manner compared to baseline conditions (paired t-test, p < 0.05). Application of the M1 agonist or PAM increased the number of spike discharges in response to the depolarizing current pulse in D1-MSNs and the number of spikes per pulse. Interestingly, these effects were not observed in D2-MSNs (paired t-test, p > 0.05). Activation of M1 altered D1-MSN Ca2+ transient frequency and amplitude compared to saline-control conditions (one-way ANOVA, p < 0.05). Compared to littermate controls, M1−/− and D1-M1−/− mice had attenuated breakpoints and biased choice allocation to the lower effort option (two-way ANOVA, p < 0.01).

**Conclusions:** M1 receptors in the NAc play an important modulatory role on D1-MSN excitability and can facilitate DA release. Together, these experiments may help to elucidate precise neural circuit dynamics that underlie aberrations in motivated behavior, which could lead to the development of safe and effective treatments for motivational dysfunctions.

**Keywords:** Motivation, D1 Dopamine Receptors, Dopamine, Muscarinic M1, Nucleus Accumbens

**Disclosure:** Nothing to disclose.

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### T133

**Visualization of AMPA Receptors in Patients With Depression, Bipolar Disorder, and Schizophrenia: The First PET Imaging Study**

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**Background:** Evidence on physiological roles of AMPA receptors in psychiatric conditions has been accumulated, which mainly derives from psychiatric disease model animals as well as post-mortem brain tissues. However, its clinical translation was limited due to lack of any tool to visualize AMPA receptors in living human brain. The development of positron emission tomography (PET) probe for AMPA receptors is expected to provide a powerful tool to tackle this problem. Here, we used the first PET probe that specifically binds to AMPA receptors and successfully visualized these receptors in living human brain of patients with depression, bipolar disorder, and schizophrenia.

**Methods:** We developed a novel PET probe for AMPARs. We radio-labelled a derivative of 4-(2-phenylsulfonylamino)ethylthio)-2,6-difuoro-phenoxyacetamide with 11C, named [11C]K-2. Male patients aged 30-49 with depression, bipolar disorder, and schizophrenia according to Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5), respectively, underwent a [11C]K-2 PET scan and an MRI scan for co-registration of the PET image and received clinical assessments for symptomatology, including the Montgomery-Asberg Depression Rating Scale (MADRS), the Young Mania Rating Scale (YMRS), and the Positive and Negative Syndrome Scale (PANSS) (registration number: UMIN000025132).

[11C]K-2 was synthesized at Yokohama City University Hospital in accordance with GMP ordinance and was certified by the Japanese Society of Nuclear Medicine. PET imaging was performed with a TOSHIBA Aquiduo scanner (TOSHIBA Medical), which provided an axial FOV of 240 mm, and 80 contiguous 2.0 mm thick slices.

Nine patients with depression (age, 41.7 ± 6.0 years; duration of illness, 6.9 ± 4.2 years; MADRS total score 10.6 ± 6.6), 9 patients with bipolar disorder (age, 42.2 ± 3.9 years; duration of illness, 14.4 ± 6.8 years; MADRS total score 10.6 ± 10.1; YMRS total score 7.4 ± 7.5), and 10 patients with schizophrenia (age, 43.1 ± 3.1 years; duration of illness, 16.8 ± 8.0 years; PANSS total score 61.7 ± 13.4) participated in this study.

**Results:** Standardized uptake value ratio (SUVR)30-50 min with the white matter as a reference decreased over a wide range of brain areas in patients with depression. According to a voxel-wise analysis of the correlation between the SUVR30-50 min and MADRS total scores, the prefrontal cortex, supplementary motor area, and cerebellum exhibited the most significant negative correlations. In patients with bipolar disorder, the voxel-wise...
analysis found significant negative correlations between the SUVR30-50 min with the whole brain as a reference and MADRS total scores in the frontal gyrus and straight gyrus, and the SUVR30-50 min and the YMRS total scores in the cerebellum. Patients with schizophrenia exhibited negative correlations between SUVR30-50 min with the whole brain as a reference in the parahippocampal and cingulate gyrus and PANSS total scores according to the voxel-wise analysis.

Conclusions: [11C]K-2 has revealed distinct distribution patterns of AMPA receptors for each of these major psychiatric disorders. Thus, [11C]K-2 is proven to be a potent PET tracer that can be used to visualize AMPARs with high contrast, leading to the elucidation of the molecular mechanisms of neuropsychiatric diseases by trans-species approaches, thereby facilitating novel drug discovery and the alteration of clinical practice.

Keywords: AMPA, AMPA Receptors, Depression, Bipolar Disorder, Schizophrenia, Synaptic Aberrations

Disclosure: Nothing to disclose.

T134

Developing and Validating Short Forms of the Youth Self-Report GBI Mania and Depression Scales

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Background: Clinical trials for mood disorders often use interviews as the basis for measuring symptom severity and outcomes (Cerineme, Goldberg, Miller, Gabrielson, & Fortney, 2019). These have limitations in terms of requiring training to ensure consistent calibration and prevent drift across raters or sites (Mackin, Targum, Kalali, Rom, & Young, 2006), and they do not transport easily to clinical practice, where they may not be reimbursed by payers and may not fit into medication check visits. A validated, brief self-report rating of hypomanic and depressive symptoms could play key roles in screening, enrolment in trials, calibration across sites, quantifying symptom severity, and tracking progress and outcomes in larger systems of care (Guo et al., 2015; Youngstrom et al., 2013).

Our goal was to develop and externally validate short forms of adolescent self-report. We report psychometric information of three GBI youth self-report short scales, one for mania when predicting any bipolar spectrum, and two for depression when predicting the presence of any mood disorder. We use two independent samples that differ in terms of demographic and clinical characteristics to evaluate generalisability of discriminative validity of these scales (Youngstrom, Halverson, Youngstrom, Lindhiem, & Findling, 2018).

Methods: The academic sample (n = 427) consisted of families seeking outpatient services at an academic medical center. The community sample (n = 313) consisted of families presenting to a large urban community MH center. All families were seeking outpatient MH services for youth between 5 and 18 years old; 160 youth (37%) from the academic sample received a BP diagnosis, as did n = 41 (13%) in the community sample. KSADS-PLW interviews with the caregiver and youth determined diagnoses, and both completed the GBI inventory, a 73 items scale that uses a 4-point Likert scale, and other mood severity ratings.

Descriptive statistics and effect sizes compared samples. Reliability, factor analyses and a series of correlations between short forms and various severity rating tested content and criterion validity test. We conducted ROC analyses to evaluate discriminant validity, computing multilevel DiLRs to provide practical guidelines for using these new short scales. All procedures were IRB reviewed and approved.

Results: There were more White and affluent families in the Academic sample, with youth averaging a year older in age when compared to those in the Community sample. They also showed higher scores on the mania and the depression rating scales, consistent with having a specialty mood clinic. Contrary, CBCL Externalizing scores were higher in the Community sample. However, we did not observe significant differences between samples, when comparing scale scores from youth self-report measures. Rates of CD, Anxiety and PTSD all were higher in the Community sample.

In terms of factor structure, all AGBI items showed adequate to strong loadings on the hypothesized factors and modest cross loadings in the short form scales. Reliability alphas in the Academic and Community samples were higher than projected (alphas > 0.899). IRT analysis showed that all of the short forms maintained good conditional reliability through the bulk of the latent trait. Content coverage was excellent, with r = 0.94 to 0.96 with corresponding full length scale. In terms of criterion correlations, convergent values for the depression scales and interview ratings were good for depression (r = 0.38 to 0.41) and showed good divergence with mania (r = 0.10 to 0.17 with YMRS scores, and lower with bipolar diagnoses). The 10M showed moderate agreement with YMRS scores, low divergent correlations with depression and internalizing scores, and higher correlation with externalizing.

When testing discriminative validity, the 10M AUC was 0.62 in the Academic and 0.63 in the Community sample, without difference when compared with the full-length form. Both forms of the depression scales performed similar in both samples and when compared with full length form, with AUCs of the same magnitude than the AUCs observed for the 10M. Finally, we observed diagnostic likelihood ratios for all scales greater than 2, confirming that information from these scales could be clinically useful in combination with other information.

Conclusions: These brief self-report rating scales of hypomanic and depressive symptoms reduce scale length by 64% and 72%, while retaining strong reliability, content coverage, and convergent, discriminant, and discriminative validity. The reduced length makes them attractive for use in clinical trials and measurement based care, where they have shown sensitivity to treatment effects in masked RCTs (Findling et al., 2012; Youngstrom et al., 2013). Also, parent report on the same scales is likely to be more diagnostically discriminating, having brief self-report scales will be valuable when caregiver data is unavailable. It also may more directly predict motivation for treatment, therapeutic alliance, and adherence than collateral reports might be able to do. Because the full length GBI is available in Spanish, Portuguese, and Korean translations, the short forms are also available. Prior work comparing the embedded and extracted item formats of administration found negligible changes in psychometric performance in an overlapping sample (Freeman et al., 2012).

Keywords: Bipolar Disorder, Children and Adolescents, Assessment

Disclosure: Guilford Press, Royalties, American Psychological Association, Honoraria

T135

Generalizing the Prediction of Bipolar Disorder Onset Across Populations

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Background: Adolescence is a high risk period for psychopathology, including bipolar spectrum disorders (BPDS). Many individuals will go ten years or longer before receiving an accurate diagnosis and appropriate treatment (Drancourt et al., 2014; Lish et al., 1994; Marchand et al., 2006). Unfortunately, this means that many young people experience significant mood symptoms during an important developmental period, resulting in consequences in both the short- and long-term (Elanjithara et al., 2011).

The use of risk calculators to estimate risk for specific clinical outcomes – established in other medical fields – is gaining interest in psychiatry (Cannon et al., 2016; Fazel, Wolf, Larsson, Mallett, & Fanshawe, 2019; Fusar-Poli et al., 2017). Risk calculators may inform progression and treatment planning in at-risk populations; because BPDSs are highly heritable and often preceded by non-specific symptoms of psychopathology, a RC to estimate risk of BPSD could help to reduce the duration of undiagnosed BPSD and improve clinical outcomes.

A previous study produced a RC for identifying bipolar offspring (Birmaher et al., 2010) likely to meet criteria for a BPSD in the future (Hafeman et al., 2017). This investigation’s goal was to evaluate the performance of this RC in a clinical high risk population and to adapt the existing RC to achieve adequate predictive ability in both the familial high risk and clinical high risk samples, in order to best serve high risk youth broadly.

Methods: Participants (aged 6-12 at baseline) from the Longitudinal Assessment of Manic Symptoms (LAMS) study (N = 473) were evaluated semiannually for six years on average. Evaluations included a KSADS semi-structured diagnostic interview. The majority of participants were recruited based on elevated symptoms of mania (Parent General Behavior Inventory 10-item mania scale; Youngstrom et al., 2008). After testing a RC that approximated the original as closely as possible (predictors were: scores from the KSADS depression and mania modules, score from the Screen for Child Anxiety Related Disorders self report [SCARED], the C-GAS, and age), we made a number of modifications in order to improve model prediction, based on characteristics of the LAMS sample. Models were initially trained in the familial high risk sample and tested in LAMS.

Results: Over the follow-up period, 65 youth newly met criteria for BPSD. The RC originally developed in a familial high-risk sample did not discriminate well between youth who developed BPSD and those who did not in the LAMS sample (5-year AUC = 0.63). Eliminating assessments from the baseline and first follow-up assessments, during which time participants were likely to be highly symptomatic, which could bias predictions, resulted in improved accuracy (5-year AUC = 0.68).

Next, we removed measures of anxiety and general functioning from the model (retaining the baseline and first follow-up assessment) in order to focus on bipolar-specific symptoms (5-year AUC = 0.68). This model accomplished the goal of limiting the bias associated with intense, non-specific symptoms and is more practical than the RC that eliminated the first two evaluations. A similar model including only core symptoms associated with the prodrome to BPSD (Van Meter, Burke, Youngstrom, Faedda, & Correll, 2016) performed equally well. As a last step, we removed age from the model (5-year AUC = 0.68), then trained the model in LAMS and externally-validated it in the familial high risk sample, in order to get an estimate of potential generalizability in new samples. The model that included only the core symptoms of mania and depression fit well (5-year AUC = 0.72).

Conclusions: Risk calculators have the potential to improve diagnostic accuracy and to reduce the duration of undiagnosed BPDS. However, the results of our investigation make clear that the clinical circumstances under which the assessment of early symptoms occurs impacts prediction accuracy. This study built on the RC previously tested in a familial high risk sample, broadening its utility to include treatment-seeking youth at clinical high risk. Greater generalizability was achieved by focusing solely on symptoms related to the onset of BPSD, but greater generalizability came at the cost of reduced accuracy. Integration of additional diagnostically-specific factors may improve the generalizability and accuracy of future risk calculators. Validation of the RC under clinically-realistic circumstances will be an important next step in determining whether psychiatric services can be supported with this RC in the way that other fields have benefited (Hafeman et al., 2017).

Keywords: Bipolar Disorder, Predictive Models, Computational Psychiatry, Children and Adolescents

Disclosure: Nothing to disclose.

T136

Appraising Esketamine Nasal Spray for the Management of Treatment Resistant Depression in Adults: What is the Number Needed to Treat, Number Needed to Harm, and Likelihood to be Helped or Harmful?

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Background: A novel approach to the treatment of major depressive disorder (MDD) is the targeting of glutamate receptors. The current study aim is to review the evidence-base for esketamine nasal spray for the management of treatment-resistant depression (TRD) using the metrics of evidence-based medicine: number needed to treat (NNT), number needed to harm (NNH), and likelihood to be helped or harmed (LHH).

Methods: Data sources were four completed Phase III randomized, double-blind, placebo-controlled, studies, including the two pivotal studies of intranasal esketamine in TRD in nonelderly adults (acute flexible-dose study NCT02418585 and maintenance study NCT02493868) that were supportive of its approval in the United States. Efficacy outcomes included acute response (>50% decrease from baseline on the Montgomery-Asberg Depression Rating Scale [MADRS] total score), acute remission (MADRS scores ≤12, as well as other thresholds using the MADRS and Clinical Global Impressions-Serious [CGI-S] scales), categorical shifts in MADRS and CGI-S scores, and avoidance of relapse/recurrence (observed relapse rates). Tolerability outcomes included commonly occurring adverse events (AEs) and rates of discontinuation because of an AE. NNT and NNH are calculated for esketamine nasal spray plus a newly initiated oral antidepressant (esketamine+AD) vs. placebo nasal spray plus a newly initiated oral antidepressant (placebo+AD, active comparator) in patients with TRD.

Results: In the acute flexible-dose study of esketamine nasal spray (56-84 mg twice-weekly for 4 weeks), MADRS response with esketamine+AD vs. placebo+AD at endpoint (71/112 [63.4%] vs. 54/109 [49.5%]) yielded a NNT value of 8 (95% CI 4-111), and MADRS remission at endpoint (54/112 [48.2%] vs. 33/109 [30.3%]) resulted in a NNT vs. placebo+AD of 6 (95% CI 4-19). AEs where the NNH values vs. placebo+AD were <10 include dissociation (30/115 [26.1%] vs. 4/109 [3.7%]), vertigo (30/115 [26.1%] vs. 3/109 [2.8%]), nausea (30/115 [26.1%] vs. 7/109 [6.4%]), dizziness (24/115 [20.9%] vs. 5/109 [4.6%]), and dysgeusia (28/115 [24.3%] vs. 13/109 [11.9%]) with NNH values of 5 (95% CI 4-8), 9 (95% CI 7-33), 6 (95% CI 4-10), 7 (95% CI 5-13), and 9 (95% CI 5-41), respectively.


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Discontinuation rates because of an AE (8/115 [7.0%] vs. 1/109 [0.9%]) yielded a NNH value of 17 (95% CI 10-95). LHH comparing MADRS remission vs. discontinuation because of an AE is 17/6, or approximately 3. The pattern of results was similar for the other acute studies and for the pooled data combining all 3 acute studies. Maintenance use of esketamine (dose 56–84 mg once-weekly or once-every-other-week) plus an oral AD demonstrated NNT values regarding relapse and/or maintenance of remission that were in favor of esketamine+AD vs. placebo+AD, with NNT values <10 and as robust as 4 (95% CI 3-7), the latter observed for the outcome of relapse in patients with stable response at the time of randomization (relapse rates for these patients were 16/62 [25.8%] vs. 34/59 [57.6%, respectively). In the maintenance study, discontinuation rates because of an AE (4/152 [2.6%] vs. 3/145 [2.1%]) yielded a NHH value of 178 (not statistically significant). Indirect comparisons of the above results in TRD with that of the effect sizes seen in positive studies of antidepressant mono-therapy vs. the placebo condition in patients who are not treatment resistant demonstrate similar values for NNT for response and avoidance of recurrence, further supporting the robustness of esketamine+AD for TRD.

Conclusions: In general, efficacy outcomes for esketamine+AD vs. placebo+AD in persons with TRD as assessed in the pivotal Phase III demonstrated NNT values <10 and denote that esketamine+AD is a potentially efficacious intervention for TRD both acutely and in maintenance use. LHH is also favorable: esketamine+AD is 3 times as likely to result in an acute remission vs. discontinuation because of an AE.

Keywords: Esketamine Nasal Spray, Treatment Resistant Depression, Evidence-Based Approach


T137

Hepatic CYP450 Isoforms Activity Contribute to the Sustained Antidepressant-Like Response to Ketamine

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Background: (R,S)-ketamine has a rapid and persistent antidepressant activity following administration of a single sub-anesthetic dose to treatment-resistant depressed (TRD) patients and in rodent models. It is a racemic mixture containing equal parts of (R)- and (S)-enantiomers, which are metabolized by multiple cytochrome P450 (CYP) enzymes to norketamine, and then to 12 different hydroxynorketamines (HNKs). Of these HNKS, (2S,6S;2R,6R)-HNK is the most abundant circulating HNK ketamine metabolite. However, currently, there is a controversy regarding (2S,6S;2R,6R)-HNK’s antidepressant activity in rodents (Collingridge et al., 2017; Zanos et al., 2017; Abdallah, 2017). To better understand the selective antidepressant actions of (R,S)-ketamine and its metabolites, we assessed whether pre-treatment with a CYP inhibitor (CYP1) would decrease (R,S)-ketamine’s metabolism and impact its antidepressant-like efficacy.

Methods: The antidepressant-like activity of an acute systemic administration of saline or (R,S)-ketamine (10 mg/kg, intraperitoneally, i.p.) was examined in male BALB/cJ mice pre-treated either with saline or the CYP1 inhibitor (Fluconazole (10 and 20 mg/kg, i.p.) 1 h before (n = 10 mice per group). First, we established pharmacokinetic parameters in the plasma over the first 2 hours following administration of (R,S)-ketamine alone versus [Fluconazole + (R,S)-ketamine (20 and 10 mg/kg, respectively). Then, twenty-four hours after (R,S)-ketamine (t24h), the forced swim test (FST), the open field test (OFT) and in vivo microdialysis in the medial prefrontal cortex (mPFC) were performed. Cortical extracellular levels of glutamate, GABA and glutamine (Glut, GABA-ext, Gln-ext) were concomitantly measured. Plasma and brain levels of (R,S)-ketamine, norketamine, and HNKS were also measured at this time point by high performance liquid chromatography (HPLC) coupled to mass spectrometry.

Results: Fluconazole dramatically changed the pharmacokinetic profile of (R,S)-ketamine, increasing plasma levels of (R,S)-ketamine at 0, 30 min, and 1 h, while markedly reducing levels of (2S,6S;2R,6R)-HNK at these time points. Fluconazole pre-treatment prevented the sustained antidepressant-like activity of (R,S)-ketamine evidenced by a decrease in FST swimming duration [F(1,54) = 22.35, p < 0.001], as well as cortical Glut-ext [F(2,36) = 2.44, p < 0.01] and GABA-ext [F(2,36) = 3.109; p 0.01] in a dose-dependent manner. In the OFT, no statistically significant differences were found in the locomotor activity among the 6 groups studied indicating that the FST effects are not being mediated by locomotor changes. Only trace concentrations of (R,S)-norketamine and (R,S)-HNK were present in the plasma and the brain at t24h in mice pre-treated with fluconazole 10 and 20 mg/kg, relative to the (R,S)-ketamine-treated group.

Conclusions: Overall, these findings suggest a pre-treatment of mice with a CYP1 blunted (R,S)-ketamine’s antidepressant-like efficacy by decreasing its metabolism, emphasizing a key role of (2S,6S;2R,6R)-HNK in the sustained antidepressant-like activity of (R,S)-ketamine. These results should also encourage clinicians to avoid concomitant use of (R,S)-ketamine and a CYP1 to maintain its sustained antidepressant activity in TRD patients.

Keywords: Hydroxynorketamine, Fast-acting Antidepressant, Glutamate GABA, Medial Frontal Cortex, Balb/c Mouse

Disclosure: Nothing to disclose.

T138

Time to Antidepressant Response in Double-Blind, Randomized, Placebo-Controlled Trials of Brexanolone Injection in Postpartum Depression


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Background: Across the United States (US), estimates of new mothers experiencing symptoms of postpartum depression (PPD) range from 8 to 20%, with an overall average of 11.5%, making PPD one of the most common medical complications during and after pregnancy. Under- or untreated PPD is associated with significant short- and long-term impacts, including increased maternal mortality due to suicide, highlighting the need for effective and rapid treatment. However, for many patients, current antidepressant...
therapies have a latency to response of 4-8 weeks. Dysfunctional GABAergic signaling has been implicated in the pathophysiology of PPD by animal and human studies, and neuroactive steroid (NAS) positive allosteric modulators (PAMs) of GABA-A receptors (GABA-A-Rs) are being explored as potential PPD therapies. Treatment of a mouse model of PPD with the synthetic NAS GABA-A-R PAM SGE-516 resulted in a decrease in depression-like behaviors. NAS GABA-A-R PAMs, such as allopregnanolone, have a pharmacology distinct from benzodiazepines, including targeting of both synaptic and extrasynaptic GABAARs and upregulation of GABA-A-R surface expression. The US Food and Drug Administration recently approved brexanolone injection (BRX), an intravenous formulation of synthetic allopregnanolone, for the treatment of adults with PPD. BRX achieved the primary endpoint of a significant reduction in depressive symptoms (measured by the Hamilton Rating Scale for Depression; HAM-D) versus placebo after Hour 60 of treatment in three double-blind, randomized, placebo-controlled trials (Study A, NCT02614547; Study B, NCT02942004; and Study C, NCT02942017). The time to antidepressant response (as assessed by HAM-D response or Clinical Global Impression – Improvement (CGI-I) response) was examined in post-hoc analyses of an integrated dataset from these three trials.

**Methods:** The three studies shared a similar protocol allowing dataset integration and analysis. Women ages 18-45, ≤6 months postpartum, with PPD and a qualifying HAM-D total score (Studies A and B: HAM-D ≥26; Study C: HAM-D 20-25) were enrolled and received a 60-hour continuous inpatient infusion of placebo or BRX, with four weeks follow-up. Patients were randomized 1:1 to placebo or BRX 90 µg/kg/h (BRX90) in Studies A and C. Patients in Study B were randomized 1:1:1 to placebo, BRX90, or BRX 60 µg/kg/h (BRX60). Efficacy endpoints were evaluated from an integrated dataset of patients receiving placebo or BRX90. The primary endpoint in each individual study and the integrated analysis was the change from baseline in HAM-D score at Hour 60. Secondary endpoints included HAM-D scores at all other time points, CGI-I throughout the study, and categorical assessments of HAM-D response (reduction in score ≥50%) and CGI-I response (a score of ‘1 – very much improved’ or ‘2 – much improved’). Post-hoc analyses of time to onset (i.e. first time point of a subject’s response by HAM-D or CGI-I) was assessed by Kaplan-Meier analysis. Safety and tolerability were examined by adverse event reporting and standard clinical assessments from an integrated study population including all placebo, BRX90, and BRX60 patients.

**Results:** BRX was administered to 140 patients (102 BRX90 and 38 BRX60), and placebo was administered to 107 patients. BRX90 achieved the primary endpoint of a statistically significant reduction in HAM-D score at Hour 60 compared with placebo (−17.0 vs. −12.8, diff. −4.1, p < 0.0001) that was sustained throughout the study (Day 30 −16.9 vs. −14.3, diff. −2.6, unadjusted p = 0.0213). At Hour 60, the cumulative HAM-D response rates for BRX90 and placebo were 81.4% and 67.2%, respectively. Through Day 30, BRX90 achieved a cumulative HAM-D response rate of 88.2% versus 75.7% for placebo. BRX90 demonstrated a greater cumulative CGI-I response rate compared with placebo at Hour 60 (81.4% vs. 61.6%) and Day 30 (88.1% vs. 78.5%). In Kaplan-Meier analysis, BRX90 demonstrated statistically significant decreases in time to onset of HAM-D (p = 0.0265) and CGI-I (0.0058) response versus placebo. The median HAM-D response onset time was 24 hours (95% CI 24 to 36) for BRX90 versus 36 hours (95% CI 24 to 48) for placebo. The median CGI-I response onset time was 24 hours (95% CI 24 to 36) for BRX90 versus 36 hours (95% CI 24 to 60) for placebo. Adverse events occurring in all BRX groups (BRX90 and BRX60) in ≥5% of BRX and two or more times the rate of placebo were sedation/somnolence, dry mouth, loss of consciousness, and flushing/hot flush.

**Conclusions:** BRX achieved rapid (by Hour 60), sustained (through Day 30), clinically and statistically significant reductions in depressive symptoms versus placebo as assessed by HAM-D total score change from baseline. Using categorical assessments of HAM-D or CGI-I response, BRX90 was associated with significantly faster median time to onset versus placebo. BRX was generally well tolerated in the all BRX population. These results suggest the potential for rapid antidepressant response rates with BRX and support brexanolone as a meaningful treatment option in adult women with PPD.

**Keywords:** GABA, GABA-A Receptors, Postpartum Depression, Brexanolone, Neuroactive Steroid

**Disclosure:** MedScape, Consultant, Janssen, Grant, Cala Health, Consultant, Sage Therapeutics, Inc

T139

**Analysis of Brain Microglia and Behavior After LPS-Induced Systemic Inflammation in Mice**

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**Background:** There is a growing body of literature that would suggest that inflammation plays some role in most CNS diseases including neurodegenerative and neuropsychiatric disorders. Although the sequelae of peripheral inflammation leading to clinical symptomatology is unclear, it is becoming apparent that microglia and neuroinflammation play a critical role in the pathophysiology of such disorders. To model the consequences of elevated inflammation in the periphery, we challenged mice with LPS intraperitoneally to study consequences on brain microglia and neuroinflammation.

**Methods:** Adult mice received intraperitoneal injections of LPS (0.8 mg/kg). Changes in plasma and brain homogenates cytokines/chemokines levels were measured at 1, 4 and 24 hr using Luminex® multiplex technology. The 4 hr timepoint was chosen to assess the effect of blocking P2X7-NLRP3 signaling on cytokines release. In addition, consequences on microglia phenotypes were analyzed 48h ex-vivo using FACS analysis and immunohistochemistry; or in-vivo using 2-photon imaging microscopy. 2-photon imaging was performed on anesthetized C57GFP+embryonic mice between 50–200 µm deep in the sensory cortex through a thin skull cranial window using a Thorlabs Bergamo® microscope.

**Results:** Systemic injection of LPS lead to a transient increase in cytokine/chemokines levels in both plasma and brain, with P2X7 and NLRP3 mechanisms showing distinct functional signatures. 48 hr post LPS, we observed increased microglial density and changes in microglia morphology reflecting microglia activation ex-vivo. Flow-cytometric assessment of whole brain microglia was sensitive and reliable enough to detect changes in microglia activation. To monitor changes in microglia phenotype over time in-vivo, we performed 2-photon imaging and confirmed a clear phenotypic change in microglial morphology upon LPS that was tractable in vivo.

**Conclusions:** Our data unequivocally demonstrates neuroinflammation after peripheral LPS treatment, setting up a model to reliably test neuroinflammatory targets under systemic inflammation.

**Keywords:** Neuroinflammation, Microglia, Two-Photon Microscopy, P2X7, NLRP3

**Disclosure:** Janssen R&D, Employee
T140

Social Instability Stress and Chronic Non-Discriminatory Social Defeat are Effective Chronic Stress Paradigms for Both Male and Female Mice

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Background: Despite stress-associated mood disorders having a higher incidence rate in females, historically preclinical research in rodents mainly utilizes males. Chronic stress paradigms, such as chronic social defeat and chronic corticosterone administration, were mainly designed and validated in males and subsequent attempts to use these paradigms in females has demonstrated sex differences in the behavioral and HPA axis response to these stressors. Here, we describe the behavioral and neuroendocrine response to two novel social stress paradigms, social instability stress (SIS) and chronic non-discriminatory social defeat stress (CNDS).

Methods: SIS exposes adult mice to unstable social hierarchies for 7 weeks. The CNDS model simultaneously introduces male and female C57BL/6J mice into the home cage of resident CD-1 aggressors for 10 daily 5-minute sessions, with CD-1 aggressors attacking males and females indiscriminately.

Results: SIS effectively induces negative valence behaviors and hypothalamus-pituitary-adrenal (HPA) axis activation in both males and females. Additionally, the effects of SIS on negative valence behaviors are reversed by chronic antidepressant treatment with fluoxetine (FLX) in both males and females. In the CNDS paradigm, stress resilient (RES) and susceptible (SUS) subpopulations emerge in both sexes, with SUS mice displaying increased negative valence behaviors relative to RES and control mice in both sexes. Furthermore, SUS male and female mice displayed HPA axis activation following CNDS exposure.

Conclusions: Overall, these data demonstrate that the SIS and CNDS paradigms are novel and streamlined approaches to effectively induce chronic stress in both adult male and female mice.

Keywords: Chronic Stress, Sex Differences, Stress Resilience and Susceptibility, Mouse Behavior

Disclosure: Nothing to disclose.

T141

Neuronal Activity Directs Stress Effects on Microglia Function: Implications for Synaptic Deficits and Associated Behavioral Consequences

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Background: Chronic stress induces neuronal atrophy and synaptic loss in the medial prefrontal cortex (PFC), and this leads to behavioral and cognitive impairments. Recent findings indicate that microglia contribute to neuronal remodeling and synaptic loss via increased colony stimulating factor (CSF)-1 in the medial PFC. Despite these findings it is unclear how chronic stress increases CSF1 signaling in the medial PFC and what mechanisms drive microglia-mediated neuronal remodeling. Interestingly, work from other labs shows that chronic stress drives aberrant neuronal activity in the medial PFC, and that neuronal hyperactivity increases CSF1 signaling and alters microglia function. In this context, we conducted studies to determine the role of neuronal activity in stress-induced CSF1 signaling and microglia-mediated neuronal remodeling in the medial PFC.

Methods: In these studies, male mice were exposed to 14 days of chronic unpredictable stress (CUS) or handled intermittently as controls, and received daily injection of vehicle or diazepam (1 mg/kg). In initial studies mice were assessed in the forced swim test (FST), novelty-suppressed feeding test (NSFT), and temporal object recognition (TOR) on subsequent days. Following behavioral testing the medial PFC and hippocampus were collected for gene expression analyses. To further examine neuron-microglia interactions transgenic Thy1-GFP(M) mice, in which a subset of layer V pyramidal neurons express green fluorescent protein (GFP) were used. Brains were collected for immunohistology to assess microglia morphology and dendritic spine density. Engulfment of GFP+ pyramidal neuron components (synaptic or dendritic) by microglia was determined in lamina I of the medial PFC. In follow-up studies mice received bilateral infusion of the adeno-associated virus encoding the activating DREADD (CamKIIa-hM3d(Gq)) in the medial PFC. Following recovery mice were injected with clozapine-n-oxide (CNO) for 14 days to drive pyramidal neuron activity in the medial PFC. Behavioral testing, molecular, and immunohistological analyses were conducted as described above.

Results: Consistent with prior results, diazepam administration diminished CUS-induced neuronal activity (FoB immunolabeling) in the medial PFC. In addition, CUS-induced behavioral despair and cognitive deficits were attenuated by diazepam. Further diazepam administration normalized Csf1 and C3 mRNA levels in the medial PFC of mice exposed to CUS, and prevented increases in Csf1r and Cd11b in sorted frontal cortex microglia. Confocal imaging in Thy1-GFP(M) mice demonstrated that diazepam limited microglial engulfment of GFP+ neuronal elements, which corresponded with reduced CUS-induced dendritic spine loss on pyramidal neurons in the medial PFC. Follow-up studies showed that protracted activity of pyramidal neurons in the medial PFC (DREADD: CamKIIa-hM3d(Gq)) was not sufficient to cause behavior or cognitive impairments, nor did it alter Csf1 mRNA levels. Notably, DREADD-induced pyramidal neuron activation caused pronounced changes in microglial morphology, and significantly increased Csf1r expression in frontal cortex microglia.

Conclusions: These studies indicate that chronic stress-induced neuronal activity directs functional changes in microglia leading to microglia-mediated neuronal remodeling in the medial PFC, and subsequent behavioral and cognitive deficits.

Keywords: Stress, Prefrontal Cortex, Synapse, Neuroimmune, Microglia

Disclosure: Nothing to disclose.

T142

Multidimensional Predictors of Susceptibility and Resilience to Social Defeat Stress: Role of Acetyl-L-Carnitine

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Background: While previous literature characterized susceptible and resilient phenotypes after exposure to stress, the mechanisms predicting the development of those phenotypes are less known. Previous work from our and other groups has mostly implicated single risk factors in stress susceptibility. Increased
anxiety-like behavior induced by a glucocorticoid overactivation has been reported as a risk factor for development of stress-induced glutamatergic dysfunction in the ventral hippocampus with corresponding depressive-like traits in susceptible mice (Nasca et al., Mol Psychiatry 2015). Heightened interleukin-6 (IL-6) release has been shown to increase susceptibility to social defeat stress (SDS) (Hodes et al., PNAS 2015). Furthermore, a growing literature from our group and others suggested acetyl-L-carnitine (LAC) as a novel rapid-acting glutamatergic agent to ameliorate stress-induced neurobiological and behavioral impairments (Nasca et al., Neuron 2017, PNAS 2013). Here we aimed at testing whether (i) multidimensional biomarkers spanning behavioral, systemic and brain domains predicted susceptibility or resilience to social defeat stress (SDS) in mice, and (ii) administration of acetyl-L-carnitine (LAC) promoted resilience at the SDS paradigm.

Methods: We used computational, molecular, behavioral and pharmacological techniques to test our hypotheses. Mice identified at the light-dark test (LDT) as high anxious phenotype (HS) and less anxious phenotype (LS), as we previously reported (Nasca et al., Mol Psychiatry 2015), were subjected to 10 days of SDS or used as social defeat unstressed controls (SDC). Before the beginning of the SDS paradigm, submandibular blood was collected for immunological assessment by flow cytometry and IL-6 measurements as we previously reported in Hodes et al., PNAS 2015 as well as to assess LAC levels by liquid chromatography–mass spectrometry (LC-MS) as we previously described in Nasca et al., PNAS 2018. Mice received administration with either LAC or a control water solution 3 days before the end of SDS. All groups of mice were tested for social interactions (SI) and sacrificed at the end of the SDS paradigm. Separate cohorts of mice were used to collect brains from the HS and LS phenotypes for brain imaging and ex vivo magnetic resonance imaging (MRI) scans. RNAseq and bioinformatics analysis for the ventral dentate gyrus was performed as we previously described in Nasca et al., Neuron 2017. In addition, we developed an algorithm in R to test predict if a given animal developed SDS-induced social withdrawal or remained resilient based on the LDT and IL-6 scores.

Results: We found that increased anxiety-like behavior, decreased hippocampal volume and aberrant systemic functions of LAC synthesis and IL-6 release characterized the HS phenotype as compared to the LS phenotype at baseline. Specifically, mice designated as HS at the LDT (i.e.: increased time spent in the dark box of the LDT, p < 0.001) showed both elevated IL-6 levels (p < 0.01) when stimulated ex vivo with LPS and decreased LAC levels (p < 0.01), as compared to mice designated as LS at the LDT (i.e.: increased time spent in the light box of the LDT). HS mice also displayed decreased hippocampal volume (p < 0.05) measured at MRI, as compared to LS mice. After exposure to 10d SDS, the HS phenotype developed behavioral deficits as shown by decreased social interaction ratio at the SI (p < 0.05) as opposed to the LS phenotype and unstressed control groups. The susceptible phenotype also showed impaired transcriptomic-wide changes in ventral dentate gyrus after SDS (FC > 1.3, p < 0.05). Enrichment pathway analyses identified gene pathways related to acyltransferase and fatty acids composition. At the individual level, a computational approach using integrative in vivo brain-body measures predicted if a given animal developed SDS-induced social withdrawal, or remained resilient, with a sensitivity of 80%. Finally, we found that 3 days administration of LAC promoted resilience at the SDS paradigm as shown by increased SI ratio (p < 0.01).

Conclusions: These findings identified multidimensional brain-body predictors of susceptibility versus resilience to SDS, and showed initial evidence that administration of LAC can serve to promote behavioral resilience at the SDS paradigm. This work provides a starting point for in vivo models of mechanisms predisposing apparently healthy individuals to develop the neurobiological and behavioral deficits resulting from stress exposure. This framework of brain-body communication can lead to novel therapeutic strategies to promote resilience in susceptible phenotype.

Keywords: Glutamate, Inflammation, Acetyl-L-carnitine LAC, Multidimensional Predictors of Susceptibility, Resilience

Disclosure: Nothing to disclose.
Replicating Altered Global Brain Connectivity as Marker of Depression – Investigation of Preprocessing Strategies and Placebo Controlled Ketamine Treatment

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Background: Significantly altered global brain connectivity (GBC) – a measure of the brain's network hubs – in the prefrontal and cingulate cortices and cerebellum was demonstrated in patients with treatment resistant depression (TRD). Altered GBC of patients resembled values of healthy controls (HC) after treatment with ketamine, however, placebo-controlled evidence is missing. Moreover, GBC is strongly affected by global signal regression (GSR). The impact of physiological noise on GBC, which was demonstrated to impact on the global signal, is unclear. To validate previous work, we aimed to replicate altered GBC in TRD with identical preprocessing strategies as previous studies, include physiological parameter regression and add evidence with placebo-controlled ketamine treatment.

Methods: We included 28 patients with TRD (mean MADRS: 33.6 ±/− 4.6) and 22 healthy controls in a double-blind, placebo-controlled crossover study receiving either ketamine hydrochloride (0.5 mg/kg) or placebo. An 8-minute rsfMRI scan with a 3T scanner was conducted at baseline and at about 2 days after drug/placebo with cardiac and respiration data. We calculated whole-brain GBC maps from rsfMRI data preprocessed with 3 pipelines: regressing the global-signal, physiological noise and just white-matter and cerebrospinal fluid signals (CSF). Because physiological components are contributing to the global signal, we hypothesized that regression of physiological parameters will resemble GSR. With GBC correlation (Fz) maps of all 3 pipelines, we compared baseline to post-ketamine/placebo differences with linear mixed effects models and post-hoc t-tests, at p < 0.01 or p < 0.001, family-wise error corrected.

Results: GBC Fz-maps after physiological noise removal resembled those of white matter and CSF regression (p > 0.05, corrected). Global signal regression significantly reduced GBC-values in wide-spread brain regions with an emphasis on posterior and basal brain regions (x, y, z = 42, 61, −21; 318.09 cm³, p < 0.001). In addition, GSR introduced negative correlations in the orbitofrontal cortex and occipital cortex. Out of all three pre-processing pipelines, we only obtained results after GSR (GBCr). Across all scans, we found a significant main effect of group (p < 0.01) with significantly reduced pretreatment GBC in TRD compared to HC in the middle cingulate cortex (p < 0.001) and anterior cingulate cortex (p < 0.01). We did not detect ketamine vs. placebo or ketamine vs. pretreatment differences, neither in HC nor in patients with TRD (p > 0.01).

Conclusions: These data independently replicated previous clinical rsfMRI results, which is an important step towards application of rsfMRI as clinical marker. In comparison to 2 other common preprocessing strategies, we demonstrated that altered GBC in TRD was only detectable after regressing the global signal. With identical methods as previous studies, we found significantly altered GBCr in similar brain regions as previously reported. A lack of treatment-effect is contradicting previous findings, however, our MRI-measurement two days after pretreatment might have obscured immediate treatment-effects. These results confirm previous work demonstrating that GBCr differentiates HC from TRD patients. Future studies comparing GBC across different stages of depression are warranted to examine the potential of GBC as staging marker.
Conclusions: Our study suggests involvement of m6A based epitranscriptomic modification in depression-like behavior in rats. Mostly likely, the changes were associated with dysregulated expression of demethylase FTO. Mechanistically, the FTO related changes are controlled by miRNA-124-3p via TF-Cebp-expression of demethylase FTO. Impaired functionality of FTO gene may significantly alter metabolic fate of coding transcripts critical in depression pathophysiology.

Keywords: FTO, Depression, Hippocampus, Epigenetics

Disclosure: Nothing to disclose.

T146

The National Pregnancy Registry for Psychiatric Medications: Effects of Fetal Exposure to Atypical Antipsychotics on Risk for Major Malformations

Abstract not included.

T147

NV-5138 a Novel Direct Activator of the Mechanistic Target of Rapamycin Complex 1 (mTORC1): Safety, Tolerability and Pharmacokinetics (PK) in Plasma and Cerebrospinal Fluid (CSF) Following Oral Administration in Healthy Volunteers

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Background: NV-5138 is a novel, orally bioavailable small molecule that directly and transiently activates mTORC1, the master modulator of cellular metabolism, which is suppressed in the brain of patients suffering from severe depression (Sengupta, S. et al., (2019) Sci. Rep.9:4107). Preclinical data demonstrate that NV-5138 produces rapid upregulation of key synaptic proteins and synaptic remodeling in the prefrontal cortex and hippocampus leading to sustained antidepressant behavioral responses in multiple animal models (Kato, T. et al., (2019) J. Clin. Invest. 130:2542). Within 2 hours post-dose, changes in metabolism (PK) in the single ascending dose (SAD) study (ClinicalTrials.gov: NCT03606395) are presented in addition to the PK in plasma and cerebral spinal fluid (CSF) from a second study in healthy volunteers receiving a single oral dose of NV-5138.

Methods: Both studies were double-blind, placebo-controlled assessments of NV-5138 in healthy volunteers. In the SAD study 48 subjects were randomized 6:2:1 to a single oral dose of NV-5138 from 150 to 2400 mg or placebo across 6 cohorts. The primary objective of the study was to assess the safety and tolerability of NV-5138, including assessment of psychiatric symptoms using the Brief Psychiatric Rating Scale, positive sub-scale (BPRS+) and Clinician-Administered Dissociative States Scale (CADSS). Assessment of plasma PK was a secondary objective. In the second study, 12 subjects with indwelling spinal catheters were randomized 8:4 to a single oral dose of 2400 mg NV-5138 or placebo. The objectives of the study were to assess safety and tolerability, PK in CSF and plasma, and to conduct exploratory biomarker analysis. Collection of CSF and plasma samples for PK, metabolomic, and proteomic analysis continued over a 36-hour period.

Results: In the SAD study, NV-5138 was safe and well tolerated up to 2400 mg with no serious adverse events reported and all adverse events considered mild. No worsening of BPRS+ or CADSS scores was observed in either treatment group and no somnolence or dissociative adverse events were reported. The maximally tolerated dose was not achieved. NV-5138 was rapidly absorbed and exposure was dose-dependent with a Tmax of approximately 0.5–1 hour and half-life approximately 13 hours. In both studies, a single oral dose of NV-5138 2400 mg had a similar safety, tolerability, and plasma PK profile. NV-5138 appeared in the CSF with a time course that lagged slightly behind that seen in plasma samples taken over the same time, with a Tmax of approximately 4 hours. The maximal exposure in CSF following a single dose of 2400 mg reached the same magnitude as observed in a rodent study following the administration of the minimally pharmacologically effective dose (160 mg/kg) in several models of stress-induced depressive behavior (Kato, T. et al., (2019) J. Clin. Invest. 130:2542). Within 2 hours post-dose, changes in metabolites known to be controlled by mTORC1 activity were evident.

Conclusions: In both studies, single oral doses of NV-5138 up to 2400 mg were safe and well tolerated, and exhibited PK supportive of once daily administration. Human CSF exposures established brain penetrance at levels consistent with effective target engagement as evidenced by changes in metabolites consistent with mTORC1 activation and fully effective doses demonstrating rapid pharmacological effects in multiple preclinical models. These results support the conclusion that a single 2400 mg dose of NV-5138 achieves effective concentrations in the brain and impacts brain metabolism rapidly post-dose with favorable safety and tolerability. Further development of NV-5138 is warranted as a potential therapy for depression.

Keywords: mTORC1, Cerebrospinal Fluid, Pharmacokinetic and Pharmacodynamic, Tolerability, Safety

Disclosure: Navitor Pharmaceuticals, Board Member, Navitor Pharmaceuticals, Stock / Equity

T148

Significant Weight Gain in Patients With Depression Taking Antipsychotics


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Background: Only approximately one-half of patients with major depressive disorder (MDD) respond to their first antidepressant medication, and 20% have side effects. Risks for side effects increase with each medication trial and result in non-adherence, overall lack of remission, and relapse. Weight gain is a common side effect of some antidepressants and antipsychotics; it creates a major health risk and is a concern for patients and their long-term health. There is tremendous individual variability in propensity for weight gain with medications. Most research on medication weight gain has been conducted for antipsychotics in patients with psychosis and/or schizophrenia, but data from patients with MDD, in spite of its high prevalence, are limited. Here, our primary aim was to understand changes in the risk of weight gain among different medication combinations for patients with MDD who have failed at least one medication. We also sought to determine if bupropion was effective at mitigating risk of weight gain from high-risk medications, since this medication occasionally is used as an augmentation strategy for patients with MDD.

Methods: The Genomics Used to Improve DEpression Decisions (GUIDED) randomized, controlled trial included 1,167 patients with MDD who had failed at least one medication trial. In a post hoc analysis from this study, we analyzed weight changes throughout the study for the 1,039 patients with weight information available.
at baseline, 12 weeks, and 24 weeks. Patient weight changes were based on ≥3% weight gain as being clinically significant, and patients who were on the medications for at least four weeks were analyzed. We sought to compare weight gain throughout the study to understand the clinical effect of specific medication groups/classes, or combinations thereof, used to treat MDD, and to see if whether augmentation with bupropion appeared to mitigate any weight gain. Medications were grouped into weight-gain risk categories based on Stahl’s Essential Prescriber’s Guide. High-risk drugs included amitriptyline, chlorpromazine, clomipramine, clozapine, desipramine, doxepin, iloperidone, imipramine, lurasidone, nortriptyline, mirtazapine, olanzapine, paliperidone, perphenazine, quetiapine, risperidone, and thioridazine. Bupropion, nortriptyline, mirtazapine, olanzapine, paliperidone, weight gain was mitigated in patients taking high-risk medications with the following risk assessment (ordered highest to lowest) for patients taking the following drug risk categories: “high-risk only” or “low-risk only,” taking only medication(s) in that category; “high-risk” or “low-risk,” taking medication in that category plus other drugs.

Results: In this study, patients taking antipsychotics were more likely to have clinically significant weight gain by weeks 12 and 24 compared with patients who were not on these medications (30.8% versus 19.0%, p = 0.01; 49.7% versus 27.7%, p < 0.0001, respectively). Some combinations showed higher risk than did others. Patients on medications currently recognized as having high-risk weight-gain liability gained significantly more weight at 12 and 24 weeks than did those not on these medications (29.7% versus 18.5% p = 0.003 at 12 weeks; 37.2% versus 28.3% at 24 weeks, p = 0.04). Patients taking only high-risk, weight-gain-liable medications saw significant weight gain at 12 weeks, but not at 24 weeks (33.5% versus 19.5%, p = 0.03; 32.7% versus 29.6%, p = 0.68). Patients on weight-gain-liable medications and bupropion were more likely to have significant weight gain than were patients not on these medications at week 24, but not by week 12 (56.8% versus 30.6%, p = 0.01; 36.3% versus 27.9%, p = 0.38). Overall, among patients with ≥3% weight gain, we saw a trend toward weight gain with medications currently considered liable for weight gain, with the following risk assessment (ordered highest to lowest) for patients taking the following drug risk groupings: high-risk > low-risk only > high-risk only > high-risk > other > low-risk only > low-risk.

Conclusions: This study illustrates the real-world risk of weight gain for patients with depression taking medications with high risk for weight gain. Weight gain was highest for patients on antipsychotics. Bupropion augmentation does not appear to mitigate this risk.

Keywords: Major Depressive Disorder (MDD), Antipsychotic Induced Weight Gain, Bupropion

Disclosure: Myriad Neuroscience/Myriad Genetics, Inc., Employee, Myriad Genetics, Inc., Stock / Equity

T149

Impact of Comorbid PTSD on Outcome of Repetitive Transcranial Magnetic Stimulation (rTMS) for Veterans With Depression

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Background: Numerous studies have demonstrated the efficacy and effectiveness of repetitive Transcranial Magnetic Stimulation (rTMS) for Major Depressive Disorder (MDD) in the general population as well as Veterans (Carpenter et al., 2012; Gaynes et al., 2014; George et al., 2010; Kozel et al., 2017). A recent randomized controlled trial of rTMS for MDD in Veterans, however, raised the question of whether co-morbid Posttraumatic Stress Disorder (PTSD) negatively impacted the outcome of rTMS in Veterans (Yesavage et al., 2018). In this study, comorbid PTSD was significantly related to the remission rate (OR, 0.47; 95%CI, 0.24–0.94; P = 0.03) with only 13 of 40 (32.5%) participants with PTSD achieving remission with active stimulation, while 20 of 41 (48.8%) participants without PTSD achieved remission with active stimulation.

Methods: The clinical outcome and characteristics of all Veterans suffering from MDD who were treated with TMS at the James A. Haley Veterans’ Hospital as outpatients from October 2013 to September 2018 were retrospectively analyzed using an existing quality database. All patients were initially evaluated to determine the safety and appropriateness of rTMS for their symptoms that included a detailed evaluation of mood and other psychiatric comorbidities by an experienced psychiatrist. At the start of treatment, and after every five treatments, patients were assessed with self-report (Quick Inventory of Depressive Symptomatology, Self-Report – QIDS-SR) and clinician rating scales (Montgomery-Asberg Depression Rating Scale - MADRS) of depression. The MADRS was added to the routine clinic monitoring starting in April 2015. Response was defined as ≥50% reduction for both scales. Remission was defined as ≤5 for QIDS-SR and ≤10 for MADRS. A significant difference was defined as p < 0.05 (2-sided) using the Pearson Chi-square test.

Results: Among the 118 patients who were treated with TMS for depression, 55 (47%) had co-morbid PTSD and 63 (53%) had no comorbid PTSD. Based on the QIDS-SR, patients with comorbid PTSD did not have a significantly different rate of response (p = 0.704; 19/50 for 38.0% with PTSD versus 20/58 for 34.5% without PTSD) or remission (p = 0.952; 11/50 for 21.2% with PTSD versus 12/58 for 20.7% without PTSD) compared to patients without comorbid PTSD. Similarly, for the MADRS, patients with comorbid PTSD did not have a significantly different response (p = 0.905; 21/40 for 52.5% with PTSD versus 21/39 for 53.8% without PTSD) or remission (p = 0.603; 18/44 for 40.9% with PTSD versus 16/45 for 35.6% without PTSD) compared to patients without comorbid PTSD. No seizures or persistent adverse effects were observed or reported in either group.

Conclusions: Co-morbid PTSD did not impact the outcome of rTMS for depression in this sample of Veterans. Future studies should include formal ratings of PTSD to determine if the severity of PTSD impacts the outcome.

References:


Depression in US Veterans: A Randomized Clinical Trial. JAMA psychiatry, 75(9), 884–893.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Major Depressive Disorder (MDD), Posttraumatic Stress Disorder

Disclosure: Nothing to disclose.

T150

Fish Oil Supplementation Alters Emotion-Generated Functional Connectivity Within Corticolimbic Networks of Depressed Bipolar Offspring: A 12-Week Placebo-Controlled fMRI Trial

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Background: The pathoetiiological mechanisms contributing to corticolimbic connectivity abnormalities observed in patients with mood disorders remain poorly understood. We previously reported that adolescents with major depressive disorder (MDD) and a biological parent with bipolar I disorder exhibit robust blood deficits in omega-3 polyunsaturated fatty acids (n-3 PUFAs), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Emerging preclinical evidence suggests that n-3 PUFAs promote synaptogenesis and synaptic plasticity in the developing brain. In adult non-human primates, a neuroimaging study found that developmental n-3 PUFA insufficiency reduced resting-state functional connectivity among prefrontal and temporal cortical networks. These and other data support a potential link between n-3 PUFA insufficiency and corticolimbic connectivity abnormalities associated with mood dysregulation. To evaluate this, the present study investigated the effects of n-3 PUFA (fish oil, FO) supplementation on emotion-generated functional connectivity within corticolimbic networks in depressed youth with a biological parent with bipolar I disorder.

Methods: Thirty-nine antidepressant-free youth (ages 9-20 years) with a current diagnosis of MDD or Depressive Disorder NOS and a biological parent with bipolar I disorder were randomized in a double-blind manner to 12-week treatment with FO (2,100 mg/d) or placebo. Depression (CDRS-R), Mania (YMRS), and global (CGI-S) symptom ratings, erythrocyte fatty acid levels, and fMRI scans were acquired at baseline and endpoint. fMRI (4 Tesla) scans were obtained while performing a continuous performance task with emotional and neutral distractors (CPT-END). Seed-based functional connectivity analyses were performed using the amygdala, insula, anterior cingulate cortex (ACC), and orbital frontal gyrus (OFG) as seed regions. The emotion – square and emotion – neutral contrasts were used to evaluate functional connectivity related to emotional regulation.

Results: Patients supplemented with FO, but not placebo, exhibited a significant baseline-endpoint increase in erythrocyte n-3 PUFA (EPA+DHA) levels. Baseline-endpoint reductions in CDRS-R (p = 0.15) and YMRS (p = 0.39) scores did not differ between treatment groups, and FO produced significantly greater reductions in CGI-S (p = 0.002) and CGI-I (p = 0.036) scores. Compared with placebo, the FO group exhibited a significantly greater baseline-endpoint increase in functional connectivity between the left OFG (seed) and left superior temporal gyrus (STG) (cluster size = 563 voxels, p < 0.001, FWE corrected), and a significantly greater reduction in functional connectivity between the right amygdala (seed) and right inferior temporal gyrus (ITG) (cluster size = 573 voxels, p < 0.001, FEW corrected). No significant differences were observed for the insula and ACC seed regions. Among patients treated with FO, but not placebo, the baseline-endpoint change in OFG/STG functional connectivity was positively correlated with changes in erythrocyte EPA levels (r = 0.52, p = 0.03), and change in AMY/ITG functional connectivity was inversely correlated with change in YMRs scores (r = −0.54, p = 0.01) and positively correlated with change in CGI-S scores (r = 0.54, p = 0.01).

Conclusions: FO supplementation alters emotion-generated functional connectivity within frontal-temporal and amygdala-temporal networks in depressed youth with a biological parent with bipolar disorder. Associations with YMRS and CGI-S scores within the FO group suggest that the observed changes in amygdala-temporal connectivity are relevant to symptom severity. These findings add to a growing body of evidence implicating n-3 PUFAs in cortical circuit plasticity and encourage additional research into the potential link between n-3 PUFA insufficiency and corticolimbic connectivity abnormalities associated with mood dysregulation.

Keywords: Omega-3 Fatty Acids, Bipolar Disorder, Brain Structural Connectivity

Disclosure: Nothing to disclose.

T151

Estimating the Potential Impact of CYP2C19 and CYP2D6 Genetic Testing on Protocol-Based Care for Depression in Canada and United States

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Background: The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) algorithm is the most recognized protocol-based care approach for moderate to severe depression. However, its implementation results in one-third of individuals receiving modest to no symptom remission. One possible explanation is the inter-individual differences in antidepressant metabolism due to CYP2C19 and CYP2D6 genetic variation.

Methods: To estimate the proportion of individuals with an actionable phenotype for medications with CYP2C19- or CYP2D6-based prescribing guidelines, we used the most current population estimates by age and ethnicity for each Canadian province/territory and US state from the Statistics Canada and US Census Bureau websites, respectively. Frequencies of CYP2C19 and CYP2D6 genotype-predicted metabolizer phenotypes (i.e., poor, intermediate, normal, rapid/ultrarapid) by ethnicity were obtained from phenotype frequency tables available from the Clinical Pharmacogenetics Implementation Consortium. We then estimated the potential impact of pairing CYP2C19 and CYP2D6 genetic testing with the STAR*D algorithm via simulating each step of the algorithm in each province/territory/state.

Results: The estimated proportion of individuals that could benefit from CYP2C19 or CYP2D6 genotyping was highest at Steps 1 and 2 of the STAR*D algorithm but then considerably decreased in Steps 3 to 5 across all US states and Canada provinces/territories. We estimated that about one-third of individuals entering the STAR*D algorithm in the US (mean = 32.2%, sd = 1.4%, range = 23.3%–33.2%) and Canada (mean = 29.4%, sd = 4.5%, range = 19.3%–34.5%) could benefit from CYP2C19 genotyping. Whereas, approximately one-fifth of individuals progressing to Step 2 in the US (mean = 22.6%, sd = 1.0%, range 16.4%–23.3%) and Canada (mean = 20.6%, sd = 3.2%, range 13.6%–24.2%) would benefit from CYP2C19 or CYP2D6 genotyping.

Conclusions: Our findings suggest a potential benefit of combining protocol-based depression care with CYP2C19 and CYP2D6 genotyping in the US and Canada. The potential impact...
of CYP2C19 genotyping was clearly seen within the first two steps of the STAR*D treatment algorithm, whereas, the potential impact of CYP2D6 genotyping was most notable in Steps 3, 4, and 5.

**Keywords:** Pharmacogenetics, Pharmacogenomics, Depression, STAR*D

*Disclosure:* Nothing to disclose.

**T152**

**(R)-Ketamine Exerts Antidepressant Actions Partly via Conversion to (2R,6R)-Hydroxynorketamine, While Causing Adverse Effects at Sub-Anesthetic Doses**

Abstract not included.

**T153**

**Self-Reported Childhood Abuse and Serotonin 1A Receptor Gene Methylation are Independently Associated With Gray Matter Volume in Major Depressive Disorder**

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**Background:** The serotonin 1A (5-HT1A) receptor is implicated in the pathophysiology of major depressive disorder (MDD) and mediates the neurotrophic effects of serotonin. Environmental stress, including early life adversity, is a risk factor for the development of depression. Stress in mice causes methylation of the −681 site of the 5-HT1A receptor promoter region and increases expression. We examined relationships between reported early childhood abuse, 5-HT1A receptor promoter methylation, and gray matter volume (GMV) in unmedicated MDD.

**Methods:** Peripheral blood methylation of 5-HT1A receptor promoter sites −681 and −1007 was assayed in 63 individuals with MDD, including 20 with a reported history of childhood abuse. T1-weighted structural magnetic resonance imaging (MRI) scans were acquired in the same subjects on a GE 3T SignaHDx scanner. Voxel-based morphometry (VBM) analysis was used to assess GMV. A priori regions of interest (ROIs) included amygdala, hippocampus, insula, occipital lobe, orbitofrontal cortex (OFC), temporal lobe and parietal lobe, as we previously observed associations between GMV in these regions with 5-HT1A receptor binding assessed by positron emission tomography imaging. At the ROI level, a linear mixed-effects model was employed, with GMV as the dependent variable. Subject was employed as a fixed effects model. Random effects were included and reported history of childhood abuse. In whole-brain analyses, patients reporting childhood abuse had higher GMV in pre-central and post-central gyri (pFWE < 0.001) and lower GMV in the intracalcarine cortex, occipital fusiform gyrus and lingual gyrus (pFWE = 0.001) compared to patients without abuse. There was no significant interaction between reported abuse and DNA methylation on GMV (for −681: p = 0.25, for −1007: p = 0.337).

**Conclusions:** Although we found observed effects of 5-HT1A receptor promoter methylation and of reported history of childhood abuse on GMV, we did not find a relationship between reported childhood abuse and DNA methylation, and DNA methylation did not mediate the effects of reported childhood abuse on GMV. These findings suggest at least partially independent effects of 5-HT1A receptor promoter methylation and reported history of childhood abuse on GMV. Given findings of −681 methylation leading to 5-HT1A receptor upregulation in animal studies (through antagonism of the Sp4 repressor element), the currently observed methylation-GMV correlation may reflect enhanced trophic effects of serotonin signaling via the 5-HT1A receptor. Localization of this finding in OFC may be due to its late maturation, which may render it particularly sensitive to epigenetic effects. Decreased GMV in occipital subregions in individuals with reported childhood abuse is consistent with other findings and may indicate persistent effects of adverse sensory experiences on visual processing regions, which may alter sensitivity to recurrent traumatic exposure. Understanding the different pathways by which adverse early life exposures and epigenetic changes lead to alterations in brain structure could potentially help elucidate meaningful subtypes of MDD as well as possible adaptive responses to stress.

**Keywords:** Serotonin 1a Receptor, Gray Matter Volumes, Depression, Childhood Adversity

*Disclosure:* Nothing to disclose.

**T154**

**Mechanisms Contributing to the Sustained Antidepressant-Like Response of L-655,708**

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**Background:** Selective modulation of hippocampal transmission by systemic administration of α5-GABAA receptor negative allosteric modulator, namely L-655,708, is capable of producing a sustained antidepressant-like effect in the absence of any psychotomimetic or abuse-related effects.

**Methods:** We utilized conventional pharmacological, electrophysiological (whole-cell, patch clamp), and behavioral approaches to examine the mechanisms by which L-655,708 produces plasticity within the ventral hippocampus (vHipp) to account for its sustained antidepressant-like effect in adult male Sprague-Dawley rats. To test whether or not acute L-655,708-induced activation of CamKII within the vHipp at the time of drug administration is necessary for the sustained AD-like effects of L-655,708, rats were implanted with stainless steel cannulae targeting the vHipp and administered either with the CamKII inhibitor KN-93 (0.5 μg, 0.5 μl) or vehicle (αCSF) directly into the vHipp followed (after 10 min) by the systemic administration of L-655,708 (3 mg/kg, i.p.) or vehicle (50% PG in saline; 1ml/kg). Twenty four hours following drug treatment, rats underwent a 5-min Forced Swim Test (FST), and videos were scored for the number of 5 second bins in which rats were immobile. The scorer was blind to the treatment. An electrophysiological approach was used next to study functional changes in pyramidal neurons in the vHipp produced by L-655,708. Twenty four hours after a single i.p. injection of L-655,708 (3 mg/kg, i.p.) or vehicle (50% PG...
in saline; 1mL/kg), patch clamp recording of vHipp CA1 neurons in brain slices were made using a extracellular solution consisted of (in mM) 124 NaCl, 2 KCl, 2 MgSO4, 1.25 NaH2PO4, 2 CaCl2, 26 NaHCO3, 10 Dextrose, and 0.4 Vitamin C. Synaptic currents were recorded using a low chloride intracellular solution (6 mM) to allow coincident assay of glutamate-mediated cation currents (ENa+K+ of ~0 mV), and GABA-mediated chloride currents (ECI of ~78 mV) using a -40 mV holding potential. Action potentials were recorded with an intracellular solution of (in mM) 120 K-glucuronate, 10 HEPEs, 0.1 EGTA, 20 KCl, 2 MgCl2, 2 Na2ATP, 0.25 Na2GTP, pH 7.4. Cells were held with a holding current to maintain a resting voltage of -80 mV and action potentials were evoked by a 200 pA current injection. All data were expressed as mean ± SEM. Number of animals per experiment varied from 4 to 10 per group. The behavioral data were analyzed by two-way ANOVA followed by Student-Newman-Keuls post-hoc analysis. The electrophysiology data were analyzed by Student's t-test. All tests were two-tailed, and significance was determined at p < 0.05.

Results: L-655,708-induced CamKII activation within the ventral hippocampus doesn't prevent the sustained effects of L-655,708 in the FST. Twenty-four hours after systemic administration of L-655,708 a reduction of the IPSC half-width was found which promoted increased excitability in CA1 neurons. There was an approximately 2.5-fold decrease in IPSC half-width, accompanied by a smaller decline of EPSC half-width and reduction of IPSC amplitude. The potential consequences of reduced inhibitory currents was investigated on a separate cohort of rats using physiological solutions to record changes in voltage and action potential properties. The most profound effect was an increase of input resistance, which resulted in an approximately two fold increase in action potential frequency.

Conclusions: These results are consistent with reduced tonic and evoked chloride currents in ventral hippocampal CA1 neurons as a consequence of L-655,708 administration, indicating that the sustained antidepressant-like effects of L-655,708 may be mediated by changes in GABAA receptor gating properties, with resultant enduring changes in ventral CA1 neuronal excitability. By identifying the mechanisms by which systemic administration of α5-GABAA receptor negative allosteric modulators recapitulate the therapeutic effects of ketamine without its psychotomimetic and abuse-related effects, it should be possible to provide novel, safe, and effective approaches for treating patients suffering from refractory depression.

Keywords: Ventral Hippocampus, L-655,708, α5-GABAA Receptor Negative Allosteric Modulator

Disclosure: Nothing to disclose.

T156

Cognitive Control Across the Spectrum of Major Depressive Disorder

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Background: Major depressive disorder (MDD) is a chronic neuropsychiatric disease, comprised of multiple heterogeneous symptoms, that affects overall functionality. Prior research has produced conflicting information regarding the impact of MDD on neurocognitive function, particularly the executive function cognitive control. For example, some research has suggested that cognitive control is impaired in MDD and is negatively associated with depression severity. However, other research has found cognitive control to be intact in MDD and unrelated to depression severity. Such findings may have been inconsistent due to methodologic limitations including diffuse diagnostic criteria, use of neurocognitive measures with poor psychometric properties or lack of available normative data, and lack of a comparison reference group. As cognitive control is a core neurocognitive function that moderates other neurocognitive abilities (e.g., planning, working memory, episodic memory), treatment outcome, and functional outcome, research is warranted to inform the status of cognitive control across the MDD spectrum. Thus, the purpose of this preliminary analysis was to characterize and determine the impact of MDD on cognitive control in adults.

Methods: The study was conducted across two academic medical centers (UT Southwestern Medical Center, Duke Uni-
versity School of Medicine) in the United States. Participants included healthy adults, adults with MDD, and adults with MDD referred for treatment with electroconvulsive therapy (which comprised the treatment-resistant depressed (TRD) cohort). All participants provided written informed consent for this IRB approved investigation before participating in study procedures. This was a cross-sectional study designed to examine the psychometric properties of neurocognitive tests, and the effects of MDD and TRD on neurocognitive function. Psychiatric diagnosis was made with the Mini International Neuropsychiatric Interview (MINI). Depression severity was assessed with the clinician-rated and self-report versions of the Inventory of Depressive Symptomatology (IDS-C, IDS-SR). Multiple cognitive domains were assessed with a comprehensive paper-and-pencil and computerized neurocognitive battery. For this preliminary analysis, the focus was on the executive function - cognitive control measures that included the CNS VitalSigns Stroop Test and Shifting Attention Test, Delis-Kaplan Executive Function System (DKEFS) Verbal Fluency – Category Switching Test, Trail Making Test Part B, and the Wisconsin Card Sorting Test (WCST; computerized version). The paper-and-pencil neurocognitive measures were administered and scored by a trained research assistant. For the computerized neurocognitive measures, administration and scoring was computer automated. Neurocognitive test scores were converted to standard scores with available demographic-normative data. Descriptive statistics were used to describe the sociodemographic and clinical characteristics of the study cohorts. Means and standard deviations are presented for continuous variables, and frequency distributions are presented for discrete variables. As the data were non-normally distributed, non-parametric analyses were used to examine for significant differences in neurocognitive test performance among the three cohorts (healthy control, MDD, TRD). Statistical significance was defined as a two-sided p-value of less than 0.05.

Results: On average, all three cohorts (healthy, MDD, TRD) showed intact performance across all cognitive control measures, though on many tests, the standard deviations were wide. Nevertheless, across the three cohorts there were statistically significant differences in performance on the cognitive control measures. Specifically, relative to the healthy control cohort, the MDD cohort showed poorer performance on Trail Making Test Part B (p = 0.01) and greater non-perseverative errors on the WCST (p = 0.01). Also, relative to the healthy control cohort, the TRD group showed less accuracy on the Stroop Test (p = 0.006). Relative to the MDD cohort, the TRD subjects showed lower accuracy on the Stroop Test (p = 0.03), lower accuracy on the Shifting Attention Test (p = 0.04), and lower switching accuracy on the DKEFS Verbal Fluency Category Switching Test (p = 0.02).

Conclusions: This preliminary study characterized cognitive control in adults with MDD and TRD with multiple neurocognitive measures of executive function. Whereas there were statistically significant differences among the healthy control, MDD, and TRD cohorts, the cognitive control scores across the cohorts were relatively similar and fell within the intact range. As the standard deviations were large on select tests, there was variability in performance within each cohort. Collectively, these findings are consistent with prior research that found many patients with MDD and TRD have intact cognitive abilities, including intact cognitive control. As such, cognitive control function may be similar across the spectrum of MDD. Future research is warranted to confirm the impact, if any, of MDD on cognitive control, as well as other executive functions including planning, problem solving, inhibition, and initiation. Such future work would benefit from the integration of multiple units of analysis such as neuroimaging and neurophysiology to identify subgroups of patients with MDD and TRD with cognitive control impairment.

Keywords: Major Depressive Disorder, Neurocognition, Cognitive Control
Disclosure: Pearson, Consultant

T157

Interleukin 6-Antibody Treatment Attenuates Social Defeat Stress-Induced Avoidance in Adult Male and Female Mice

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Background: Major depressive disorder (MDD) is characterized by a host of mental and physical manifestations. Individuals with MDD often have compromised immune system promoting dysregulated inflammatory signaling. Recent work has shown that activity of certain cytokines, in particular interleukin 6, is correlated with life history of mood disorders it is possible that maintaining the right balance of IL-6 could attenuate maladaptive stress responses.

Methods: Adult male and female mice were used in these experiments. A modified social defeat paradigm was used to defeat female mice but briefly. CS7/b6 mice were exposed to male aggressors for 5–10 minutes for 10 days. Prior to being exposed to the defeat bout, mice were administered 8ug of monoclonal IL-6 (mIL6Ab) antibody (R&D systems). Mice were treated every other day with either mIL6Ab or saline. Mice were then tested in the social interaction test and sacrificed for blood sampling. A separate group of mice were administered mIL6Ab and exposed to an acute defeat to assess neutralization efficiency of mIL6Ab after acute stress exposure.

Results: Intermittent mIL6Ab treatment was sufficient to buffer CSDS-induced social avoidance in both male and female mice. Interestingly, antibody treatment was able to attenuate the increase in IL-6 after an acute defeat but did not neutralize IL-6 after a chronic defeat schedule.

Conclusions: Intermittent mIL-6Ab treatment is sufficient to induce behavioral resilience to chronic social defeat stress in both male and female mice. Interestingly, mIL-6Ab treatment does not neutralize increases in circulating IL-6 after chronic social defeat suggesting that the therapeutic mechanism of IL-6 antibody treatment is beyond the effect it has on its ligand. Future studies should delve into the intricacies of mIL-6Ab kinetics as they pertain to antidepressant mechanisms.

Keywords: Chronic Social Defeat, Interleukin-6, Female
Disclosure: Nothing to disclose.

T158

Cerebrospinal Fluid Alpha-Synuclein in Late-Life Depression and Neurobiological Correlates

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Background: Misfolding of the pre-synaptic protein, α-synuclein (α-Syn), and formation of abnormal cytoplasmic aggregates, have been implicated in the progressive degeneration and synaptic
dysfunction associated with Parkinson’s disease (PD) and a number of related disorders. Several lines of evidence have also implicated disturbances in α-synuclein in the pathophysiology of depression. For example, depression is highly prevalent in PD and other synucleopathies, and often precedes motor symptoms. In preclinical experiments overexpression of human α-Syn has been linked to a depressive-like phenotype, and with factors implicated in depression including disruption of monoamine transporters, degeneration of dopamine neurons and microglial and HPA-axis activation and release of proinflammatory cytokines. Despite these findings, there are no studies that have examined CSF α-Syn concentration in depression and its relationship to symptoms, monoaminergic indices and markers of synaptic dysfunction such as neurogranin (Ng) and neuroinflammation (IL-6, IL-8).

Methods: A total of 51 individuals participated in a 3-year longitudinal study examining possible Aβ disturbances in depression and agreed to have a lumbar puncture. Of these 51 individuals, 47 showed (28 MDD and 19 controls) no MRI evidence of synucleinopathy. Forty-seven participants were also enrolled in the Translational Pregnancy and Mental Health Study at the Medical University of South Carolina, Charleston, South Carolina, USA, and completed assessments (p values ≤ 0.05). In depressed subjects only, CSF α-Syn was significantly positively correlated with Ng (ρ = 0.35; p < 0.01), and negatively correlated with cortisol (ρ = −0.355; p = 0.064). Interestingly, there was no significant relationship with depressive symptoms at Baseline (p = .36). α-Syn was not correlated with memory performance on the selected assessments (p values ≥ 0.400). Ng displayed a trend correlation with Rs (ρ = 0.482; p = 0.009, adjusted p = 0.081), consistent with expectations, but was not otherwise correlated with the other variables (adjusted p values ≥ 0.350).

Conclusions: In depressed subjects only, CSF α-Syn was significantly positively related with CSF Ng. Similar correlations have been reported in PD and together with reductions in CSF levels ascribed to impaired synaptic activity in that population from increased brain deposits. Given that CSF α-Syn did not correlate with cognitive and depressive symptoms, the significance of this finding, if any, remains to be determined.


T159
An Investigation of Brain Activation to Alcohol Cues in Co-Occurring Bipolar Disorder and Alcohol Use Disorder: fMRI Results From a 2x2 Factorial Design
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Background: Bipolar disorder (BD) and substance use disorders (SUD) co-occur very frequently, and co-occurring BD and SUD are associated with devastating public health costs. There has been very little neurobiological research conducted to guide the development of effective treatments for this treatment-resistant population. However, behavioral research has consistently supported a central role for impulsivity as a potential “link” between BD and SUD. The present study was designed to provide an important first functional Magnetic Resonance Imaging (fMRI) study of brain activation to visual alcohol cues in individuals with co-occurring BD and current Alcohol Use Disorder (AUD), BD alone, current AUD alone, and healthy control (HC) subjects.

Methods: One hundred and eleven individuals who met DSM-IV criteria for BD I or II and current AD (BD + AD, n = 26), BD I or II alone (BD, n = 32), current AD alone (AD, n = 26), or no psychiatric diagnosis (HC, n = 27) were recruited from inpatient and outpatient clinical settings and community advertisements. Proton magnetic resonance spectroscopy results from a subsample of these participants (n = 90) were presented at the 2016 ACNP meeting, and published in Translational Psychiatry (2017). All participants were required to demonstrate ≥ 1 week of abstinence from alcohol and drugs via serial ethyl glucuronide (EtG) and urine drug screen (UDS) testing. General exclusions included serious medical illness or history of head injury, severe co-occurring Axis I disorders, benzodiazepine/antidipsotropic use, and history of complicated alcohol withdrawal. Co-occurring drug use disorder was exclusionary for BD and HC, but not BD + AD and AD, participants. BD + AD or BD participants with substantial medication dose changes ≤ 1 week before MRI were excluded. Participants completed a baseline diagnostic visit including the Structured Clinical Interview for DSM-IV Axis I Disorders and the Obsessive Compulsive Drinking Scale (OCDS). Participants then returned approximately 4 days later for an MRI including an fMRI paradigm containing pictures of alcoholic beverages (ALC), nonalcoholic beverages (BEV), visual control images, and a cross-hair fixation stimulus. Stimuli are presented in six 120-s epochs, each consisting of four 24-s blocks of each image type. This task was administered while acquiring BOLD weighted transverse scans using a gradient echo, echo-planar imaging (EPI), fMRI sequence: Flip Angle = 81°; TE = 30 ms; TR = 2500 ms; Field of View = 224 mm; Matrix = 64 x 64; 40 slices, 3.5 mm thick with 0 mm gap; voxel size = 3.5 x 3.5 x 3.5 mm³. Data were analyzed using SPM 12 software within MATLAB R2019a. The primary contrast of interest, ALC-BEV, was evaluated in a 2x2 factorial ANOVA model with a height threshold of F = 6.88 (p < 0.01), FWE-corrected for multiple comparisons at the cluster-level (α < 0.05).

Results: Participant groups did not significantly differ on age (p = 0.21) or sex (p = 0.29). There were marginal group differences in smoking (p = 0.09), but smoking was not associated with brain activation to the ALC-BEV contrast. 2x2 factorial ANOVA analysis of whole-brain activation to ALC-BEV cues demonstrated a significant BD×AD interaction, with a cluster-extent of 306 voxels and a peak
voxel located at (MNI) x = 51, Y = 14, Z = 2, signifying uniquely low levels of activation to alcohol cues in the BD+AD participant group in a cluster encompassing the right Inferior Frontal Gyrus (rIFG; the brain region centrally implicated in the response inhibition facet of behavioral impulsivity). Supplementary correlational analyses in BD+AD and AD participants demonstrated a significant inverse association between OCDS total scores and activation to ALC-BEV cues in the identified rIFG cluster, such that lower activation to ALC-BEV cues in the rIFG was significantly associated with higher OCDS scores (r = −0.47, p < 0.001).

Conclusions: In sum, these data demonstrate that BD+AD and AD have abnormally low levels of rIFG activation to alcohol cues, and that low levels of rIFG activation to cues are associated with elevated self-reported alcohol craving. Given that rIFG is principally involved in inhibiting pre-potent behavioral responses, decreased rIFG activation to alcohol cues in BD+AD may represent a novel biomarker of disinhibited behavioral activation in response to cues of alcohol reward in this patient group.

Keywords: Bipolar Disorder, Alcohol and Substance Use Disorders, Functional MRI (fMRI), Comorbidity, Inferior Frontal Gyrus

Disclosure: Nothing to disclose.

T160
Pharmacogenomics of Anti-Epileptic Drug Mood Stabilizer Response in Bipolar Disorder

Abstract not included.

T161
A Randomized Trial of the NMDAR Glycine Site Antagonist Prodrug 4-Chlorokynurenine in Treatment-Resistant Depression

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Background: In the past decade, the emergence of glutamate modulator ketamine and its rapid, robust and sustained antidepressant effects in treatment-resistant depression has caused a paradigm shift in the field, as well raised significant concerns due to its dissociative and psychotomimetic adverse effects. In this line, significant efforts have been made to develop ketamine-like antidepressant drugs with minimal side effects. 7-chlorokynurenine acid (7-CI-KYNA) is a potent and selective non-competitive antagonist at the glycine site of the NMDA receptor. Its prodrug, L-4-chlorokynurenine (4-CI-KYN (AV-101)), is a brain-permeable pro-drug that is converted to 7-CI-KYNA by astrocytes in the brain. The drug was developed because 7-CI-KYNA is inefficient at crossing the blood-brain barrier. Previous preclinical data have shown that 4-CI-KYN administration results in rapid and sustained antidepressant effects in rodents, that was comparable to antidepressant effect of ketamine. Contrary to ketamine, 4-CI-KYN is devoid of psychomimetic effects in either rodents or healthy volunteers. This study examined whether 4-CI-KYN administration has rapid antidepressant effects in individuals with treatment-resistant depression (TRD).

Methods: This randomized, placebo-controlled, double-blind, crossover study was conducted from October 2015 to February 2019. After a two-week drug-free period, 19 subjects (ages 18 to 65) with severe treatment-resistant MDD were randomized to receive daily oral doses of 4-CI-KYN monotherapy (1,080 mg/day for seven days followed by/or 1,440 mg/day for seven days) or placebo for 14 days. Treatment arms were separated by a two- to three-week washout period. Depressive symptoms were rated at baseline and on Days 1, 2, 3, 7, and 13 using the Hamilton Depression Rating Scale (HAM-D) and other rating scales. Pharmacokinetic measures of 7-CI-KYNA and 4-CI-KYN were assessed in plasma and cerebrospinal fluid (CSF). In addition, pharmacodynamic measures assessments that aimed on assessing target engagement were obtained longitudinally and included 1H-magnetic resonance spectroscopy (MRS) brain glutamate levels, resting state functional magnetic resonance imaging (fMRI), and plasma and cerebrospinal fluid measures of kynurenine metabolites and neurotrophic factors.

Results: In this sample, the average number of lifetime antidepressant trials was 7.8 (SD = 5.67). The linear mixed models for total HAM-D/HDRS, MADRS, BDI and other rating scale scores showed no treatment effect. No difference was observed for any of the peripheral or central biological indices or for side effects adverse effects at any time between groups. 4-CI-KYN was safe and well-tolerated, with no treatment-related serious adverse events.

Conclusions: In this small cross-over trial, 4-CI-KYN monotherapy had no antidepressant effect or anti-anxiety efficacy in this sample of subjects with treatment-resistant MDD subjects. Furthermore, 4-CI-KYN appeared to have no biochemical effects, as no significant changes were observed in glutamate brain levels measured by 7T 1H-MRS, rs-fMRI connectivity, or peripheral and central measures (including tryptophan/kynurenic metabolites).

Keywords: 4-chlorokynurenine (AV-101), NMDA Glycine-Site Receptor, Treatment Resistant Depression, NMDA Glutamate Receptors, CNS Clinical Trials

Disclosure: Nothing to disclose.

T162
TNF-A and its Receptors Mediate the Relationship Between Number of Prior Severe Mood Episodes and Executive Function in Euthymic Bipolar Disorder

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Background: It is known that 40-60% of patients with bipolar disorder (BD) have neurocognitive deficits. It is increasingly accepted that functioning in BD is negatively impacted by these deficits, yet they have not been a successful target for treatment. The hypothesis of neuroprogression in BD postulates that cognitive deficits develop over the course of the illness and are influenced by prior severe mood episodes, leading to wear-and-tear on the brain. The biomarkers that predict cognitive deficits in BD are largely unknown, however recent evidence suggests that inflammation may be associated with poorer cognitive outcomes in BD.

Methods: We measured tumor necrosis factor alpha (TNF-α), and TNF receptors one and two (TNF-R1 and TNF-R2) in 219 euthymic BD patients. A path analysis was used for the primary purpose of assessing whether TNF markers measured by the multiple indicators TNF-α, TNF-R1 and TNF-R2, mediates the effect of number of prior severe mood episodes (number of psychiatric hospitalizations) on the latent variable “Executive Function” assessed by a set of observed variables, namely, the


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neurocognitive tests: Controlled Oral Word Association Test (COWAT), Wisconsin Card Sorting Test (WCST) and Stroop.

**Results:** The Chi-Square value of 49.6 had a non-significant p-value of 0.23, which indicated that the model fits the data acceptably. Further, the Root Mean Squared Error Approximation (RMSEA) of 0.027 and PCLOSE of 0.9 further supported that the model had a ‘close fit’. Holding covariates constant (age, sex, premorbid IQ, education, and race), the direct effect of prior severe mood episodes on executive function (EF) was −0.14, whereas the indirect effect of severe mood episodes on EF mediated by TNF was −0.03, and thus the total effect of severe mood episodes on EF was −0.17. The path coefficients above were individually significant, and by inference the direct, indirect, and total effects were also statistically significant. The direct effect of TNF markers on EF in this model is −0.2. Thus, this estimated model was consistent with peripheral TNF markers partially mediating a causal effect of severe mood episodes on executive cognitive function.

**Conclusions:** Our results indicate that TNF variables partially mediated the relationship between prior severe mood episodes and executive function in BD. These results may implicate TNF variables in the neuroprogressive course of BD.

**Keywords:** Mood Disorder, Inflammation, Cytokine, Cognition

**Disclosure:** Nothing to disclose.

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**T163**

**Gamma Visual Stimulation Induces Neuroimmune Signaling Cascade**

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**Background:** Interactions between the brain and immune system play critical roles in neurological and neuropsychiatric disorders (Calsolaro and Edison, 2016; Wohleb et al., 2016). The neuroimmune system including glial cells and immune signaling molecules, offers a unique target to treat disease and improve brain health. However, little is known about how to non-pharmacologically manipulate the brain’s immune system. Recent research has shown that exogenous stimulating neural electrical activity at gamma frequency (30–50 Hz), through sensory stimulation, recruits microglial activity, a component of the neuroimmune system (laccarino et al., 2016; Adaiikkan et al., 2019). While this recent research shows that neural electrical activity manipulates a component of the neuroimmune system, a broader understanding of how exogenous stimulation of neural activity affects neuroimmune function is required.

**Methods:** In the present study, we determined how 40 Hz visual stimulation affects expression of key cytokines involved in the brain’s immune response as well as synaptic plasticity and neuronal health. Furthermore, since cytokine expression is regulated by intracellular signaling, we assessed if NFkB and MAPK phospho-signaling change prior to increases in cytokine expression in adult wild-type male mice exposed to 40 Hz visual stimulation compared to control visual stimuli. We tested this by exposing mice to varying durations (5, 15, 60 min) of LED lights flickering at 40 Hz, which is known to induce gamma neuronal activity, as well as several control conditions (Gray et al., 1989; laccarino et al., 2016; Singer et al., 2018; Adaiikkan et al., 2019). Cytokine and phosphoprotein quantification was determined using Milliplex Mouse MAP Mouse Cytokine/Chemokine Magnetic Bead Panel 32-Plex and Milliplex MAP MAPK/SAPK Signaling Magnetic Bead 10-Plex, respectively.

Data was z-scored before analysis. A discriminant-partial least squares discriminant analysis (PLSDA) was performed in MATLAB (Mathworks) using the algorithm by Cleiton Nunes (Mathworks File Exchange) (Eriksson, L.; Byrne, T.; Johansson, E.; Trygg, J.; Vikström, 2006). To identify latent variables (LVs) that best separated conditions, an orthogonal rotation in the plane of the first two latent variables (LV1-LV2 plane) was performed. Error bars for LV1 figures represent the mean and standard deviations after iteratively excluding single samples, one at a time, and recalculating the PLSDA 1000 times. For multi-plex analysis, either a one-way analysis of variance (ANOVA) (more than two groups) or two-tailed unpaired t-test (two groups) was used to determine if there was a significant LV1 separation between groups. The top correlated cytokines and/or phosphoproteins on the LV1 were isolated and an ANOVA or two-tailed unpaired t-test was performed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA) to determine statistical significance between groups. These tests were followed by a post-hoc Dunnett’s multiple comparisons test to determine differences between specific groups or a Tuckey’s multiple comparisons test to compare differences in phosphorylation levels across time. Levels of significance were set to *p < 0.05, **p < 0.01, ****p < 0.001.

**Results:** We found that 40 Hz flicker leads to significant increases in the expression of cytokines, key immune signals known to recruit microglia, in the visual cortex. Furthermore, we found that 40 Hz flicker rapidly changes phosphorylation of proteins in the NFkB and MAPK pathways, both known to regulate cytokine expression. Our findings are the first to delineate a specific rapid immune signaling response following 40 Hz visual stimulation, highlighting both the unique nature and therapeutic potential of this treatment.

**Conclusions:** Our results show that 40 Hz flicker drives rapid signaling within the NFkB and MAPK pathways followed by upregulation of cytokines. The diverse functions regulated by these pathways together with the diversity of cytokine and growth factors expressed in response to 40 Hz stimulation suggests that minutes of flicker stimulation may induce changes in various cell and tissue functions that include immune activity and neuronal and synaptic health. Furthermore, different forms of visual stimulation induced unique cytokine profiles. Thus, flicker stimulation may be used to rapidly and non-invasively manipulate signaling and expression of genes that go beyond neural immune activity. Importantly, all of our analysis was conducted in wild-type animals, helping establish the effects of 40 Hz flicker stimulation independent of disease pathology. Our work provides a foundation for flicker’s therapeutic potential to disorders involving the neuroimmune system such as psychiatric disorders.

**Keywords:** Microglia, Cytokines, Visual Stimulation, Brain Stimulation

**Disclosure:** Nothing to disclose.

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**T164**

**Gene Expression and Ketamine Response in Subjects With Treatment-Resistant Depression**

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**Background:** Treatment resistant depression (TRD) occurs in approximately one-third of all subjects with major depressive
disorder, representing a substantial public health burden associated with long-lasting morbidity and elevated risk of suicide. The recent discovery that ketamine, a prototypic glutamatergic modulator, leads to rapid and sustained antidepressant effects in subjects with TRD has emboldened novel drug discovery efforts and has led to an increasing focus on the role of glutamatergic transmission in severe depressive disorders. However, while ketamine’s mechanism of action has been extensively studied in animal models, there are far fewer comparable studies in humans and a relative dearth of data on potential predictors of this rapid improvement in symptoms.

Methods: Using a repeated measures longitudinal design, we have performed whole blood RNA-sequencing of 20 subjects with treatment-resistant MDD and 14 healthy controls who were enrolled in a double-blind, placebo-controlled, randomized cross-over trial looking at the ketamine mechanism of action of (Ket-MOA (NCT00088699)) at the National Institute of Mental Health. Subjects were required to have a score a score ≥20 on the Montgomery-Asberg Depression Rating Scale (MADRS) at screening and before each infusion.

RNA was extracted from whole blood before and 230 minutes after single intravenous 0.5mg/kg ketamine infusion. Whole blood RNA was globin depleted and sequenced using a strand specific protocol on an Illumina HiSeq2500 sequencer to an average depth of 60M Million Reads. Fragments were aligned to the human genome using STAR, followed by differential expression analysis using edgeR and network analyses with weighted co-expression network analyses (WCGNA).

Results: After a single IV ketamine infusion, there was an average drop in the MADRS score of 11 points, with approximately half of all treated subjects with TRD meeting traditional response criteria. A percent decline in MADRS was calculated for each subject and standardized across subjects for comparison. The standardized percent decline in MADRS demonstrated FDR adjusted significant associations with PLIN1, ADORA3, and TRIM17 genes, among others. Gene Ontology analysis reported binding and catalytic activity as the two most abundant molecular functions in the significant gene set. A weighted gene co-expression network analysis with post-treatment samples indicated a gene module to be associated with the standardized MADRS score. Additional ongoing analyses, including weighted co-expression and context specific eQTL analyses, will be also presented.

Conclusions: This initial study represents one of the first comprehensive genomic assays of ketamine treatment in subjects with treatment-resistant MDD using blood as a surrogate tissue. We will provide a context for these initial results by integrating the available evidence from other biomarker-based assessments of ketamine response in subjects with TRD as well as provide potential translational links to mechanistic studies being performed in animal models.

Keywords: Biomarker, Ketamine, Treatment Resistant Depression

Disclosure: Johnson and Johnson, Grant

T166

Ovarian Steroid-Related Alterations in Subgenual Cingulate Resting Regional Cerebral Blood Flow in PMDD: Preliminary Evidence for an Association With Expression of the ESC/E(Z) Family of Genes

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Background: Premenstrual dysphoric disorder (PMDD), a prevalent and debilitating condition that affects 3–15% of women of reproductive age, is characterized by the appearance of mood and behavioral symptoms confined to the luteal (post-ovulatory/premenstrual) phase of the menstrual cycle. There is substantial evidence that circulating ovarian steroids modulate neural
function differently in women with PMDD than in those without this disorder (Rubinow and Schmidt, 2018). This abnormal neurofunctional responsivity to the natural fluctuation of ovarian hormones in PMDD could reflect an intrinsic cellular abnormality in response to ovarian steroids. Indeed, in lymphoblastoid cell lines from women with PMDD, we recently observed increased expression of a majority of the genes in the ESC/E(Z) complex, an essential gene-silencing complex containing 13 gene members that regulates transcription (Margueron R, Reinberg D, 2011).

However, the relationship between hormonally-triggered differential neural response and altered gene expression in PMDD has not been studied. Here, we investigated the effects of estradiol (E) and progesterone (P) on resting regional cerebral blood flow (rCBF) using oxygen-15 water positron emission tomography (PET). Additionally, in an exploratory analysis including a subset of the women who were studied with PET, we tested whether resting rCBF in brain regions modulated by PMDD and by hormones was related to gene expression in the ESC/E(Z) complex.

Methods: Forty-three healthy control women (age [mean ± SD] = 33.9 ± 8.2) and 20 women with prospectively confirmed PMDD (37.6 ± 8.3; t(61) = 1.8; P = 0.07) underwent two eyes-open resting PET scans (10 mCi oxygen-15 water per scan, GE Advance [Milwaukee] three-dimensional PET scanner [septa retracted, 4.25 mm slice separation, 35 slices, axial field of view 15.3 cm]) during each of three hormone conditions: ovarian suppression induced by the gonadotropin-releasing hormone agonist leuprolide acetate (Lupron), Lupron plus E replacement, and Lupron plus P replacement. After preprocessing, the two resting rCBF scans of each woman for each hormone condition were averaged together and entered into a voxel-wise random effects analysis to determine the interaction between diagnosis and hormone conditions, masked by main effects of hormone condition, at a threshold of P < 0.05, FDR corrected. rCBF values were extracted from identified regions and were further explored in post-hoc analyses and in preliminary correlations with gene expression data.

Gene expression data from lymphoblastoid cell lines were obtained from RNA-sequencing performed in a previous study (Dubey N et al., 2017). These data were available for a subset of the PET cohorts - eight control women and eight with PMDD - and were used in a principal component analysis on expression values for each of the 13 genes. Principal components (PCs) were calculated using the prcomp() package in R and were visualized using the ggplot2 package (Wickham, 2016). In both controls and patients, we tested for a relationship between gene expression and rCBF extracted from voxels that showed a significant diagnosis-by-hormone interaction. The relationship between extracted rCBF values and the first ESC/E(Z) complex PC (PC1) were then examined with Pearson correlation coefficients (using SPSS) for each group separately.

Results: In the rCBF PET data, there was a significant interaction between diagnosis and hormone condition in the subgenual cingulate (SGCC), PFDReq=0.05. In PMDD, post-hoc analyses showed a differential modulation of rCBF in SGCC by ovarian steroids that was not observed in control women. Specifically, decreased resting rCBF was present during E (P = 0.02) and P (P = 0.0002) replacement (when PMDD symptoms are at risk of recurring) compared with hypogonadism (when PMDD symptoms are in remission). Because of these differences in resting SGCC rCBF between the Lupron alone condition and the E and P replacement conditions in the PMDD group, values for rCBF change between P and Lupron alone as well as between E and Lupron alone were used in the subsequent gene expression correlation analyses.

In PMDD, ESC/E(Z) complex PC1 significantly correlated with the change in resting rCBF between P and Lupron alone (Pearson r = −0.807, P = 0.016), whereas no significant correlation in these values was observed in controls (Pearson r = −0.296, P = 0.476). Additionally, there were no significant correlations between PC1 values and changes in resting rCBF between Lupron alone and E in either PMDD or healthy women (Pearson r’s ≤ −0.461, P’s ≥ 0.2).

Conclusions: These data demonstrate that in PMDD, ovarian hormone exposure differentially impacts resting rCBF in the SGCC, a region that is involved in affective regulation and that, therefore, could contribute to the risk for mood destabilization. Several ESC/E(Z) complex genes are expressed in the SGCC (Akula et al., 2019), and our data suggest the possibility that altered expression of these genes in the SGCC could contribute to the ovarian steroid-induced alterations in resting rCBF that were observed in PMDD. This work provides a preliminary framework for investigating genetic modulation of the neural control of affective state and the propensity for mood destabilization in PMDD.

Keywords: PET Imaging, Human Genetics, Ovarian Hormones, Mood Disorders, Estradiol

Disclosure: Nothing to disclose.

T167

Use and Impact of Psychotropic Medications During Transcranial Magnetic Stimulation for Major Depressive Disorder

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Background: Transcranial Magnetic Stimulation (TMS) is a non-invasive neuromodulatory therapeutic modality that is FDA approved for treatment-resistant Major Depressive Disorder (MDD). Many earlier regulatory trials leading to the FDA approval required participants to be medication free, but in clinical practice, the majority of patients undertaking a course of TMS are on concurrent psychotropic medications from a variety of pharmacological classes. Limited data exist to help clinicians understand the role of medications on MDD treatment outcomes during a standard course of TMS.

Methods: De-identified data from 307 patients treated in the Butler TMS Clinic from 2009–2018 were analyzed. Concomitant psychotropic medications were coded into 11 commonly referenced categories based on their pharmacological actions (or class) to explore effects on TMS Therapy outcomes. The Inventory of Depressive Symptoms Self-Report (IDSSR) and Patient Health Questionnaire-9 item (PHQ9) scales at baseline and at various timepoints including treatment endpoint were evaluated, with “response” characterized by ≥50% change in score (relative to baseline) on PHQ9 or IDSSR from baseline to endpoint and “remission” defined by endpoint scores (<14 on the IDSSR and <4 on PHQ9). Percent change (relative to baseline) was used as a continuous predictor variable. All patients completed a standard course of treatment (typically 36 sessions over 6-9 weeks) with stimulation delivered to the left dorsolateral prefrontal cortex. Chi-square tests and linear regression model analyses were performed using SPSS with significance p < 0.05.

Results: 14 patients (4.5%) of the sample were on no concurrent medications during TMS and 293 (95.5%) were taking at least one psychotropic agent. Using IDSSR categorical outcomes only, TMS patients on concurrent atypical antipsychotics had lower response (23%, p = 0.032) and remission rates (17%, p = 0.042) compared to those not on atypical antipsychotics. However, neither atypical antipsychotics, nor any of the other 11
medication categories we examined was found to be a significant predictor of outcome in models controlling for clinical variables such as past history of ECT, prior psychiatric hospitalizations, sex, and baseline severity. Additionally, the number of concurrent medication classes (varying from 0 to 6) was also not found to be related to TMS outcomes.

**Conclusions:** This analysis of the effects of concurrent psychopharmacology on TMS therapy outcomes for MDD indicated that no specific category or number of medications predicted better or worse therapeutic outcomes from TMS. Although, not found in our sample, there has been some hypothesis regarding the concurrent use of anticonvulsant agents or benzodiazepines portending to worse TMS therapy outcomes, perhaps because those agents tend to diminish excitability of the motor cortex. While a trial involving prospective, randomized assignment of patients to specific concurrent medication classes during their course of TMS is not likely to be a feasible approach, a larger sample size and greater division of medication into subgroups based on their pharmacological mechanisms may have detected effects or uncovered significant associations not revealed by our medication classes. In summary, our data do not suggest that patients with MDD undertaking a course of TMS should preferentially initiate or discontinue certain medications to get the best possible effect from TMS therapy.

**Keywords:** Repetitive Transcranial Magnetic Stimulation (rTMS), Major Depressive Disorder (MDD), Neuromodulation, Psychotropic Medications

**Disclosure:** Nothing to disclose.

**T168**

**Dissociation Between Food and Monetary Reward Responses Under Stress in Major Depressive Disorder**

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**Background:** Major Depressive Disorder (MDD) remains a major public health concern, with the majority of individuals failing to respond to treatments or relapsing after partial recovery. A better understanding of the neural mechanisms underlying this condition is of main interest for the development of innovative treatments. Neuroimaging studies have shown that MDD is associated with altered reward circuitry, characterized by hypoactivation of mesolimbic circuitry during anticipation and receipt of rewards. Moreover, stress may contribute to HPA-axis dysregulation, which preclinical and clinical studies have demonstrated to interact with the reward system to promote symptom persistence. However, the underpinnings of these mechanistic pathways remain poorly understood. Further data are needed to determine whether these alterations are specific to certain reward modalities (primary reinforcers such as food, secondary reinforcers such as money), and whether reward circuitry activation to different reward modalities is impacted by stress. The aim of this study was to examine the relationship between HPA-axis hormones and mesolimbic circuitry activity during monetary and food reward processing in response to stress in MDD.

**Methods:** Forty individuals with current Major Depressive Disorder (MDD) (28.38 ± 6.00 years; 25.84 ± 4.52 BMI; 18 females) and 33 Healthy Control (HC) subjects (26.24 ± 5.44 years; 26.80 ± 5.98 BMI; 18 females) participated in our study. They completed the Maastricht Acute Stress Test (MAST). Post-MAST, participants were scanned during a Monetary Incentive Delay (MID) task and a Food Incentive Dealy (FID) task. Brain activity was recorded during anticipation and receipt phases for each task. Plasma cortisol was collected pre- (T0) and at serially post-MAST (T20, T80, T105, T140, T190). fMRI data were analyzed with SPM12 (small-volume correction; initial voxel-wise height threshold: p < 0.05 uncorrected). Results were reported as significant if they additionally met the peak level threshold of p < 0.05, FWE-corrected). Predefined ROIs for a priori hypotheses were: nucleus accumbens (NAcc); ventral tegmental area (VTA), hypothalamus, amygdala, caudate, putamen, medial prefrontal cortex (mPFC). Average beta estimates were extracted from anatomical ROIs using REX, and relationships between beta estimates and hormones were examined with SPSS v19.

**Results:** Analyses of cortisol data yielded a main effect of Time (F(1, 69) = 92.53, p < 0.001). For both groups, cortisol level increased during T0-T20 (p < 0.001), decreased between T20-T105 (p < 0.001), and increased between T140-T190 (p < 0.05). No Time x Condition interaction was found [F(1, 69) = 0.15, p = 0.70]. During anticipation of reward, there was a Group x Condition interaction in the right VTA. Post-hoc analyses showed that this interaction was driven by greater activity in MDD during anticipation of money compared to food [t(39) = -3.29, p < 0.005]. Results also revealed a main effect of Reward in VTA, bilateral caudate nucleus, and left putamen. Post-hoc analyses determined that these main effects were driven by greater activity during anticipation of money compared to food for both HC and MDD groups. During the outcome of reward, results depicted a Group x Condition interaction in the hypothalamus. Post-hoc analyses showed that this interaction was driven by greater activity in MDD during receipt of money compared to food [t(39) = -2.455, p = 0.02], higher activity in HC during receipt of food compared to money [t(32) = -3.22, p < 0.005], higher activity in MDD compared to HC during receipt of money [t(71) = 0.48, p < 0.005], and higher activity in HC compared to MDD during receipt of food [t(32) = -3.22, p < 0.005]. Cortisol at T0, T20, T80 and T105 and right VTA activity during anticipation of money were positively correlated in the MDD group (T0: r = 0.38, p = 0.02; T20: r = 0.38, p = 0.02; T80: r = 0.34, p = 0.04, T105: r = 0.32, p = 0.04), and inversely correlated in the HC group for T0 (r = -0.38, p = 0.03), and unrelated at T20 (r = -0.19, p = 0.29), T80 (r = -0.29, p = 0.09) and T105 (r = -0.29, p = 0.10). Cortisol at T0, T20, T80 and T105 and left putamen activity during anticipation of food were positively correlated in the MDD group (T0: r = 0.36, p = 0.02; T20: r = 0.33, p = 0.04; T80: r = 0.33, p = 0.04, T105: r = 0.33, p = 0.04), and inversely correlated in the HC group for T80 (r = -0.37, p = 0.03) and T105 (r = -0.41, p = 0.02), and unrelated at T0 (r = -0.24, p = 0.19) and T20 (r = -0.21, p = 0.25).

**Conclusions:** These preliminary results showed that under stress, individuals with MDD exhibit different neurophysiological responses to food and money. MDD was associated with mesolimbic reward circuitry alteration, characterized by decreased activity anticipating and obtaining food, and increased activity anticipating and obtaining money. HC depicted higher activity when anticipating money compared to food, but showed the opposite pattern of activity when obtaining reward. Interestingly, HC showed greater activity during receipt of food compared to MDD. Results suggest a strong association between HPA-axis dysregulation and mesolimbic activity dysfunction (specifically, increased activity) during anticipation and receipt of reward in MDD. Future analyses will examine whether similar patterns are observed in distinct MDD appetitive phenotypes (increased vs. decreased appetite/weight).

**Keywords:** Mesolimbic Reward Circuitry, Major Depressive Disorder (MDD), Stress, HPA Axis

**Disclosure:** Nothing to disclose.
**T169**

**Electroconvulsive Stimulation Changes Behavior and Hippocampal GFAP Expression in Mice Subjected to Chronic Social Defeat Stress Compared to Controls**

Abstract not included.

**T170**

**Effects of Amphetamine on Motivation for Reward in Human “Workers” vs. “Slackers”**

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**Background:** The ability to weigh effort against reward and select which rewards to pursue is critical to adaptive functioning. Indeed, effort-related decision-making is abnormal in several psychiatric disorders. Research in laboratory animals suggests that dopamine underpins effort-related decisions, and that increasing dopamine using a stimulant drug can increase exertion of effort for reward. Our previous study in healthy adults confirmed that the stimulant amphetamine (20mg) increases willingness to exert effort for rewards in humans. However, studies in laboratory animals also suggest that baseline effort predicts stimulant effects. Specifically, rats who are low effort “slackers” at baseline work harder when given amphetamine, while high effort “workers” slack off. Identifying which individuals are likely to see increases in reward motivation from a stimulant could help individualize treatment of disorders involving low motivation for reward (e.g. depression), and also, might help identify those vulnerable to abuse of stimulants as “study drugs”. Thus, in the current study we examined whether baseline effort predicts the effect of amphetamine on willingness to exert effort for reward, in healthy adults.

**Methods:** Healthy adults (N = 30) completed a four-session within-subject study. Participants first completed a drug-free session to measure baseline effort. Next, they completed three sessions at which they received placebo, 10 mg and 20 mg of amphetamine in a counterbalanced order, to measure effects of amphetamine on effort. Our measure of effort was the Effort Expenditure for Rewards (EER) task, in which participants decide repeatedly between low effort/low reward vs. high effort/high reward options. Size of reward and probability of receiving a reward vary, to provide a wide range of expected values for the high effort choice.

**Results:** We replicated our prior findings that 20 mg of amphetamine robustly increases exertion of effort for reward in healthy adults (p = 0.006). There was a small-to-moderate relationship between baseline effort and enhancement of effort by amphetamine (r = 0.25), such that individuals working less at baseline showed greater increases in effort under amphetamine, but this effect was not significant (p = 0.12).

**Conclusions:** We replicated our previous findings, showing that amphetamine robustly increases willingness to exert effort for reward in healthy adults. However, baseline levels of effort did not predict response to amphetamine as strongly in our study, compared to a previous study in animals. Aside from species differences, one potential reason could be type of effort, as the current study used physical effort, while the rodent study used cognitive effort. Future directions include testing the ability of other potential indicators of dopaminergic functioning (e.g. working memory) to predict effects of amphetamine on reward functioning, and testing amphetamine effects on cognitive effort in humans. In conclusion, this study extends key findings about the basis of effort from animals into humans, informing the applicability of these models to human behavior.

**Keywords:** Reward-Based Decision-Making, Effort Based Decision Making Task, Amphetamine, Dopamine Agonists, Translational Neuroscience

**Disclosure:** Nothing to disclose.

**T171**

**Characterization of Suicidality in Bipolar Disorder Patients**

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**Background:** Suicides claim over 800,000 lives per year worldwide. About 50% of bipolar disorder patients have history of suicide attempt (Jamison, 2000). The etiology of suicide is highly complex.

**Methods:** We characterized suicide attempt history and suicidal behaviour severity in our bipolar disorder sample (N = 260) using measures of personality (Eysenck Personality Questionnaire), life events, sleep disorder, alcohol use disorder, depressive episodes, manic episodes, mixed episodes, and demographic information (feature selection using R and cluster analysis using SPSS).

**Results:** After feature selection, three and five EPQ items were found to be confirmed or tentative attributes for suicide attempt and suicidal behaviour severity scores. We performed cluster analysis using these selected variables to explore potential subtypes of suicide attempters and suicidal behaviour in our bipolar disorder sample.

**Conclusions:** Better characterization of suicide may help design future neurobiological studies of suicidal behaviour.

**Keywords:** Suicidality, Suicidal Behavior, Bipolar I & II Disorder, Cluster Analysis, Phenotypes

**Disclosure:** Patent applications, Patent

**T172**

**Sex-Specific Molecular and Cellular Adaptations to Chronic Stress in NAc- and VTA-Projecting Pyramidal Neurons of the mPFC**

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**Background:** Males and females respond differently to chronic stress. The medial prefrontal cortex (mPFC) is part of a complex circuit controlling stress responses. The mPFC sends projections to various limbic structures involved in stress responses including the nucleus accumbens (NAc) and ventral tegmental area (VTA). However, whether these pathways are differently involved in the expression of depressive-like behaviors following chronic stress in males and females is still unclear.

**Methods:** In this study, we used chronic variable stress (CVS) to induce depressive-like behaviors in males and females. We used retrograde adeno-associated to label mPFC neurons projecting either to the NAC or the VTA in males and females. Through this approach we recorded electrophysiological measures and compared the functional impact of CVS on the activity of mPFC neurons
projecting to both brain regions in males and females. We also used fiber photometry in freely behaving males and females to record the activity of both neuronal populations simultaneously during the administration of CVs. We performed neuronal reconstruction to determine whether CVs in males and females induces morphological changes in a pathways specific fashion. Chemogenetic approaches were used to validate behavioral contribution of both pathways to the expression of depressive-like behaviors in males and females. Finally, using a conditional viral approach in ribotag transgenic mice we isolated RNA from the mPFC neurons projecting to both brain regions and used RNAseq to screen pathways specific transcriptional profiles in males and females following CVs.

Results: CVs induced depressive-like behaviors defined by a complex spectrum covering anxiety, behavioral despair and anhedonia in both males and females. Our viral approach allowed us to differentiate mPFC neurons according to their projection. Our electrophysiological recordings show that CVs in females increases the frequency of EPSCs in NAc and VTA projecting neurons and decreases IPSC frequency only in NAc projecting mPFC neurons. On the contrary, in males, CVs raise the frequency of EPSCs only in VTA projecting mPFC neurons but decreases the frequency of IPSCs in NAC projecting mPFC neurons only. We supplemented these findings through Ca2+ imaging recordings showing sex-specific dynamic changes in both projections throughout the duration of CVs. These functional changes were accompanied by sex-specific alterations in dendritic arborization and spine density in both projections. Consistent with these changes, we showed that the chemogenetic activation of mPFC neurons projecting to the NAc increases stress susceptibility while the inhibition of this pathway reverses stress-induced behavioral deficits in females. Finally, using RNAseq, we screened the transcriptional profiles of NAc and VTA projecting neurons in stressed males and females. Our analysis revealed pathway-specific transcriptional alterations in males and females that could underlie the sex-specific behavioral, functional and morphological alterations induced by CVs.

Conclusions: Our results suggest that chronic stress impacts the cortico-striatal and -tegmental pathways differently in males and females through sex-specific functional and morphological alterations in mPFC pyramidal neurons.

Keywords: Chronic Stress, Sex Differences, Depressive-Like Behavior

Disclosure: Nothing to disclose.

T173
Small Molecule Allosteric Modulators of Voltage-Gated Sodium Channels


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Background: The ability of neurons to fire action potentials depends upon the pore-forming alpha subunit of the voltage-gated Na+ (Nav) channel. Growing evidence indicates that activity of the Nav channel in the brain requires a complex nexus of protein:protein interactions (PPI) that extends beyond the membrane spanning pore and incorporates intracellular scaffold-ing and signaling molecules. Recent studies indicate that disruption of these PPI might contribute to the etiology of psychiatric disorders. From a drug development standpoint, PPI interfaces are highly specific and flexible, and could provide ideal scaffolds for targeted probe and lead development. Here, we present recent progresses of a drug discovery campaign based on an integrated in vitro-to-ex vivo pipeline targeting the PPI interface of the Nav1.6 channel and its regulatory protein, fibroblast growth factor 14 (FGF14). The FGF14:Nav1.6 complex is a molecular determinant of firing in medium spiny neurons (MSNs) in the nucleus accumbens (NAC). Thus, compounds from this campaign could lead to the development of PPI-based antidepressants and/or mood stabilizers.

Methods: We employed medicinal chemistry, chemoinformatics, the split-luciferase assay (LCA), surface plasmon resonance (SPR), patch-clamp electrophysiology in heterologous expression systems and ex vivo brain slices to synthesize and validate the activity of hits in vitro, in-cell and ex vivo preparations.

Results: We conducted an in-cell high-throughput screening against the FGF14:Nav1.6 complex using LCA. Following development of a double-stable HEK293 cell line expressing LCA constructs, we screened ~50,000 small molecules, peptides and rationally-designed drug-like analogues. Of these, 640 compounds failed to achieve significance during triplicate validation screening and counter-screenings against full-length luciferase resulted in the exclusion of an additional 149 compounds due to significant inhibitory effects (Z ≤ -3). Thus, the primary set of hits was reduced to 151 inhibitors and 20 enhancers, which were then stratified by structural and chemical parameters including predicted permeability (logP). An initial 8-point dose response (n = 4 per concentration) was conducted for the top 50 compounds with the greatest potential for blood-brain barrier permeability. From those 23 compounds (17 inhibitors and 6 enhancers) were then selected for further analysis based upon their potency (EC50/IC50) using a 10-point dose response (n = 8 per concentration). For inhibitors, in-cell IC50s ranged from 0.95 µM–35 µM, while EC50s ranged from 0.77–11 µM. These hits were then validated by orthogonal screening in vitro using surface plasmon resonance (SPR) and the protein thermal shift (PTS) assay to determine drug binding affinity to purified FGF14 and Nav1.6 C-tail protein. For FGF14, all hits by PTS also displayed measurable binding affinity by SPR, and a total of 8 compounds demonstrated binding affinity of <10 µM. For Nav1.6, four PTS hits were confirmed by SPR, and a total of 9 compounds demonstrated binding affinity of <10 µM. One top hit was compound 5674 (cmp5674), which had an in-cell IC50 of 0.95 µM and bound to both FGF14 and Nav1.6 by PTS (ΔTm = −2.05 and −3.54 °C for FGF14 and Nav1.6, respectively) as well as SPR (KD = 940 nM and 2.5 µM for FGF14 and Nav1.6, respectively). We selected cmp5674 for whole-cell patch-clamp electrophysiology in acute NAc slices to characterize activity of the compound on MSNs firing. We found that cmp5674 significantly increased the number of action potentials (100µM cmp5674, 24.33 ± 2.08) when compared to control (0.01% DMSO, 12.40 ± 4.43, n = 5-6, p < 0.05 with Student t-test), while no changes were observed in passive electrical properties.

Conclusions: These results not only demonstrate the validity of centering a drug discovery campaign on PPI in the brain, but also provide an innovative strategy in neuropharmacology that could lead to new medications for psychiatric disorders.

Keywords: Drug Discovery - New Approaches, Ion Channels, Intracellular Signaling, Excitability, Mood Disorder

Disclosure: Nothing to disclose.

T174
Resting-State Brain Network in U.S. Veterans With Subconcussive Blast Exposure

Abstract not included.
Subgenual Cingulate, Dorsal Lateral Prefrontal Cortex, and Dorsal Nexus Interactions Provide Predictors and Mechanisms for the Clinical Response to Transcranial Magnetic Stimulation in Depression

Tracy Barbour*, Kristen Ellard, Joan Camprodon

Background: Transcranial magnetic stimulation (TMS) is a noninvasive and nonconvulsive neuromodulatory therapy that uses powerful, rapidly changing magnetic fields to cause changes in neural excitability when applied over the surface of the skull. rTMS treatment is thought to modulate activity at the target site of stimulation and within functionally connected downstream circuits. However, it is not yet clear how pre-treatment network connections and treatment related changes in network dynamics are related to clinical outcomes. Previous work has linked abnormal functional connectivity in the cognitive control and affective networks with core symptoms of depression, and rTMS treatment for depression directly targets a key node of the cognitive control network, the dorsolateral prefrontal cortex (DLPFC), and indirectly targets a node of the affective network, the subgenual cingulate (SCC). The cognitive control and affective networks are functionally connected to a region of the dorsal medial prefrontal cortex (DMPFC) called the dorsal nexus. Functionally connected to several brain regions/networks, the dorsal nexus is thought to serve a key role in the simultaneous presentation of heterogeneous, depressive symptoms, thought to arise from distinct neural networks. To better understand the role of the dorsal nexus in TMS treatment of depression, we examined measures of functional connectivity in the SCC, DLPFC, and DMPFC before and after an rTMS treatment course for depression to determine whether baseline patterns of functional connectivity and changes in functional connectivity are related to antidepressant response.

Methods: 20 outpatients with major depressive disorder were enrolled from the Massachusetts General Hospital TMS Clinical Service and received a treatment course of rTMS consisting of up to 36 treatments. Each treatment consisted of 3000 pulses delivered at 10 Hz frequency and 120% of the resting motor threshold to an individually determined left prefrontal cortex target. All subjects were scanned before and after TMS, with two resting state fMRI scans and a high-resolution multi-echo MPRAGE T1 image. Prior to treatment, the SCC was manually identified on each subject’s T1 MRI scan using anatomical landmarks, and this was used as the seed region to generate whole brain functional connectivity maps. The target of stimulation was identified as the location within the left prefrontal cortex most highly anticorrelated with the SCC. A functional DMPFC ROI was created by applying a whole brain regression of baseline functional connectivity with the changes in DMPFC to SCC connectivity following rTMS between our regions of interest (ROI): the SCC, the left DLPFC target of stimulation, and the DMPFC.

Results: At baseline we found that weaker negative functional connectivity between the left DLPFC target and SCC (p = 0.01) and higher positive connectivity between the left DLPFC target and DMPFC (p = 0.02) predicted response to TMS. Furthermore, the interaction between baseline functional connectivity of the left DLPFC target and SCC with baseline functional connectivity between the DMPFC and SCC predicted clinical response (p = 0.02); weaker negative functional connectivity between the left DLPFC target and SCC in conjunction with weaker positive connectivity between the DMPFC and SCC correlated with more clinical improvement. Following treatment, we found that clinical improvement was related to reductions in DLPFC connectivity with the SCC (p = 0.015) and DMPFC (p = 0.01). We also found that depressive symptom improvement was related to the relationship between the changes in DMPFC to left DLPFC target functional connectivity with the changes in DMPFC to SCC functional connectivity (p = 0.02). Specifically, greater clinical improvement was related to larger reductions in functional connectivity between the DMPFC and left DLPFC target relative to DMPFC and SCC functional connectivity.

Conclusions: Using a personalized approach to TMS, we show that treatment response is predicted by the left DLPFC target’s functional connectivity relationships with both the SCC and DMPFC. We also show that functional connectivity changes between the left DLPFC target and the SCC, as well as between the left DLPFC target and the DMPFC are related to treatment response. Overall, our results suggest that the therapeutic effect of TMS involves modulation of functional relationships between neural networks. While a larger study is needed to verify our results, future TMS studies using fMRI resting state connectivity in personalized TMS treatment targeting approaches may want to consider using a method for identifying the treatment target that accounts for the functional relationships between the left DLPFC, the dorsal nexus, and the SCC.

Keywords: TMS, Depression, Resting-State fMRI

Disclosure: Nothing to disclose.
along with multiple Institutes across the NIH, built a collaborative infrastructure of therapeutic development programs designed to enhance our understanding of the development and prevention of chronic pain. These programs span the discovery process from target validation through clinical trials.

**Results:** Programs in the infrastructure include: (1) A Preclinical Screening Platform for Pain (PSPP) focused on the identification and profiling of non-addictive/non-opioid therapeutics for pain, and (2) an Early Phase Pain Investigation Clinical Trial Network (EPPIC-Net), to test new therapies for pain conditions in adults and children. PSPP will be a one-stop resource for preclinical evaluation of potential pain therapeutic agents that lack abuse liability, including small molecules, biologics, devices and natural products. EPPIC-Net will test new pain treatments in early stage trials and provide proof-of-concept testing of potential biomarkers and new non-addictive treatments.

**Conclusions:** This presentation will describe the two NINDS/NIH-HEAL Initiative programs and efforts to test new therapies for pain conditions.

**Keywords:** Pain Therapeutics, Pre-Clinical Screening, Clinical Development, Non-Addictive

**Disclosure:** Nothing to disclose.

**T177**

**Serotonin Release and Increased Blood Flow in the Occipital Cortex in Response to Visual Stimuli**


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**Background:** Endogenous serotonin (5-HT) release can be measured non-invasively using high-resolution positron emission tomography (PET) imaging in combination with certain serotonergic radiotracers. This allows us to study the living human brain during both pharmacological and non-pharmacological interventions. Here we studied the neural responses to a visual stimulus using hybrid PET/MR imaging.

**Methods:** In a cross-over design, 11 healthy individuals (10 females, 1 male) were PET/MR scanned with the 5-HT1B receptor radioligand [11C]AZ10419369, which is sensitive to changes in HT1B receptor binding and CBF in the occipital cortex. We speculate that using more powerful physiological stimuli would cause larger 5-HT release and consequently increase the changes in binding and CBF. The association between the changes in receptor binding and CBF suggest a mechanism in which 5-HT is involved in either attention or the processing of visual material. To the best of our knowledge, this is the first time changes in 5-HT levels in response to non-pharmacological stimuli has been measured with PET.

**Key Words:** Hybrid PET/MR, Serotonin, Visual Stimulation, 5-ht1b

**Disclosure:** Nothing to disclose.

**T178**

**Auto-Antibodies Against NMDA Receptor Induced Encephalitis Phenotypes in Marmosets**

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**Background:** Anti-NMDA receptor encephalitis is a severe encephalitis attributed to the presence of autoantibodies against the N-methyl-D-aspartate receptor (NMDAR) in the brain. The patient’s manifestation starts with psychiatric symptoms and neurologic complications such as movement abnormalities and hyperventilation, and progresses to long term impairments in executive dysfunction and impulsivity. Recently patients seropositive for anti-NMDAR autoantibody were also reported in schizophrenia, bipolar disorders, and autism spectrum disorders, suggesting that the pathogenic antibody causes psychiatric symptoms and cognitive impairments in some of patients diagnosed with neuropsychiatric disorders. Novel therapeutics targeting these autoantibodies could be a new treatment option for the encephalitis as well as related neuropsychiatric disorders. Unfortunately, there are no appropriate preclinical animal models of anti-NMDAR encephalitis. To establish the foundation of drug discovery, we used non-human primate, common marmoset, because non-human primates exhibit much more similar sensitivity to NMDA hypofunction than rodents do. We have constructed a new animal model of anti-NMDAR encephalitis by infusing recombinant pathogenic antibody in common marmosets.

**Methods:** The binding affinity for NMDAR and the activity of NMDAR internalization were assessed to find potent artificial pathogenic auto-antibodies. For constructing preclinical animal model of encephalitis, we used three common marmosets (two males, one female). Under isoflurane anesthesia, marmosets were placed on stereotaxic apparatus, and a bolus injection needle was inserted into the third cerebroventricle to infuse the pathogenic anti-NMDA receptor. To record the body temperature and locomotion, simple telemetry system, nano tag™, was implanted under the abdominal skin. Once animals gained consciousness, they were housed singly, and behavioral and ketamine sensitivity assessments were performed by video recording at 24 hours after surgery. The agility, and ambidexterity were calculated by latency to first, and motor and mental abnormality were assessed by

rating scales. The temperature and locomotion were calculated by software, nanotag Viewer.

**Results:** Two types of recombinant pathogenic antibodies showed potent binding affinities for NMDA receptors. These auto-antibodies induced clear internalization activities for NMDA receptors. All animals quickly recovered after the surgery and showed normal behavioral and physical conditions prior to the behavioral assessments. However, at 24 hr after the surgery, marmosets infused with pathogenic antibody began to show reduction in ambidexterity and worsening in mental and motor rating scale scores. In ketamine challenge, these marmosets displayed altered spontaneous locomotion and reduced body temperature (post/pre-treatment).

**Conclusions:** This is the first report attempting to establish anti-NMDAR encephalitis model in non-human primate, common marmoset. Our results demonstrate that the pathogenic auto-antibodies cause abnormal behaviors, mental disruption, and higher sensitivity to an NMDA antagonist, ketamine. These phenotypic alterations could be useful for assessment of the efficacy of novel drug candidates and clarification of pathophysiology in anti-NMDAR encephalitis.

**Keywords:** Autoimmune Encephalitis, NMDA Receptor, Non-Human Primate, Antibody, Marmoset

**Disclosure:** Nothing to disclose.

**T179**

Translational Programs and Initiatives Within NINDS and Across the NIH

Abstract not included.

**T180**

Association and Modulation of Functional Connectivity-Guided Cortical Gaba Levels and Clinical Pain in Fibromyalgia Syndrome: A Spectroscopic Imaging Pilot Study

Abstract not included.

**T181**

Progressive Functional and Structural Neurodegeneration in Veterans With Mild Traumatic Brain Injury

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**Background:** Mild traumatic brain injury (mTBI) may predispose individuals to progressive neurodegeneration and the development of dementia. This study aimed to identify evidence of neurodegeneration through longitudinal evaluation of structural and functional changes in the visual and central nervous system in Veterans with a history of mTBI. Our long-term objective is to identify biomarkers that can be used for early identification of those Veterans at risk for future functional decline.

**Methods:** 69 veterans with mTBI (mean age = 50.0 years; 7 women) and 70 age-matched control veterans (mean age = 49.9 years; 15 women) received evaluations at 6 month intervals of visual function, cognition, and Optical Coherence Tomography (OCT), and of magnetic resonance imaging (MRI) at 18 months. A linear trend statistical model was used to estimate slopes of change over time in subjects’ retinal nerve fiber layer (RNFL) thickness, contrast sensitivity, and tests of cognitive function. Changes in structural MRI (cortical thickness) over 18 months was completed in 100 of the subjects. Slopes from the linear models fit to each outcome measure were used in statistical analyses.

**Results:** Compared to controls, Veterans with mTBI showed significantly greater thinning of the retinal nerve fiber layer (RNFL) in the retina. There was an average loss of 1.47 microns/year in mTBI and of 0.31 microns/year in controls (F(1, 122) = 8.42, p = 0.004, Cohen’s d = 0.52). This was associated with a small, but significant reduction in high spatial frequency (12 cycles/degree; cpd) contrast sensitivity (p = 0.02) compared to controls. A significant difference in the change over time of the number of errors committed during the Groton Maze Learning Test (GMLT) was found between groups (F(1,127) = 4.43, p = 0.04, d = 0.37), with a greater decrease in errors in controls compared to mTBI. Greater RNFL tissue loss was significantly correlated with mTBI severity (Spearman’s ρ = −0.25, p = 0.006). The more severe the mild TBI (larger severity score derived via consensus from a semi-structured TBI interview), the faster the reduction in RNFL thickness (i.e. the more negative the slope) across time. Greater RNFL tissue loss was also significantly correlated with the worsening performance on the GMLT (Spearman’s ρ = −0.20, p = 0.03). Finally, a significant difference in percent change in cortical thickness in primary visual cortex (V1) was found for the mTBI vs. control group (F(1, 98) = 5.01, p = 0.028, d = 0.45), with a larger decrease in V1 thickness in mTBI over time.

**Conclusions:** We have found longitudinal evidence for significant, progressive neural degeneration over time in Veterans with mild TBI, as indicated by greater RNFL tissue loss and V1 cortical thinning in mTBI vs Controls. Functional tests of vision showed small, but significant changes over the current time period of study. Results suggest that these longitudinal measures may be useful biomarkers of neurodegeneration. Continued evaluation of subjects will determine if the extent of functional deterioration in the visual and cognitive pathways follow structural loss in the retina and visual cortex.

**Keywords:** Mild Traumatic Brain Injury, MRI, Retina

**Disclosure:** Mild Traumatic Brain Injury, MRI, Retina

**T182**

Neural Correlates of Apathy in Myotonic Dystrophy Type 1

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**Background:** Apathy is a known feature of Myotonic Dystrophy 1 (DM1); however, few studies have investigated possible mechanisms. Previous structural MRI has demonstrated that amygdala volume is abnormal in individuals with DM1. The amygdala is central to the motivational salience network, along with the putamen, medial orbitofrontal cortex (MOF), and anterior cingulate cortex. Dysregulation of this circuitry may be associated with apathy in DM1.

**Methods:** Neuromaging data was acquired in 68 controls (mean age = 43.5 years, SD = 12.9) and 50 individuals with adult-onset DM1 (mean age = 45.7 years, SD = 11.4). Apathy was assessed using the self-report version of the Apathy Evaluation Scale (AES) and the motivational salience network was evaluated by calculating ratios of the amygdala volume and each of the regions of interest involved in the network.
**Results:** There were no significant sex differences between groups (Welch’s t(2) = 6.62, df = 1, p = 1). The DM1 group exhibited significantly higher apathy scores (t(112) = 4.8, p < 0.0001) than did controls. CTG repeat length predicted higher apathy scores in the DM1 group (t(39) = 2.2, p = 0.029). Two significant group by ROI interaction effects were detected: in DM1 only, increased amygdala-to-caudate ratio (t(111) = 1.8, p = 0.06) ratio.

**Conclusions:** Apathy in DM1 may be related to dysregulation of the amygdala-striatal circuitry, a hypothesis that can be explored further using functional connectivity analyses. The amygdala-striatal circuit may be a tangible target for clinical trials addressing apathy in DM1.

**Keywords:** Myotonic Dystrophy, Human Neuroimaging, Apathy

**Disclosure:** Nothing to disclose.

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**T183**

**Neurosteroids in TBI: Biomarkers to Therapeutics**

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**Background:** Neurosteroids demonstrate pleiotropic actions that may be relevant to the pathophysiology and treatment of traumatic brain injury (TBI). Common sequelae of TBI include anterior pituitary hypofunction, which could adversely impact neurosteroid regulation and lead to neurosteroid deficits. Characterizing neurosteroid signatures post-TBI could thus have biomarker potential. If neurosteroids are decreased post-TBI, then ameliorating neurosteroid deficits post-injury with exogenous supplementation could be clinically therapeutic. Preclinical models support this possibility. For example, dehydroepiandrosterone sulfate (DHEAS) mitigates cognitive and behavioral symptoms in rodents post-TBI, enhances functional recovery, and exhibits neuroprotective actions. In its unsulfated form, dehydroepiandrosterone (DHEA) decreases gliosis and astrocytic proliferation following brain injury. We have previously demonstrated that administering DHEA quadruples downstream androsterone levels (Sripada et al. 2013). Androsterone is a metabolite of DHEA (which, like allopropynoglanone, has neuroprotective actions in rodent models of TBI and also positively modulates GABA-A receptors). Androsterone may therefore be a promising biomarker candidate in TBI. We thus quantified DHEA, DHEAS, and androsterone levels in Iraq-Afghanistan veterans with a history of TBI.

**Methods:** Androsterone levels were quantified in serum samples from 485 male Iraq/Afghanistan-era veterans by mass spectrometry. DHEAS and DHEA levels were quantified in 660 male Iraq/Afghanistan-era veterans by radioimmunoassay. Regression analyses were conducted using age and smoking as predetermined covariates to determine if participants with a history of TBI demonstrate altered neurosteroid levels. To assess depression, the Beck Depression Inventory (BDI) was utilized (cut-point > 20); to assess suicidality, the Beck Scale for Suicide Ideation Scale (BSS) was utilized (cut-point ≥ 3).

**Results:** Iraq/Afghanistan-era veterans with a history of TBI (n = 132) had significantly reduced serum androsterone levels compared to veterans with no history of TBI (n = 297), controlling for age and smoking (p = 0.023). When the TBI group was circumscribed to only those with loss of consciousness (LOC) associated with TBI (n = 94), DHEAS was significantly decreased (p = 0.033). Androsterone levels were significantly decreased in veterans with depression (n = 92) compared to those without depression (n = 393), p = 0.0005; DHEAS levels tended to be reduced in veterans with depression (n = 130) compared to veterans with no depression (n = 529). DHEAS levels were also significantly lower in veterans reporting more suicidality (BSS ≥ 3; n = 45) compared to veterans reporting less or no suicidality (BSS < 3; n = 614), p = 0.035.

**Conclusions:** Androsterone and DHEAS are significantly decreased in Iraq/Afghanistan-era veterans with a history of TBI. Furthermore, androsterone levels are also markedly reduced in veterans reporting depression, and DHEAS is decreased in veterans reporting suicidality. DHEAS and androsterone may thus be promising biomarker candidates. Since administering DHEA quadruples androsterone, treatment with DHEA could represent a precursor loading strategy for enhancing downstream androsterone levels while also ameliorating DHEA reductions. Administering exogenous DHEA could therefore represent a multipronged therapeutic approach with the potential to mitigate numerous TBI sequelae.

**Keywords:** Neurosteroid, Neuroactive Steroid, Traumatic Brain Injury, Androsterone, Dehydroepiandrosterone

**Disclosure:** Nothing to disclose.

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**T184**

**DBS-Related Apathy in Parkinson’s Disease is Associated With Differential Stimulation of Limbic, Associative and Motor White Matter Tracts**

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**Background:** Subthalamic Deep Brain Stimulation (STN DBS) is a well-established and effective treatment for motor symptoms of Parkinson’s disease (PD). However, STN DBS is associated with apathy, i.e. lack of motivation. The mechanism of DBS-related apathy is presently unknown.

**Methods:** We compared tractography stimulation models between 9 patients with postsurgical apathy (Starkstein Apathy Scale (SAS) ≥ 14) and 9 patients without apathy (SAS < 14). DBS contact locations were identified by postoperative CT image fused with pre-surgical MRI. Patient-specific DBS activation volumes (Volume of Tissue Activated: VTA) were generated. Probabilistic tractography from estimated VTA to cortical targets (motor, associative, and limbic cortex) were performed to identify patterns of white matter connectivity from DBS locations associated with and without apathy.

**Results:** Patients with apathy showed significantly less stimulation of prefrontal limbic and associative relative to motor tracts, compared to patients without apathy.

**Conclusions:** This finding suggests that apathy during STN DBS for PD may be a manifestation of insufficient limbic and/or associative stimulation. This finding has implications for the use of tractography in DBS targeting and programming towards better engagement of non-motor circuits in PD.

**Keywords:** Deep Brain Stimulation, Apathy, Parkinson’s Disease, Diffusion Tractography

**Disclosure:** Nothing to disclose.
[18F]FDG Uptake in Globus Pallidus is Positively Correlated With Impairment of Cognitive and Behavioral Functioning and a Neuroinflammation Biomarker in Veterans With Blast-Related Mild Traumatic Brain Injury

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Background: Blast-induced mild traumatic brain injury (mTBI) is the “signature injury” of the wars in Iraq (OIF) and Afghanistan (OEF). Blasts account for 70-88% of mTBIs sustained in OEF/OIF, with affected Servicemembers usually experiencing multiple blast mTBIs. Even years after returning from deployments, many Veterans with blast-related mTBI report postconcussive cognitive symptoms, behavioral symptoms, and somatic symptoms, leading to substantial disability and interference with job and family relationships. In particular, cognitive symptoms, including executive functioning, and behavioral symptoms, including irritability, are particularly difficult to treat clinically and their pathobiology remain poorly understood.

We have previously reported a 1) consistent pattern of less [18F]FDG uptake in infratentorial structures and medial temporal cortex in male OIF Veterans with mTBI compared to cognitively and medically healthy community controls; 2) hypometabolism in bilateral parietal lobes, left somatosensory cortex, and right visual cortex, as well as in parahippocampus for Veterans with 20 or more blast mTBI compared to deployed control Veterans with no lifetime history of mTBI; and 3) a strong correlation between glucose metabolism in the cerebellum and number of blast exposures, which we have replicated in a much larger cohort. We have also found that [18F]FDG is significantly greater in globus pallidus of Veterans with mTBI compared to deployed controls. Given that this region has efferent and afferent projects to the prefrontal cortex and limbic system, we considered if this region could have an underappreciated involvement in cognitive or behavioral symptoms in Veterans with blast mTBI, and if the increased [18F]FDG could be attributed to neuroinflammation.

Methods: Methods: The local institutional review boards approved all procedures and all the participants provided written informed consent before enrollment into the study. Veterans with blast-related mTBI (n = 79; average blast mTBI exposures 22), with history of military deployment and no lifetime history of any TBI (n = 29) underwent thorough history and clinical characterization as part of our ongoing longitudinal study. A standard clinical 15-minute PET acquisition was performed 60 minutes after injection 8-10 mCi of [18F]FDG on either a GE Advance scanner or Philips Gemini PET/CT, and data underwent OSEM reconstruction. Three 5 minute frames were averaged following motion correction, smoothed 8x8x8mm, and transformed directly into MNI standardized space using SPM12. Individual images were scaled for intensity using a VOI applied to parenchyma, yielding fractional uptake (unitless) as the measured value in PMOD. The AAL VOI library was applied for regional analysis. Executive functioning was assessed using the Behavior Rating Inventory of Executive Function® - Adult Version (BRIEF-A), for which greater t-scores correspond to greater impairment (mTBI n = 50, controls n = 10). Emotional dysregulation was measured by both depressive symptoms using PHQ-9, and irritability using an index of irritability subscores from the PTSD Checklist – Military (PCL-M; irritability) and Neurobehavioral Symptom Inventory (NSI; irritability, feeling overwhelmed) (mTBI n = 60; control n = 17). Cerebrospinal fluid was obtained by lumbar puncture (mTBI n = 28, controls n = 12), and IL-6 and IL-7 measured by antibody-based electrochemiluminescence (Meso Scale Discovery, Rockville, MD).

Results: As previously reported, compared to deployed controls, [18F]FDG uptake in left pallidum was significantly higher in Veterans with mTBI (p < 5 x 10^-15). While BRIEF-A t-scores were positively correlated with [18F]FDG uptake in left pallidum for the combined groups (r2 = 0.21; p < 0.005) it was primarily driven by those with mTBI (r2 = 0.41; p < 5 x 10^-7). Similarly, PHQ-9 scores were positively correlated to left pallidum uptake in the combined group (r2 = 0.23; p < 1 x 10^-3) and primarily driven by those with mTBI (r2 = 0.39; p < 1 x 10^-4). The irritability index score was positively correlated to left pallidum uptake in the mTBI group (r2 = 0.08; p < 0.05) and not significantly correlated in controls. IL-7 was correlated to left pallidum uptake in the mTBI group only (p < 0.05), and IL-6 was not significantly correlated to left pallidum uptake in either group.

Conclusions: Veterans with history of blast mTBI demonstrate greater [18F]FDG uptake in globus pallidus compared to those with no history of TBI. This region of greater uptake is correlated to impairments in executive functioning, increased symptoms of depression, increased irritability, and a neuroinflammatory biomarker. This suggests that global pallidus involvement in cognitive and behavioral deficits, either by increased glucose use in compensation or due to neuroinflammation, and warrants further investigation for its involvement and networked connections in the pathobiology of blast mTBI.

Keywords: PET Imaging, Mild Traumatic Brain Injury, Combat Veteran}

Disclosure: Nothing to disclose.

T186

Serotonergic IL-1Rs Support CNS Inflammatory Cytokine Production and Neuronal Activation

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Background: A host of neuropsychiatric disorders, ranging from anxiety and depression to autism spectrum disorder and schizophrenia, have been found to display immune system alteration. Importantly, experimental studies in animals and humans demonstrate that peripheral modulation of the immune system triggers behavioral changes, suggesting that, for some subjects, and in some disorders, immune system dysfunction may be contributory or causal. Inflammatory cytokines have been found to impact multiple dimensions of neural signaling, from modulation of neurotransmitter metabolism, release, and transport, to neural excitability and plasticity. Forebrain-projecting serotonin (5-HT) neurons of the dorsal raphe nucleus (DRN) play a key role in regulating behaviors related to mood, fear, sleep, feeding and social interactions and dysfunction in 5-HT neurotransmission is therefore believed to contribute to multiple neuropsychiatric disorders. We have found that peripheral activation of the innate immune system rapidly increases the activity of the presynaptic serotonin transporter (SERT), which limits serotonin neurotransmission. In vitro and ex vivo studies support a role for signaling by the inflammatory cytokine, IL-1β through its receptor IL-1R1, to influence SERT via p38a MAPK. The identification of subsets of serotonin neurons modulated by IL-1Rs and the brain regions targeted by these cells may reveal key mechanisms by which immune system influences behavior and...
where dysfunction could impact risk for neuropsychiatric disorders.

Methods: Using transgenic mice that allow for conditional elimination of IL-1R1 (IL-1R1loxP/loxP) or restoration of IL-1R1 on an otherwise IL-1R1 knockout background (IL-1R1r/r), we assessed the distribution of serotonergic IL-1R1 expression, as well as the necessity and sufficiency of serotonergic IL-1R1 in mediating inflammation-induced changes in 5-HT neurotransmission. To assess whether the peripheral immune system’s effects on serotonergic neurons are mediated by IL-1R1s, we acutely administered LPS (0.2 mg/kg i.p.) or saline to adult (8–12 wks) male ePet1:Cre:IL-1R1fl/fl or ePet1:Cre:IL-1R1r/r mice. Brains were fixed as above and sliced at 40 µm. 100µm slices containing dorsal raphe were immunolabeled with 5-HT (Immunostar #20079, 1:1000) and cFos (Abcam #ab190289, 1:5000) and cleared using an established glycerol gradient, with cell counts obtained by a blinded observer. 40 µm slices were similarly immunolabeled but went through an increasing ethanol gradient and Citrisolv incubation for dehydration and clearing, respectively. An additional cohort was sacrificed by rapid decapitation one hour after LPS injection, followed by RNA isolation of midbrain and hippocampus. qPCR was conducted using a Taqman gene expression assay, with Taqman probes for 18S RNA (HS99999901) and IL-1β (Mm00434228). In vivo chronoamperometry was utilized to determine the effect of local IL-1β (2 ng) on 5-HT clearance in wild type mice.

Results: Using the il1r1 restore transgene, we demonstrate the existence of subsets of serotonergic neurons that express IL-1R1s, with enrichment evident in the dorsal and dorsolateral sub sections of the DRN. Acute 1-hour treatment with LPS (0.2 mg/kg) caused an upregulation of IL-1β mRNA expression in the midbrain and hippocampus. qPCR was conducted using a Taqman gene expression assay, with Taqman probes for 18S RNA (HS99999901) and IL-1β (Mm00434228). In vivo chronoamperometry was utilized to determine the effect of local IL-1β (2 ng) on 5-HT clearance in wild type mice.

Conclusions: These data provide support serotonergic neurons as significant contributors to CNS immune response after peripheral immune activation, both via IL-1β induction and neuronal activation in the raphe as well as serotonergic targets. Moreover, our studies with novel transgenic models reveal an unexpected, region-dependent functional role of serotonergic IL-1R1s. The contribution of serotonergic IL-1R1 to NAc core versus shell activation following peripheral LPS supports a role for IL-1R1 activation in behavioral outcomes, whose definition is underway. We are now working to identify the target projections of serotonergic IL-1R1-expressing neurons, and to activate and inhibit these projects in behaving animals. Our work has the potential to clarify links between elevated inflammatory markers and mechanisms that in humans drive risk for neuropsychiatric disorders. If corroborated, they may provide a rationale for the development of novel strategies to target anatomically and functionally discrete, immunosensitive serotonergic projections, including the intracellular targets of neuronal IL-1R1 signaling.

Keywords: Serotonin, Interleukin 1beta, Immune System, Interleukin 1 Receptor

Disclosure: Nothing to disclose.
FPN connectivity in pedOCD and greater DMN-SAL connectivity in adultOCD were associated with lower C/YBOCS scores post-CBT.

Conclusions: We found different patterns of connectivity imbalances between task positive and task negative networks across pediatric and adult OCD. Reduced DMN-FPN connectivity in pediatric OCD patients parallels the connectivity patterns detected in both OCD and HC adults, perhaps reflecting an accelerated maturation of these circuits in pediatric OCD, that may, in turn, facilitate response to treatment, consistent with our finding that more reduced DMN-FPN connectivity predicted better treatment response. In contrast, greater DMN-SAL connectivity in adult OCD patients was associated with better response, perhaps pointing to different network dynamics underlying pediatric and adult OCD. We are currently assessing effects of age of onset on connectivity and treatment response and analyzing follow-up fMRI data from these samples to assess changes in connectivity that coincide with changes in OCD symptoms following ERP Therapy. Nevertheless, these preliminary findings set the stage for the development of novel strategies to target these networks in the service of improving control processes (e.g., via cognitive control training) to augment the effects of ERP therapy.

Keywords: Resting-State fMRI, Obsessive-Compulsive Disorder (OCD), Large Scale Networks
Disclosure: Nothing to disclose.

T188
A Pilot Survey of Cannabis Use in Individuals With Obsessive Compulsive Disorder

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Background: Obsessive-compulsive disorder (OCD) affects 1%–3% of the general population [1]. It is a chronic condition and is associated with significantly lower quality of life and major societal costs, which have been estimated to be over $8 billion annually [1]. Standard treatment for OCD involves pharmacotherapy in the form of antidepressants, as well as cognitive behavioural therapy (CBT) with a focus on exposure and response prevention (ERP) [2]. Approximately 40–60% of OCD patients continue to experience impairing symptoms following first-line treatment [3]. As a result, patients have turned to alternative treatment methods, including cannabis. Research examining the efficacy of cannabis as a treatment for mental health is in its infancy. However, cross-sectional data indicates that cannabis is commonly used in the community for the treatment of a variety of mental health conditions [4]. As found in a recent meta-analysis of 31 studies, not only was cannabis commonly used as a treatment modality for anxiety disorders (including OCD), but the amount of cannabis used was positively associated with symptom severity [5]. Although another survey of young adult cannabis users found that OCD severity was unrelated to general cannabis use, a significant and positive association linked OCD symptom severity and cannabis misuse [6]. To date, no known randomized controlled trials have investigated the efficacy of cannabis products in treating OCD. Only one case report has been published to describe the efficacy of dronabinol, a synthetic THC product, in two patients with treatment-refractory OCD [7]. In considering these gaps in the literature, the current study examines the prevalence and the perceived efficacy of cannabis for individuals with OCD.

Methods: Adults aged 18 and over completed an online survey assessing their mental health status and substance use. After signing an electronic informed consent, respondents were asked whether they were formally diagnosed with OCD by a healthcare professional and completed a questionnaire battery. The battery contained the Obsessive-Compulsive Inventory, Short Version (OCI-R) to confirm their OCD symptom severity, questions related to their overall substance use, specific details regarding cannabis use, as well as the Generalized Anxiety Disorder-7 (GAD-7) to assess anxiety severity and Patient Health Questionnaire (PHQ-9) to assess depression severity. Data collection is currently ongoing.

Results: Twenty-four participants have completed the survey to date, with 79.2% being female. The mean age was 39.8 ± 13.3 years. The mean OCI-R score of the sample was 29.9 ± 13.4, indicating significant OCD. Moderate levels of depression (mean PHQ-9 = 11.3 ± 7.1) and anxiety (mean GAD-7 = 11.1 ± 6.1) were also found. Over 1/3 (37.5%) of respondents reported receiving current pharmacological treatment for their OCD, 4.2% were receiving non-pharmacological treatment, and 29.2% were receiving combined treatment. 66.7% of respondents reported using cannabis at some point within their lifetime. Of these respondents (n = 16), 12.5% reported using cannabis once or twice over 6 months, 12.5% reported monthly use, 6.3% reported weekly and 12.5% used it almost daily. Among those who reported using cannabis over the past 6 months (n = 7), the most commonly used types of cannabis product were dried cannabis flower/leaf (100%), cannabidiol (14.3%), and edibles (14.3%). Most cannabis users (85.7%) reported consuming approximately 0.125 grams each time they used cannabis. Only 8.3% of respondents reported being prescribed medical cannabis for a medical problem. Within the whole sample (N = 24), 25% of respondents attempted to treat OCD with cannabis at some point in their lifetime. Among the those who did use cannabis for their OCD (n = 11) most (63.3%) found cannabis to be helpful for OCD symptoms. Cannabis was found to be most helpful in allowing patients to retain their train of thought (36.4%), reducing the discomfort or anxiety brought on by intrusive thoughts (36.4%), reducing the number of intrusive thoughts (27.3%), decreasing compulsions (36.4%), and reducing anxiety in general (36.4%). Cannabis did not improve OCD symptoms in 18.2% of these respondents. Additionally, cannabis was reported to exacerbate OCD symptoms, including difficulties falling asleep (54.5%), easily losing their train of thought (54.5%), and elevated their general anxiety (45.5%). There was no worsening of OCD symptoms in 36.4% of the sample. Of all respondents who reported using cannabis for their OCD symptoms, one individual (14.3%) reported using cannabis in place of a prescribed OCD treatment on weekends only.

Conclusions: Although more individuals who used cannabis to treat their OCD perceived cannabis to be helpful, a substantial proportion of individuals found cannabis to exacerbate their OCD symptoms. Rates of substituting prescribed OCD treatment with cannabis were very low. This preliminary data supports the need for well-controlled clinical trials of cannabinoids for the treatment of OCD.

Keywords: Obsessive Compulsive Disorder, Cannabis Use, Alternative Medicine
Disclosure: Allergan, Advisory Board, Almatica, Advisory Board, Lundbeck, Advisory Board, Otsuka, Advisory Board, Purdue Pharma, Advisory Board, Brainsway, Advisory Board, Hamilton Academic Health Sciences Organization Innovation Grant, Grant, Purdue Pharma, Allergan, Consultant, Lundbeck, Pfizer, Purdue Pharma

T189
The Transcriptome Landscape Associated With Disrupted-In-Schizophrenia-1 Locus Impairment in Early Development and Adulthood

Abstract not included.
T190

Modulation of Brain Function and Behavior by Circulating Extracellular Vesicles

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Background: Extracellular vesicles (EVs) circulate throughout the body and deliver biomolecules such as RNAs and proteins from one cell to another. EV-associated molecules such as microRNAs have been implicated as useful biomarkers for various disorders, including brain disorders. In addition, multiple studies showed that EVs could modify the phenotypes of cells in an organ distant from their origin. In this study, we have addressed if circulating EVs influence brain function and behavior.

Methods: Mice deficient for adaptive immune cells (immuno-deficient mice, e.g., Rag1 or Rag 2 knockout mice) were used as a model to study the effects of EVs from specific cell types (adaptive immune cells) on brain cellular phenotypes and behaviors. Circulating EVs in the peripheral blood were analyzed with electron microscopy, nanoparticle tracking assay, and biochemical methods. Mouse behavioral phenotypes were also examined. Changes in brain cellular phenotypes were assessed by immunohistochemistry and qRT-PCR experiments.

Results: We found that serum EVs contained substantial amount of EVs from adaptive immune cells (T and B cells). As expected, immunodeficient mice lacked these EVs, which were recovered by the transfer of wild-type (WT) mouse T and B cells. Immunodeficient mice also displayed social behavioral deficits, accompanied by enhance c-Fos immunoreactivity in the excitatory neurons in the medial prefrontal cortex (mPFC). Notably, an injection of serum EVs from WT mice rescued the behavioral deficits in immunodeficient mice. Further analysis on the target(s), key molecules, and distribution of adaptive immune cell-derived EVs is in progress.

Conclusions: Our data suggest that circulating EVs may modify mouse behaviors by influencing brain function. This study provides a novel biological insight into the mechanisms underlying peripheral-to-brain communications and may have a broad impact on psychiatric disorders and other brain diseases.

Keywords: Extracellular Vesicles, Immune Cells, Social Behavior, Mice

Disclosure: Nothing to disclose.

T191

Identification of Disease- and Treatment-Associated Metabonomic Biomarkers Within the Cerebrospinal Fluid and Serum of First-Episode Psychosis Patients

Abstract not included.

T192

Meta-Analysis of Janssen Disease and Omics Database for Bipolar and Schizophrenia Disease Signatures

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Background: Transcriptomics technologies such as high-throughput next-generation sequencing and microarray platforms, provide exciting opportunities for improving diagnosis and treatment of complex diseases by facilitating the development of pharmacotherapies. Despite their great promise, individual studies may generate results that are not reproducible due to a variety of reasons including, small sample size, confounding factors, differing platforms and differing bioinformatics pipelines. A clear understanding and unified picture of many complex diseases remains elusive, emphasizing an urgent need to effectively integrate multiple transcriptomic studies for discovering and validating disease signatures. In this study, we utilize multiple meta-analysis algorithms to combine a total of 30 genome-wide gene expression studies to provide an illustrative example of the effectiveness of meta-analysis and to elucidate transcriptomic signatures of bipolar disorder and schizophrenia.

Methods: Since 2012, we have been compiling high-quality transcriptomics studies in normal and disease conditions from both public and internal sources. By applying consistent preprocessing, QC, normalization and statistical inference procedures to these studies, and integrating them into a collective database, we created the Janssen BodyMap database. In the presented study, a total of 30 RNA Sequencing or Microarray-based datasets on bipolar disorders and 25 studies on schizophrenia were included. Using r-th ordered p-value (rOP) and random effect model (REM) meta-analysis algorithms we generated a list of differentially expressed (DE) genes. We then conducted pathway enrichment, genetic association and literature analysis to prioritize genes and select for gene targets.

Results: DE gene list based on our meta-analysis were created for each disease. In bipolar disorder, 328 genes were identified (FDR < 0.05) in any brain regions and 204 in the prefrontal cortex. In schizophrenia, 340 genes were identified in any brain region and 219 in the prefrontal cortex. Genes between the two diseases are significantly overlapped in either any brain regions or in the prefrontal cortex. Among shared gene families were potassium channels, integrins, and metallothioneins. Pathway enrichment analysis of shared genes revealed a role for GPCR signaling, glutamate metabolism, immune system, apoptosis, and epileptic seizure. Further genetic association and literature analysis identified the Reelin pathway (RELN/ApoER2/DAB1) as a potential target.

Conclusions: We show that our workflow can be applied in multiple disease areas to create a unified picture of the disease signature providing potentially new drug targets and pathways. Using this workflow we have collected comprehensive transcriptomics datasets across Janssen’s therapeutic areas, established systematic meta-analysis pipelines for disease signature and target identification in selected diseases and created a more comprehensive picture of bipolar disorder and schizophrenia.

Keywords: Schizophrenia, Bipolar Disorder, Genomics, Postmortem Human Brain, Data Mining

Disclosure: JNJ, Stock/Equity

T193

Influence of Sex and Antipsychotic Drug Treatment Effects on mRNA Differences Between Patients With Schizophrenia and Controls

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Background: Some of the biochemical abnormalities underlying schizophrenia, studied in both brain and peripheral blood, involve differences in methylation and methylating enzymes, as well as other genes uncovered by chip seq or RNA seq analysis. Of enzymes and some of the target genes. However, few of these studies have examined the effect of sex and drug treatment on the differences between chronic schizophrenics and controls. We present results of a larger study measuring differences in mRNA in lymphocytes of chronic schizophrenics (CSZ) and non-psychotic controls (NPC) emphasizing the differential effect of sex and antipsychotic drug treatment on the different biochemical findings.

Methods: We studied mRNA in lymphocytes of 61 CSZ and 49 NPC subjects using qPCR assays with TaqMan probes for multiple genes to assess mRNA levels for DNMT, TET, GABAergic genes, Glucocorticoid receptor, BDNF, and several genes with high hits from RNA sequence analysis. Statistical analysis tested the effects of diagnostic status (CSZ vs. NPC) on these mRNA levels and investigated the effects of sex differences and differences in antipsychotic drug treatment on the mRNA levels in CSZ and NPC.

Results: DNMT1 and DNMT3A mRNAs were significantly (P < 0.01) higher only in male CSZ subjects, with the small sample of females showing no statistical difference between CSZ and NPC, but a trend in the opposite direction from the male CSZ vs NPC comparison. Several other mRNA’s differences between CSZ and NPC showed a trend for a greater diagnostic difference in males than in females. The GAD1, glucocorticoid receptor (NR3C1) and CNTNPA2 mRNAs were significantly (P < 0.01) higher in CSZ than NPC. The FPRF3 mRNA was significantly (P < 0.03) lower in CSZ vs NPC, and the GAD67 mRNA showed a trend in the same direction for males (P < 0.10). In CSZ currently treated with clozapine, GABAergic mRNAs (GAD1, GAD67, GAD25) mRNA were significantly higher than in patients not treated with clozapine. CSZ treated with clozapine had significantly lower TET1 mRNA. There was a trend (P < 0.10) for NR3C1-B mRNA to be higher in clozapine patients. When we did analysis of differences between CSZ and NPC subjects, incorporating the clozapine treatment variable, it was clear that for the GABAergic mRNAs (GAD1, GAD67, GAD25), and for NR3C1-B, CNTNPA2, and IMPA2 mRNAs, that clozapine treated CSZ were primarily responsible for the difference from NPC, whereas non-clozapine treated CSZ had mRNA values more similar to NPC controls.

Conclusions: It is important to consider sex and antipsychotic drug treatment in comparing mRNA levels in in schizophrenic patients to controls, since some of the differences are only present in male subjects and other may be explained by clozapine treatment rather than primarily diagnosis. Many previous studies of similar differences in post-mortem brain samples, and some studies of methylation differences using peripheral blood cells, have not examined sex and drug treatment effects, and these could be possible confounds in interpreting diagnostic differences in these biochemical effects.

Keywords: mRNA, Neuropsychiatric Disorders [Schizophrenia, Parkinson's Disease, Major Depressive Disorder], GAD, DNMT, Sex Differences

Disclosure: Nothing to disclose.

T194

Treatment-Resistant Schizophrenia (TRS): Subtypes and Trajectories of Response to Clozapine

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Background: While most patients with schizophrenia are antipsychotic-responsive, as many as 30% of patients are diagnosed with treatment-resistant schizophrenia (TRS). Different criteria exist for defining TRS and while they vary somewhat, there is consensus that the definition includes failure to respond to two trials of non-clozapine antipsychotics. However, recent studies have suggested two distinct sub-types within TRS: (i) those who show no initial response to non-clozapine antipsychotic (AP) treatment (TRS from the outset), and (ii) a second group who develop resistance following relapse after past remission with non-clozapine compounds (Developed TRS). We employed a real-world, naturalistic design to investigate response to clozapine in these two TRS subtypes and assessed whether clozapine response trajectories differed between the two.

Methods: All patients met criteria for first-episode schizophrenia or schizoaffective disorder, were antipsychotic-naïve and were followed-up by a community-based first episode psychosis team. Data were collected as part of routine clinical care and analyzed retrospectively. A treatment algorithm standardizing pharmacologic management offered trials with two second-generation antipsychotics (SGA) before clozapine (Agid et al. J Clin Psy, 2011). The patient would advance to the next SGA trial when unable to meet remission criteria after 12 weeks; remission was defined as BPRS core psychotic symptoms score /= 3 (mild) on each of the four BPRS core-psychotic items. Clozapine was also offered to patients who experienced relapse and diminished overall response after having previously responded to non-clozapine SGA's. Medication adherence was assessed through patient and caregiver feedback, pharmacy reports, random pill counts and urine screens. Clinical rating scales, incorporated as part of routine clinical practice, included the Brief Psychiatric Rating Scale (BPRS) and Clinical Global Impressions (CGI).

Results: A total of 144 patients did not meet criteria for response to two first-line SGA trials and were offered a trial of clozapine of whom 120 (82.4%) agreed (77.5% male, mean age, 24.8 ± 5.9 years). Ninety percent (108/120) of patients completed a clozapine trial. 78 (65%) patients presented with TRS from the outset of their illness, while 42 (35%) developed TRS following remission with non-clozapine antipsychotics (SGA) before clozapine (Agid et al. J Clin Psy, 2011). The present findings align with two other reports (Lally et al. Psycho Med, 2016; Demjaha et al. Psychol Med, 2017) indicating that at least two types of TRS exist based on time of onset of treatment resistance. Individuals who manifest TRS from illness onset make up the majority of TRS patients. Data from the present study also suggest that there are differences in clozapine response time to clozapine treatment, as defined by 20% change from baseline BPRS total scores, revealed significant differences between the two TRS sub-types (X² = 6.131, P < 0.05). Those who developed TRS responded more rapidly (3.7 weeks ± SEM = 0.76 (95% CI. = 2.2 – 5.2)) than those who were TRS from the outset of their illness (6.1 weeks; SEM = 0.71 (95% CI. = 4.7 – 7.5)).

Analysis of response trajectory for each sub-type of TRS (outset, developed) using BPRS total scores, revealed significant time (p < 0.001, F = 120.11, df = 6) and time x illness group (p < 0.005, F = 4.03, df = 6) interaction effects, indicating greater symptomatic improvement in patients that develop TRS compared to those who present with TRS from the outset.

Conclusions: The present findings align with two other reports indicating that at least two types of TRS exist based on time of onset of treatment resistance. Individuals who manifest TRS from illness onset make up the majority of TRS patients. Data from the present study also suggest that there are differences in clozapine response time to clozapine treatment.
response based on these sub-types; those with TRS from the outset are less likely to respond to clozapine and respond more slowly than those who develop TRS over time.

These results underscore the role of relapse in contributing to the development of treatment resistance in a subset of TRS patients and the importance of initiating clozapine early in the course of illness for the majority of TRS patients who are treatment resistant from the outset. The lack of biomarkers or established endophenotypes does not permit these individuals to be identified as such at this point. These findings suggest that these two sub-types of TRS are mediated through different underlying mechanisms. Going forward, it will be critical to ensure these groups are distinguished as we seek to understand clozapine’s unique clinical profile, while at the same time working to develop alternative and more effective treatments for individuals with TRS.

Keywords: Treatment-Resistant Schizophrenia, Clozapine, Clinical Psychopharmacology, Psychopharmacology Treatment Guidelines

Disclosure: Minerva Neurosciences Inc., Honoraria, Lundbeck, Honoraria, Janssen, Otsuka, HLS Therapeutics

T195

Construct and Face Validity of Mismatch Negativity (MMN) as a Translational Biomarker in Mice

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Background: MMN is one of potential biomarkers associated with dysfunctions in auditory processing and cognitive function in schizophrenia patients. In contrast to the clinical evidence, preclinical research on MMN is far behind, especially in mice. Thus, understanding of the mechanism of MMN is essential for better translatability and higher success rate of drug development for psychiatric disorders. This study reports a newly established robust and well-reproducible MMN recording paradigm that allows us to test the effects of drug candidates.

Methods: C57BL/6 male mice were implanted with tripolar electrodes in the CA3 subregion of hippocampus. A week after the surgery, the mice were presented with auditory stimuli composed of standard and deviant stimuli, at 12 and 4 kHz, respectively. Frequency of deviant tones were 2% as sequential probability. All event related potentials were recorded after acclimation to the tones. Data was analyzed using EEGLAB. To characterize the MMN recording paradigm pharmacologically, we used 3 types of tool compounds, NMDA receptor antagonist, MK-801, Alpha 7 nicotinic acetylcholine receptor partial agonist, EVP-6124, and GABAB receptor agonist, baclofen, over a range of dosages similar to clinical studies.

Results: Mice displayed augmented negative peaks between 50 and 70 msec in response to the deviant stimuli. Similarly, the augmented negative peak was observed when the deviant and standard stimuli were flipped to 12 and 4 kHz, respectively. To mimic pathogenic features of schizophrenia, the mice were treated with an NMDAR antagonist, MK-801, and subjected to MMN recording. MK-801 significantly reduced MMN amplitude with delayed latency as expected in a dose-dependent manner. Next, we examined the effect of EVP-6124, since this compound has been studied recently in clinical trials for the treatment of cognitive impairment presented in schizophrenia patients. MK-801-induced disruption of MMN (N1 amplitude) was partially normalized by the treatment with EVP-6124; however, it was not effective to other EEG measures, such as increases in baseline gamma power. We also tested baclofen, which is well characterized for pharmacological effects on some of EEG measures in both preclinical study and clinical trials for Fragile X patients. As expected, baclofen was able to restore MK-801-induced reduction in baseline gamma. However, we did not detect the effect on the MK-801-induced MMN deficits.

Conclusions: In the present study, we established a mouse model of MMN and validated the recording paradigm with the NMDAR antagonist. We further demonstrated partial, but not full, reversal of the MMN deficits by EVP-6124. This result somewhat reflects the outcome of the clinical trial with schizophrenia patients in which therapeutic efficacy was not obtained. Thus, our preclinical MMN recording paradigm potentially presents predictivity in pharmacological efficacy of compounds in a clinical setting.

Keywords: Mismatch Negativity, EEG Biomarkers, Neuropsychiatric Disorders [Schizophrenia, Parkinson’s Disease, Major Depressive Disorder], Baclofen, Alpha7 Nicotinic Acetylcholine Receptor

Disclosure: Astellas Research Institute of America, Employee

T196

Sodium Nitroprusside Enhances the Antipsychotic-Like Effect of Lumateperone in the Conditioned Avoidance Response Test

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Background: Lumateperone is a novel first-in-class drug providing selective and simultaneous modulation of serotonin, dopamine and glutamate. The drug is currently being reviewed by the FDA for the treatment of schizophrenia. Lumateperone is an antagonist with high-affinity for the 5-HT2A receptor and it has a 60-fold higher affinity for these receptors compared to dopamine D2 receptors. As the dose is increased, it acts as a partial agonist at D2 receptors presynaptically and as a D2 antagonist postsynaptically. Lumateperone also exhibits potent serotonin reuptake inhibition and increases phosphorylation of glutamatergic N-methyl-D-aspartate (NMDA) GluN2B receptors in a mesolimbic-specific manner (Snyder et al., 2015).

A single injection of the antihypertensive nitric oxide donor sodium nitroprusside (SNP) has been found to induce a rapid (within 4 hours) and sustained (several weeks) antipsychotic effect in young treatment-resistant schizophrenic patients (Hallak et al., 2013). Moreover, we have recently shown that a low dose of SNP may significantly enhance the antipsychotic-like effect of a sub-effective dose of risperidone in rats in the conditioned avoidance response (CAR) test to a clinically relevant level (Titulaer et al., 2019).

Against this background, we have now studied the antipsychotic-like effect of lumateperone and the combination of lumateperone and SNP in rodents using the same behavioral assay.

Methods: We used young male rats in the CAR test to determine the antipsychotic-like efficacy, since this behavioral test has shown a very high predictive validity to identify drugs with clinical antipsychotic activity. The experiments were analyzed with Friedman’s analysis of variance (ANOVA) followed by Wilcoxon matched-pairs signed-ranks tests. All experiments were approved by the local animal ethics committee, Uppsala and Uppsala University, Sweden.
Results: Lumateperone caused a dose-dependent antipsychotic-like effect in the CAR test, where 10 mg/kg lumateperone significantly suppressed CAR by 77% to a clinically relevant level (≥70–80%), 3 mg/kg and 7 mg/kg significantly suppressed CAR as well, but in a sub-effective manner (6% and 54%). SNP alone had no effect on CAR suppression (0%). However, when SNP (1.5 mg/kg) was added to lumateperone (3 and 7 mg/kg) the CAR suppression was enhanced to 27% and 86% respectively.

Conclusions: The present study shows that lumateperone dose-dependently suppresses CAR and that SNP can indeed enhance the antipsychotic-like effect of a sub-effective dose of lumateperone to a clinically relevant level.

Whereas Hallak and colleagues found that additional SNP treatment significantly improved positive, negative and depressive symptoms of schizophrenia, a more recent clinical study could not replicate these findings, as SNP had no significant effect compared to placebo (Brown et al., 2019). However, significantly older patients were used, and the authors argue that SNP may be more effective in patients with recent-onset psychosis (Brown et al., 2019).

Lumateperone has already shown to be safe and well-tolerated with a safety profile similar to placebo for motor disturbances, prolactin changes, weight gain, cardiovascular and metabolic side effects in several phase 3 clinical trials.

Our current data propose that the combination of lumateperone and SNP may allow for a lower dosage of lumateperone and a potentially long lasting reduced risk of adverse side effects in young schizophrenic patients.

Acknowledgement: This work was supported by Intra Cellular Therapies Inc., New York, USA and BDD Berolina Drug Development, Berlin, Germany.

References:

Keywords: Lumateperone, Sodium Nitroprusside, Antipsychotics, Behavioral Pharmacology

Disclosure: Nothing to disclose.

T197
Longitudinal Investigation of the Relationship Between Omega-3 Essential Fatty Acids and Social Cognition in Recent-Onset Psychosis

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Background: Polyunsaturated fatty acids (PUFAs) account for approximately 35% of all cell membrane phospholipids and are critical to cellular neurotransmission. Evidence from rodent studies suggests that developmental n-3 insufficiency leads to enduring abnormalities in neurochemical systems associated with the pathophysiology of schizophrenia. Cross-sectional studies reported low n-3 PUFAs levels in erythrocytes and postmortem brain tissue of patients with schizophrenia. Abnormalities in n-3 PUFAs levels have also been identified in the early stages of schizophrenia, including individuals who are antipsychotic drug-naive and at ultra-high risk for psychosis. Despite this evidence, there is little known about the possible relationship between n-3 insufficiency and neurocognitive deficits in psychosis.

Methods: Forty-six (35M/11F) patients with recent-onset psychosis (mean age = 22.0, SD = 5.3) received neuropsychological (NP) assessments using the MATRICS Consensus Cognitive Battery (MCCB) at baseline and were then randomly assigned to receive either 16 weeks of treatment with either risperidone + fish oil (FO) or risperidone + placebo. Twenty-five patients received follow-up NP assessments at the end of the clinical trial (FO, n = 12/Placebo, n = 13).

All patients received open-label risperidone. Half were assigned randomly to receive FO and the other half to placebo in a double-blind manner. The FO and matching placebo (a soybean/corn blend) capsules contained 370 mg EPA and 200 mg DHA as well as 2 mg/g tocopherol. The total daily dose was 740 mg of EPA and 400 mg of DHA. The initial daily scheduling for risperidone was: 1 mg qhs days 1-3; 2 mg qhs on day 4 and 3 mg on day 7 and could be increased up to 6 mg, if needed. Risperidone dosage was increased until participants responded or until side effects precluded further increases.

Patient diagnoses were based on the Structured Clinical Interview for Axis I DSM-IV Disorders (SCID-I/P) supplemented by information from clinicians and, when available, family members. Patients met DSM-IV criteria for schizophrenia (n = 31), schizophreniform disorder (n = 9), psychotic disorder NOS (n = 1), schizoaffective disorder (n = 1), or bipolar disorder (n = 4). Ten patients were antipsychotic drug-naive at the time of consent and the remaining 36 patients had a median exposure of 4.5 days of antipsychotic treatment (range = 1 to 240 days). Duration of untreated psychotic symptoms was 93.7 weeks (range = 2 to 574).

Total Brief Psychiatric Rating Scale score (BPRS) was 41.6 (SD = 6.4). Whole venous blood was collected into EDTA-coated BD Vacutainer tubes and centrifuged for 20 minutes using methods described previously. Erythrocyte fatty acid composition (mg fatty acid/100 mg fatty acids) was computed for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA) at baseline and following treatment.

Results: Prior to treatment, there was a significant positive correlation between total n-3 PUFAs level (EPA + DHA + DPA) and the composite measure of overall NP functioning (r = 0.39, df = 39, p = 0.013); post-hoc tests revealed significant effects with social cognition (r = 0.49, df = 42, p = 0.0009), but not with any of the other NP domains (all ps > 0.05). Investigation of the individual n-3 PUFAs with social cognition revealed significant effects with both DHA (r = 0.47, df = 43, p = 0.002) and EPA (r = 0.44, df = 42, p = 0.004), but not with DPA (p > 0.05). Correlations between social cognition and DHA (r = 0.57, df = 31, p = 0.0006) and EPA (r = 0.35, df = 31, p = 0.044) remained statistically significant when partial correlation analysis was used to control for functioning on the other NP domains from the MCCB and total BPRS score.

Patients treated with FO + risperidone demonstrated a significant longitudinal increase in DHA (t = −4.48, df = 9, p = 0.002; 56.2% change), DPA (t = −6.69, df = 9, p = 0.0006; 72.6% change), and EPA (t = −4.91, df = 9, p = 0.001; 281.7% change). Patients treated with FO + risperidone demonstrated a significant 25.4% longitudinal increase in social cognition (t = −2.42, df = 10, p = 0.036) compared to patients treated with risperidone + placebo who demonstrated a 9.9% increase in social cognition (t
= −1.12, df = 11, p = 0.29). At the time of the follow-up NP exam patients treated with FO + risperidone scored significantly higher on the social cognition domain compared to patients treated with risperidone + placebo (t = −2.56, df = 23, p = 0.018). Furthermore, longitudinal increases in DHA correlated significantly with longitudinal increases in social cognition among patients treated with FO + risperidone (r = 0.79, df = 8, p = 0.021).

**Conclusions:** These findings provide the first evidence to our knowledge implicating a role for n-3 PUFAs in neuropsychological functioning in patients with recent-onset psychosis. Of the three n-3 PUFAs investigated greater EPA and DHA were associated with better social cognition in patients with recent-onset psychosis even after controlling for functioning on other neuropsychological domains and overall level of symptoms. Patients treated with FO + risperidone had a significant increase in social cognition in contrast to patients treated solely with risperidone. Group differences in social cognition at the follow-up timepoint were associated with a large effect size (Cohen’s d = 1.025; 95% CI = 0.191 to 1.86). Larger longitudinal studies will be required to replicate these findings.

**Keywords:** Social Cognition, Early Psychosis, Omega-3 Fatty Acids

**Disclosure:** Nothing to disclose.

**T198**

**Toxoplasma Gondii Effects on the Relationship of Kynurenine Pathway Metabolites to Cognition in Schizophrenia Versus Control Subjects**

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**Background:** Chronic infection with the common parasite, Toxoplasma gondii (TOXO) results in chronic microcysts in the brain that affect brain dopamine levels. In immune competent individuals these microcysts are kept in check, in part by compensatory changes in the kynurenine pathway thought to be set in motion by inflammatory activation. TOXO seropositivity is associated with a heightened risk of schizophrenia (SCZ; odds ratio 2.7) and with cognitive impairments both in humans and animals as per several prior analyses by our group and others. Impaired cognition, in turn, is seen in SCZ, and is associated with functional impairments in this population that have meaningful impact on the course of illness. Thus, understanding the contribution of TOXO infection to cognition is of clinical importance. The objective of this study was to tease apart the association of kynurenine pathway markers with cognition in the presence or absence of chronic TOXO infection in individuals with and without SCZ.

**Methods:** 43 subjects with SCZ and 29 healthy controls (CON) were recruited, male and female, from the Atlanta Veterans Affairs Medical Center. The following kynurenine pathway markers were measured using liquid chromatography triple quadrupole mass spectrometry: kynurenine (KYN), tryptophan (TRYP), 3-hydroxyanthranilic acid (3-OHAA), anthranilic acid (AA), and kynurenic acid (KYNA). TOXO status was determined by an ELISA that measured TOXO IgG, yielding both a dichotomous variable and a continuous serointensity value. The following tests were used to quantify psychomotor speed and cognition: Finger Tapping Task, Wisconsin Card Sorting Test (WCST), Reynolds Intellectual Screening Test (RIST), Weschler Memory Scale, Trail Making Test, Letter-Number Span, Hopkins Verbal Learning Test, Category Fluency, Symbol Coding Test, and the Reaction Time Task. Non-normal variables were natural log-transformed. T-tests and chi-square tests were performed to test for differences between diagnostic groups. Backward linear regression models were used to determine the relationship between kynurenine markers and cognition, first with all subjects analyzed together, and then with analyses stratified by TOXO status.

**Results:** As expected, SCZ subjects were worse than CON in cognitive performance. KYNA was higher in CON and significantly associated with better performance in the Trail Making Test (p = 0.02), WCST (p = 0.01), and the RIST (p = 0.01). Tryptophan was significantly higher in CON and corresponded to better scores on the Letter Number Span (p = 0.03), WCST (p = 0.01) and the Weschler Memory Scale (p = 0.01). When we stratified analyses by TOXO status (positive vs. negative), robust differences emerged. For instance, WCST scores were predicted by KYNA (p = 0.022), TRYP (p = 0.006), 3-OHAA (p = 0.037), AA (p = 0.02), and KYNA (p = 0.047) in TOXO positive subjects, but in TOXO negative subjects kynurenines did not predict WCST scores. A similar pattern was seen with the Symbol Coding test: KYNA (p = 0.04) and 3-OHAA (p = 0.005) predicted performance in TOXO positive but not TOXO negative subjects. AA (p = 0.05) predicted Weschler Memory Scale performance in TOXO positive subjects but not TOXO negative subjects. Finally, RIST performance was predicted by 3-OHAA (p = 0.028) and KYNA (p = 0.002) in TOXO positive subjects but not TOXO negative subjects. On the Trail Making Test: KYNA (p = 0.003), TRYP (p = 0.02), 3-OHAA (p = 0.013), and AA (p = 0.007) predicted performance in TOXO positive subjects, but only AA (p = 0.05) predicted performance in TOXO negative subjects. The Letter Number Span was predicted by KYNA (p = 0.001) and AA (p = 0.008) in TOXO positive subjects, but only by TRYP (p = 0.015) in the TOXO negative subjects.

**Conclusions:** These results indicate that kynurenine pathway markers are predictive of cognitive performance. Further, many kynurenines had strong associations with cognition in subjects who have chronic TOXO infections, yet very few kynurenines were associated with cognition in TOXO negative subjects. While TOXO seropositivity is found in a minority of patients with SCZ, it has been estimated that the population attributable fraction (the proportion of diagnoses of SCZ that would not occur in a population if TOXO infections were not present) is at least 21% (Smith, Preventative Veterinary Med, 2014; 117:425-435). The underlying neurobiology that leads to cognitive impairment in those who are TOXO positive seems to involve perturbations of the kynurenine pathway. Further work to understand how kynurenine pathway changes may result from TOXO infection and lead to cognitive impairment may provide a path toward novel personalized treatment for patients with SCZ who are TOXO positive.

**Keywords:** Schizophrenia, Cognition, Toxoplasma Gondii, Kynurenine Pathway

**Disclosure:** Nothing to disclose.

**T199**

**Distinct Polygenic Score Profiles in Schizophrenia Subgroups Exhibiting Different Trajectories of Cognitive Development**

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Background: Genetic, developmental and clinical heterogeneity in schizophrenia are challenges for investigations of biology and treatment. We sought to identify and characterize subgroups in a schizophrenia sample with different trajectories of early cognitive development, using adult cognitive data, and then to profile the subgroups across four polygenic score (PGS) dimensions.

Methods: At NIMH, demographic, clinical, cognitive, and academic/vocational data, and blood samples, were obtained from people with schizophrenia and controls (N = 2271). Estimates of premorbid and current IQ were inputs in cluster analyses to derive cognitive trajectory subgroups in the schizophrenia sample (N = 746) and subgroup characteristics were contrasted. For those with genotype information (total N = 1384; schizophrenia N = 540), separate PGS were derived for schizophrenia, cognition, educational attainment and ADHD. PGS profiles across the schizophrenia subgroups were tested using general linear models and logistic regression.

Results: Cluster analyses identified 3 cognitive trajectory subgroups in the schizophrenia sample. Subgrouping was based on IQ patterns but subgroups exhibited strikingly different cognitive, clinical and functional characteristics. There was evidence of pre-adolescent cognitive impairment, pre-dating psychotic symptoms, in 19% - as well as learning difficulties, low education and poor adult outcome. Forty-four percent appeared to experience an adolescent decline in cognitive development, likely coinciding with illness prodrome, which combined with high clinical symptoms and poor global functioning in adulthood. The last 37% showed a more cognitively stable developmental trajectory through adolescence, despite emerging psychosis, and had the best cognitive, clinical and functional outcomes in adulthood. In multinomial logistic regression, the four PGS predicted 7.9% of the variance in cognitive trajectory subgroup membership (chi-squared[8] = 43.83, p = 6.10E-07). Schizophrenia cases in the cognitively stable subgroup had somewhat elevated schizophrenia PGS (F[1,1012] = 45.0, p = 3.26E-11, ES = 0.042) but were otherwise genetically similar to controls. The adolescent decline subgroup had markedly elevated schizophrenia PGS relative to controls (F[1,1067] = 168.3, p = 7.66E-36, ES = .136) and unfavorable cognition PGS (F[1,1067] = 8.2, p = 0.004, ES = 0.008). The pre-adolescent impairment subgroup showed generalized, disadvantageous PGS relative to controls, with elevated schizophrenia (F[1,935] = 52.6, p = 8.62E-13, ES = 0.053) and ADHD PGS (F[1,935] = 16.6, p = 2.70E-05, ES = 0.017) and low cognitive PGS (F[1,935] = 18.6, p = 1.80E-05, ES = 0.019) and education PGS (F[1,935] = 17.8, p = 2.70E-05, ES = 0.019).

Conclusions: The study provided evidence that subgroups derived on the basis of IQ patterns represented different trajectories of cognitive development with different clinical, cognitive and functional characteristics. These findings were remarkably convergent with subgroup genetic profiles. Those who remained cognitively stable through adolescence only differed from controls on schizophrenia PGS. Those with an adolescent decline in cognition and more severe adult symptoms had the highest schizophrenia PGS and disadvantageous cognitive PGS. The subgroup whose members showed pre-adolescent impairment in cognitive and academic performance, and poor adult outcome, also had the most generalized, disadvantageous PGS relative to controls. Notably, this was the only subgroup that differed from controls on ADHD and education PGS. Simultaneous analysis of multiple PGS may contribute to clinical stratification in schizophrenia and other psychiatric disorders.

Keywords: Schizophrenia Subgroups, Cluster Analysis, Premorbid and Current IQ, Polygenic Scores

Disclosure: Nothing to disclose.
and female subjects across all pairs of clusters (a total of 136 pairs). We did this in the following manner. For each pair of clusters, we measured the ratio of the fiber cluster endpoint distances: (cortical distance - striatal distance)/(cortical distance + striatal distance). Then, for each subject, we put all ratio numbers (a total of 136 values) together and performed a PCA for dimension reduction. For each dimension value (1-136), we computed a p-value using a Hotelling T-Squared test between males and females. When the dimension value was higher or equal to 23, we found p < 0.05.

**Conclusions:** Using dMRI fiber cluster topography analysis in HSs, we show that the PFC wiring projection pattern between the PFC and the striatum deviates from a topographic, parallel, organization, due to a pattern of convergence in regionally specific anatomic clusters connecting the PFC and striatum. Of note, this was true for both left and right hemispheres. We have performed 2 further analyses at this point for the left hemisphere, alone. First, we identified the anatomic location in the PFC of these regionally specific fiber clusters with significant patterns of convergence, and found that these clusters originated in limbic (medial and lateral orbitofrontal cortex) PFC and ventral associative (pars orbitalis cortex) PFC. Second, we found a sex difference in the ratio of the fiber cluster endpoint distances such that if the dimension value, using PCA, was higher or equal to 23, there was a significant group difference. We plan to do the same, additional analyses in the right hemisphere, as well. We also plan to test for variation in the pattern of frontostriatal brain wiring in other neuropsychiatric conditions such as schizophrenia.

**Keywords:** Diffusion MRI, Brain Structural Connectivity, Frontostriatal Circuitry

**Disclosure:** Nothing to disclose.

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**T201**

**Mapping Changes in Expression of Neuroinflammatory Genes HIVEP2 and SERPINA3 in High Inflammation Biotypic Schizophrenia: Regional and Cellular Divergence Within the Dorsolateral Prefrontal Cortex**

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**Background:** Dysregulation of immune signalling in the brain and blood is evident in around 40% of people with schizophrenia. This high-inflammation biotype characterizes a subpopulation of patients who also suffer from more severe cognitive deficits and neuropathology. Although many questions remain about what triggers neuroinflammation, molecular factors upstream of cytokine expression are known to be altered in schizophrenia, suggesting that changes in the control of gene expression is involved. The transcription factor nuclear factor kappa B (NFkB) – a critical initiator of the inflammatory cascade – is increased in schizophrenia; NFkB both ‘switches on’ and ‘shuts off’ the expression of various inflammatory proteins that are abnormally expressed in schizophrenia. The NFkB activation pathway is normally suppressed via inhibitors that interfere with the ability of NFkB protein dimers to translocate to the nucleus or bind their target DNA. The expression of one such neuronal inhibitor, Human Immunodeficiency Virus Enhancing Protein 2 (HIVEP2), is downregulated in people with schizophrenia in the prefrontal cortex. Mice lacking HIVEP2 (also named Schnurri-2) display schizophrenia-like behavioural, cognitive and neuropathological phenotypes, suggesting a critical role of HIVEP2 in the pathophysiology of schizophrenia. Increased protease inhibitor SERPINA3 is a robust marker of high-inflammation biotype schizophrenia and is also the transcript that is most abnormally expressed in the prefrontal cortex of both Schnurri-2 knockout mice and human schizophrenia patients. The first aim of this study is to map the expression of HIVEP2 and SERPINA3 within the prefrontal cortex of patients in order to identify the cell populations with dysregulated immune signalling. The second aim of this study is to determine whether changes in HIVEP2 expression are specific to patients with the high-inflammation biotype.

**Methods:** Post-mortem dorsolateral prefrontal cortex samples were obtained from the NSW Tissue Resource Centre and included tissue blocks from 37 healthy controls and 37 males and females with schizophrenia predetermined as having a low (N = 23) or high inflammation biotype (N = 14) (Fillman et al., 2013). Tissue was cut into 14 μm cryostat sections mounted on gelatin-subbed slides. To map HIVEP2 and SERPINA3 mRNA within this region, in situ hybridisation was performed using radio-labeled antisense and sense (control) riboprobes for each gene, transcribed from linearised cDNA templates (s.a. = 2.3 x 109 cpm). Slides were exposed to high resolution emulsion-sidated autoradiographic film and then dipped in NTB emulsion, exposed, developed and Nissl stained. The density of mRNA in μCi/g was calculated using optical density data from 14C standards and converted to nCi/g. Silver grain microscopy was used to identify cell types expressing HIVEP2 and SERPINA3 mRNA based on nuclear morphology, and this was confirmed at the protein level with fluorescent triple-label immunohistochemistry (rabbit anti-SERPINA3 1:100, SIGMA Prestige Antibodies HPA002560; mouse anti-NeuN 1:1000, Chemicon International MAB377; goat anti-GFAP 1:1000, Abcam ab53554).

**Results:** We found that SERPINA3 hybridization was more intense in the white matter as compared to grey matter in all cases. Furthermore, there was uneven hybridization with the appearance of ‘hot spots’ of intense hybridization in some cases and a distinct pattern of intense staining following the typical pattern of blood vessels in some regions. We confirmed increased SERPINA3 mRNA expression in high-inflammation biotype schizophrenia patients compared to controls in both the grey matter (overall ANOVA F(1,60) = 5.62, p < 0.001, d = −0.89) and white matter (overall ANOVA F(2,59) = 11.80, d = −1.34, p = 0.007) of the dorsolateral prefrontal cortex. There was no effect of sex on SERPINA3 expression in white matter (F(1,56) = 2.46, p = 0.12) or grey matter (F(1,58) = 0.32, p = 0.57). At the cellular level, SERPINA3 expression was found predominately in astrocytes and was strongest in blood vessel-associated astrocytes. The pattern of protein expression matched mRNA expression; SERPINA3 co-localised with GFAP in astrocytic processes and soma around select blood vessels. In contrast, HIVEP2 mRNA was expressed in a laminar pattern typical of a neuronal expressed mRNA, with highest expression in Layers 4-6, and was decreased in high-inflammation biotype schizophrenia in Layers 2-6 compared to controls (overall ANOVA F(2,63) = 2.84, all p’s < 0.03, d = 0.67). There was no effect of sex on HIVEP2 mRNA expression in the dorsolateral prefrontal cortex (overall ANOVA F(1,60) = 0.143, p = 0.71).

**Conclusions:** Our results indicate that transcriptional controllers upstream of cytokine expression are mainly dysregulated in neurons, while inflammation is most apparent in astrocytes. This raises questions of how reduced HIVEP2 (and NFkB dysregulation more broadly) in cortical neurons could lead to astrogliosis and if neurons may be primary producers of NFkB-stimulated cytokine expression leading to a reaction in astrocytes.

**Keywords:** Astrocyte, Schizophrenia, Inflammation

**Disclosure:** Nothing to disclose.

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Effects of Concomitant Medication Use on Tardive Dyskinesia Outcomes in a Long-Term Rollover Study of Valbenazine

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Background: Tardive dyskinesia (TD) is a persistent and potentially disabling movement disorder associated with prolonged exposure to antipsychotics or other dopamine receptor blocking agents. Patients with TD often have multiple comorbidities that require concomitant medications, such as antipsychotics, antidepressants, anxiolytics, and anticholinergics. Valbenazine is a highly selective vesicular monoamine transporter 2 (VMAT2) inhibitor approved for the treatment of TD in adults. Data from a long-term, rollover study (NCT02736955) were analyzed to evaluate the effects of concomitant medications on treatment outcomes.

Methods: Completers from 2 prior long-term studies (KINECT 3 [NCT02274558], KINECT 4 [NCT02405091]) were re-initiated (open-label) at valbenazine 40 mg following washout of prior valbenazine. The dose was escalated after 4 weeks to 80 mg based on tolerability and clinical assessment of TD. Reduction to 40 mg was allowed if 80 mg was not tolerated. The rollover study was designed to include 72 weeks of treatment or until valbenazine became commercially available. 4 participants reached the Week 60 visit, and none reached Week 72 when the study was terminated due to valbenazine becoming commercially available. Stable doses of concomitant medications to treat psychiatric disorders and comorbid medical conditions were allowed throughout the study. Mean changes from baseline in Clinical Global Impression of Severity-TD (CGIS-TD) and the percent of participants with a CGI-TD score of 1 (in Clinical Global Impression of Severity-TD (CGIS-TD) and the percent of participants with a CGI-TD score of 1 (‘normal, not at all ill’) or 2 (‘borderline ill’) were analyzed at Week 48 in the overall study population and in subgroups taking the following concomitant medications: antipsychotics, antidepressants, anxiolytics, anticholinergics, antipsychotics plus antidepressants (AP+AD), antipsychotics plus anxiolytics (AP+ANX), antidepressants plus anxiolytics (AD+ANX), and antipsychotics plus anticholinergics (AP+AC). Subgroup categories were not mutually exclusive and only reflected concomitant medication use in the rollover study; concomitant medication use in the prior studies were not considered, nor was the mean daily antipsyhotic dose of the subgroups.

Results: A total of 161 participants were enrolled; 138 were receiving treatment when the study was terminated due to commercial availability of valbenazine. At Week 48 in the overall population (n = 56), the mean change from baseline in CGIS-TD score was −1.8. Similar changes were found in the concomitant medication subgroups: antipsychotics, −1.7 (n = 43); antidepressants, −1.9 (n = 39); anxiolytics, −1.5 (n = 20); anticholinergics, −1.6 (n = 17); AP+AD, −1.6 (n = 24); AP+ANX, −1.4 (n = 15); AD+ANX, −1.5 (n = 17); AP+AC, −1.6 (n = 17). Analyses at Week 48 also showed that 64.3% of participants in the overall study population had a CGI-TD score ≤2, with similar results in the concomitant medication subgroups: antipsychotics, 60.5%; antidepressants, 61.5%; anxiolytics, 60.0%; anticholinergics, 64.7%; AP+AD, 54.2%; AP+ANX, 53.3%; AD+ANX, 58.8%; AP+AC, 64.7%.

Conclusions: Substantial global improvements in TD were found with once-daily valbenazine in participants regardless of concomitant medication use at baseline. More research is needed to better understand how changes in concomitant medication use (e.g., stopping current medications, starting new medications, changing doses) might affect outcomes in patients with TD who are being treated with valbenazine.

Keywords: Tardive Dyskinesia, Valbenazine, VMAT2, Long-Term Results

Disclosure: Neurocrine Biosciences, Inc., Employee

Early Visual Processing is Associated With Social Cognitive Performance in Recent-Onset Schizophrenia

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Background: Moderately large deficits in early-stage visual processing have been reported in chronic schizophrenia. Consistent with a cascade model of information processing, whereby early perceptual processes have downstream effects on higher-order cognition and functioning, impaired visual processing is associated with deficits in social cognition in this clinical population. However, it is unclear whether early-stage visual processing is impaired early in the course of schizophrenia. Likewise, the nature of the relationship between visual processing and social cognition in the early phase of illness is unknown. Here, we present data from a study of early visual processing and social cognitive performance in recent-onset schizophrenia (i.e., within 24 months of illness onset; ROSz).

Methods: Thirty-two people with ROSz and 20 healthy comparison (HC) subjects participated in this study. All subjects completed a visual backward masking task using stimuli of real world objects (Object Masking) to assess early-stage (i.e., 0–100 ms post-stimulus onset) visual processing. Subjects also completed two tasks of social cognition, one assessing relatively low-level and automatic processes of emotion identification (Emotion Biological Motion, or EmoBio), and another assessing more complex, higher-order theory of mind abilities (The Awareness of Social Inference Test, or TASIT). The EmoBio task is comprised of 24 brief point-light-walker videos, and was used to assess emotion identification based only on limited cues from body motion (e.g., gait, limb movement, speed of movement). The TASIT consisted of 16 audio-video vignettes of adults interacting with each other, and was used to assess the ability to detect lies and sarcasm based on integration of multiple sources of social information including facial affect, vocal prosody, body motion, mental state attributions, social knowledge, and use of contextual cues. Group differences on the tasks were tested with repeated measures ANOVA and independent samples t-tests. Associations between early-stage visual processing and social cognitive performance in ROSz and HC were tested using linear regression models. The social cognition task score was regressed on three sequential predictors: 1) group, 2) Object Masking score, and 3) the Object Masking x group interaction. A significant Object Masking x group interaction indicated significant regression line slope differences between the groups. Non-significant Object Masking x group interactions were dropped from the model, and the common slope was tested for significance. Follow-up bivariate correlations quantified the strength of significant associations identified in the regression analyses.

Results: For the Object Masking task, a typical backward masking response profile was evident in ROSz and HC across the stimulus onset asynchrony (SOA) conditions [main effect of SOA, F(3.01, 350) = 136.68, p < 0.001, partial η² = 0.73], with poorer performance at early vs. later SOAs. However, the masking effect was exaggerated in ROSz [SOA x group, F(3.01, 350) = 3.04, p = 0.03, partial η² = 0.06]. ROSz were less accurate on the emotion recognition task.
identification task [EmoBio t (50) = −3.36, p = 0.001, Cohen’s d = −0.95]. For the TASIT, although ROSz exhibited overall poorer performance compared to HC [main effect of group, F (1, 50) = 38.37, p < 0.001, partial η² = 0.43], ROSz were disproportionately impaired on items assessing sarcasm detection [group x task, F (1, 50) = 4.30, p = 0.04, partial η² = 0.08]. There was a significant effect for the common slope for EmoBio performance [p = 0.01] and TASIT total score [p = 0.02], indicating a significant relationship between visual processing and performance on the social cognition tasks. None of the interaction effects were significant [p’s > 0.20], indicating similar slopes in ROSz and HC. Across all subjects, better early visual processing (Object Masking) was associated with better performance on the EmoBio [r = 0.48, p < 0.001] and the TASIT [r = 0.49, p < 0.001].

Conclusions: Early-stage visual processing, low-level social cognition, and higher-order social cognition were all significantly impaired in this sample of young adults with recent-onset schizophrenia. Across all subjects, early-stage visual processing was significantly associated with performance on the social cognitive tasks, consistent with a cascade model of information processing. These data suggest that early visual processing deficits may be an appropriate target for intervention in recent-onset schizophrenia, as improvements may yield downstream effects on social cognitive processes.

Keywords: First Episode Psychosis, Visual Processing, Social Cognition, Emotion Perception, Theory of Mind

Disclosure: WCG-MedAvante-Prophase, Employee

T204

Emotion Processing and Cognition in Comorbid Psychosis Spectrum and Cocaine Use Disorders

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Background: Psychosis spectrum disorders (PSD) are common and severe disorders including schizophrenia (SZ), schizoaffective (SAD) and bipolar disorder (BD). There is high comorbidity between PSD and cocaine use disorder (CUD). The impact on cognition, emotion processing and functioning has been studied in patients with PSD and patients with PSD-CUD separately. Patients with CUD alone or PSD alone show cognitive and emotion processing impairments compared to controls. However, little is known about the impact of comorbid PSD + CUD on cognition and emotion processing. We aimed to address this gap in knowledge by characterizing the effect of comorbid PSD + CUD on cognition and emotion processing, testing 2 alternate hypotheses: 1) Per an additive model, those with comorbid PSD + CUD would show the largest impairments in cognition and emotion processing, compared to those with PSD or CUD alone; 2) Per a self-medication model, those with comorbid PSD + CUD would outperform those with PSD alone.

Methods: The sample (n = 529) included 86 patients with comorbid PSD + CUD, and 3 control groups: 107 healthy controls, 70 CUD patients, and 266 PSD patients without CUD (PSD-CUD). Diagnoses in the PSD group included 210 BD 1; 49 BD 2; 51 SZ, 40 SAD, 2 Other. Cognitive measures: Wide Range Achievement Test-3rd Ed Reading (WRAT3) subtest, an estimate of verbal premorbid IQ; Matrix Reasoning subscale of the brief Wechsler Adult Scale of Intelligence (WASI), an estimate of current IQ. Emotion processing measures (Cambridge Neuropsychological Test Automated Battery, CANTAB): Emotion Recognition Test (ERT), yielding outcomes of accuracy and reaction times across 6 conditions: happiness, sadness, anger, disgust, fear, surprise; affective Go/Nogo (AGNG). AGNG outcomes include reaction times and computed signal detection measures d’ (a measure of perceptual sensitivity to different stimulus conditions) and criterion (β, a measure of response biases, i.e., the minimum level of internal certainty needed to decide that a particular stimulus was present). We used factor analysis of ERT and AGNG outcome variables for dimension reduction, and parallel analysis (Horn, 1965) to select the optimal number of factors. Univariate ANOVAs with Tukey’s test for multiple comparisons, and Chi-square were used to compare sociodemographic variables across groups. Significant variables were included as covariates in multivariate analyses. We used MANCOVA covarying for age, sex, race, medication (antipsychotic, mood stabilizer, antidepressant, anxiolytic), lifetime psychosis (yes/no) and symptom severity (BPRS, SANS) to compare cognition outcomes and the emotion processing factors across groups.

Results: The factor analysis of emotion processing variables identified 3 factors, which explained 0.41 of the variance: Factor 1 (Emotion Recognition Accuracy, highest loadings from ERT accuracy for negative emotions); Factor 2 (AGNG response timing/emotion sensitivity, highest loadings from AGNG reaction times and d’); Factor 3 (AGNG response bias, highest loadings from AGNG βs and reaction times).

Controls were younger than all patient groups (p < 0.05); Those with CUD were older than controls (p < 0.01), and those with PSD + CUD were older than those with PSD-CUD (p = 0.007). Those with CUD were more likely to be male (Chi square = 23.02; df = 3; p < 0.001) and African American (Chi square = 53.65; df = 6; p < 0.001). Therefore, age, sex and race were included as covariates in the models.

MANCOVA showed that: The CUD group had lower WRAT scores compared to controls (p = 0.005); The PSD group had lower WASI scores compared to controls (trend level, p = 0.058); Those with CUD scored lower than controls in emotion processing Factor 1 (emotion recognition accuracy to negative emotions, p = 0.001). Interestingly, PSD + CUD patients showed greater emotion recognition accuracy to negative emotions (Factor 1) compared to PSD patients without CUD (p = 0.02). PSD patients showed significant differences in emotion response biases (Factor 3) compared to controls (p = 0.03).

Conclusions: Our findings suggest that CUD is associated with lower premorbid IQ, and PSD with lower current IQ. Consistent with previous findings, PSD patients showed abnormal emotion response biases compared to controls. Additionally, while those with CUD alone showed emotion recognition deficits for negative emotions compared to controls, PSD patients with comorbid CUD had better emotion recognition than PSD patients without CUD. This finding appears to support the self-medication hypothesis in that cocaine’s stimulating effects may contribute to better emotion recognition in the PSD + CUD group. Therefore, comorbid CUD in PSD patients may revert emotion processing deficits found in PSD alone. These results should be interpreted with caution, due to the following limitations: 1) Not all patients with CUD were currently using cocaine, as a subset were abstaining or in remission; 2) Cocaine use was not confirmed with urine toxicology in all subjects; 3) The cross-sectional design does not allow to examine whether the less impaired emotion recognition skills in the PSD + CUD group pre-dated the cocaine use or were ameliorated by it. Another explanation could be a selection bias, in that all comorbid PSD + CUD participants have been able to successfully navigate their environment and obtain illicit substances, which may require better skills for identifying negative emotions. Therefore, those with PSD + CUD may have better emotion recognition skills compared to those with PSD alone.
Keywords: Cocaine Use Disorder, Schizophrenia, Bipolar Disorder, Emotion Processing, Cognition

Disclosure: Takeda, Grant, Merck, Grant, Al Cure, Neurocrine

T205

Maintenance of Response After 4-Week Washout in Patients With Tardive Dyskinesia who Received Long-Term Treatment With Once-Daily Valbenazine

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Background: Tardive dyskinesia (TD) is a persistent and potentially disabling movement disorder associated with prolonged exposure to antipsychotics or other dopamine-receptor blocking agents. Valbenazine is a highly selective vesicular monoamine transporter 2 (VMAT2) inhibitor approved for the treatment of TD in adults. Steady-state plasma concentration is reached within 1 week after treatment initiation; the half-life of valbenazine and its primary active metabolite is 15 to 22 hours. The long-term effects of once-daily valbenazine (40 and 80 mg) on TD have been evaluated in two Phase 3 studies in which participants received up to 48 weeks of active treatment: KINET 3 (NCT02274558), which included a 6-week double-blind placebo-controlled (DBPC) phase, followed by a 42-week blinded extension and 4-week washout; and KINET 4 (NCT02405091), which included 48 weeks of open-label treatment followed by a 4-week washout. In both studies, some participants maintained a treatment response at Week 52, 4 weeks after valbenazine was discontinued. Data from Week 48 (end of treatment) and Week 52 (end of washout) of KINET 3 and KINET 4 were pooled to assess the percentage of participants who maintained various levels of response after washout.

Methods: Data from KINET 3 and KINET 4 were pooled by dose group (40 mg, 80 mg); participants who received placebo in the DPBC phase of KINET 3 were excluded from analyses. Week 48 responders were defined as participants who met any of the following criteria after 48 weeks of once-daily valbenazine: Abnormal Involuntary Movement Scale (AIMS) total score, ≥50% improvement from baseline; AIMS total score, ≥30% improvement from baseline; Clinical Global Impression of Change (CGI TD) score ≤2 ("much improved" or better); CGI-TD score ≤3 ("minimally improved" or better); Patient Global Impression of Change (PGIC) score ≥2 ("much improved" or better); or PGIC score ≤3 ("minimally improved" or better). Six groups of Week 48/52 responders were defined as follows: 1) AIMS ≥50% improvement at Week 48 and any threshold of improvement (≥50%, ≥40%, ≥30%, ≥20%, ≥10%) after washout at Week 52; 2) AIMS ≥30% improvement at Week 48 and any improvement (≥30%, ≥20%, ≥10%) at Week 52; 3) CGI-TD score ≤2 at Week 48 and any improvement (score ≤2 or ≤3 at Week 52); 4) CGI-TD score ≤3 at Weeks 48 and 52; 5) PGIC score ≤2 at Week 48 and any improvement (score ≤2 or ≤3 at Week 52); and 6) PGIC score ≤3 at Weeks 48 and 52. Analyses were conducted only in participants with available assessments at Weeks 48 and 52.

Results: At Week 48, 76.4% (136/178) of participants had a clinically meaningful AIMS response (i.e., ≥30% total score improvement from baseline). In addition, >90% had a score ≤3 ("minimally improved" or better) on the CGI-TD (96.1% [171/178]) or PGIC (93.8% [165/176]). Over 50% of these responders maintained the same level of improvement at Week 52: AIMS ≥30% (50.7% [69/136]); CGI TD ≤3 (64.3% [110/171]); PGIC ≤3 (77.0% [127/165]). Analyses based on more rigorous thresholds of improvement showed that at Week 48, 66.3% (118/178) of participants had a robust AIMS response (i.e., ≥50% total score improvement from baseline) and >75% had a score ≤2 ("much improved" or better) on the CGI-TD (79.8% [142/178]) or PGIC (80.7% [142/176]). Among these responders, the percentage who maintained the same level of improvement at Week 52 was as follows: AIMS ≥50%, 35.6% (42/118); CGI TD ≤2, 39.4% (56/142); PGIC ≤2 (62.7% [89/142]). Among the same participants, the percentage who continued to have a less rigorous (but still clinically meaningful) AIMS response or a minimal global response was as follows: AIMS ≥30% (51.7% [61/118]); CGI-TD ≤3 (64.8% [92/142]); PGIC ≤3 (76.8% [109/142]).

Conclusions: More than 75% of all participants had a clinically meaningful AIMS response (≥30% improvement) or "much improved" global rating (CGI-TD score ≤2, PGIC score ≤2) after 48 weeks of once daily treatment with valbenazine (40 or 80 mg). More than 50% of these Week 48 responders continued to maintain some level of improvement after a 4-week valbenazine washout period. More research, including blinded trials with longer withdrawal periods, is needed to understand whether and why some patients may experience continued TD response even after treatment is discontinued.

Keywords: Valbenazine, Tardive Dyskinesia, Long-Term Treatment

Disclosure: Neurocrine Biosciences Inc., Consultant, TEVA Pharmaceuticals, Osmotica Pharmaceuticals, DisperSol Technologies

T206

Neuroinflammation Biotypes in Schizophrenia: Toward Mechanistic Validation of Shn2 Knockout Mice as an Experimental System

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Background: One of the major challenges in developing novel therapies for schizophrenia is the underlying pathophysiological heterogeneity within the broad patient population. Further, there is a paucity of tractable experimental systems that can provide predictive readouts of the patient condition and response to novel treatments. However, recent progress in subdividing patients based on discrete pathophysiological states (biotypes) suggests that immune dysfunction and neuroinflammation may contribute to the underlying etiology of disorder at least in some cases (termed high inflammatory biotype). To develop novel therapies, a desirable experimental system would enable mechanistic validation, along with a better understanding of pathophysiological progression, and enable identification of novel therapeutic targets. Most important is pathophysiological validity meaning direct comparison of key biological changes between human patients and the experimental system. NFKB is a critical and potent inducer of cytokines, while Schnurri-2 (Shn-2) encodes a nuclear factor-kB site-binding protein that normally suppresses NFKB-mediated expression. Reduced expression of the mouse homolog of Shn-2 is found in the brain of schizophrenia patients and Prof. Miyakawa and colleagues reported that deficiency of schnurri-2 in mouse induces mild chronic inflammation in the brain and behavioral phenotypes related to schizophrenia. The aim of this study is to extend the mechanistic validation of Shn-2-knockout mice as...
Background: The FDA has guided drug developers focused on cognitive improvement in schizophrenia to demonstrate not only improvement on cognitive endpoints, but also to demonstrate the functional relevance of such improvements with a co-primary measure of functional capacity. The recently developed Virtual Reality Functional Capacity Assessment Tool (VRFCAT) is a computer based measure that uses a realistic simulated environment to create a series of routine activities of daily living. Like the MATRICS Consensus Cognitive Battery (MCCB), the components of the VRFCAT were constructed to measure aspects of cognition that apply conceptually to real world tasks. The VRFCAT components involve meal preparation, travel and transit, shopping, and financial skills. The FDA requires evidence that co-primary measures show strong convergence with one or more of the MATRICS cognitive domains that were determined to be functionally relevant. To optimally evaluate the convergent validity of the VRFCAT with the MCCB, an essential first step is to determine whether components of the VRFCAT should be examined separately or should be aggregated on the basis of their latent structure.

Methods: The aims of this study were to evaluate the latent structure and the cognitive correlates of the VRFCAT. 156 patients with schizophrenia (SZ) and 166 healthy controls (HC) were recruited from three sites (University of California San Diego, University of Miami Miller School of Medicine, and University of South Carolina). All subjects completed the VRFCAT, an immersive simulation of real-world situations that require participants to complete 12 objectives that involve navigating a kitchen, catching a bus to a grocery store, finding/ purchasing food in a grocery store, and returning home on a bus. Participants also completed the MCCB and two additional commonly used functional capacity measures, one performance based; the UCSD Performance-Based Skills Assessment (UPSA-VIM), and one interview based, the Schizophrenia Cognition Rating Scale (SCoRS). We first examined the structure of each measure with principal components analyses, and then examined convergence between the VRFCAT and MCCB.

Results: In the SZ sample, the VRFCAT demonstrated a robust unidimensional structure, with the first principal component accounting for 44%. Notably, the UPSA-VIM and SCoRS demonstrated similarly robust single factor structures, suggesting that the VRFCAT’s unidimensional structure is not unique among functional capacity measures. Regarding cognition, the MCCB demonstrated a robust unidimensional factor structure, with the first principal component accounting for 55% of the variance. The latent traits for the VRFCAT and MCCB were highly inter-correlated (r = 0.44). The results were similar within the HC and the combined SZ/HC samples. Supplemental analyses within the SZ sample indicated that each of the MCCB domains showed significant, albeit smaller, correlations with VRFCAT latent trait, while VRCAT objectives also manifest significant, but smaller correlations with the MCCB overall latent trait.

Conclusions: Empirically derived factor models suggest the VRFCAT is best explained and evaluated as a unidimensional trait measure. Results further suggest the VRFCAT is not idiosyncratic in this regard, as a similar unidimensional structure was found for the other functional capacity measures examined across performance-based and interview methods. Consistent with prior studies in large clinical samples and recently published genomic data in healthy populations, the latent structure of cognition measured by the MCCB was also unidimensional. Overall, results suggest convergent validity analyses involving the VRFCAT and MCCB should focus on aggregated composite scores rather than associations between specific functional capacity and cognitive components. The robust correlation between the latent traits for the VRFCAT and MCCB supports validity of the VRFCAT as a co-primary measure.
T208

The Antipsychotic Aripiprazole has an in Vivo Functional Signature of a ‘Partial Antagonist’ as Determined by PET/fMRI

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Background: The atypical antipsychotic aripiprazole has been described as a partial agonist for the D2 receptor in functional assays [1,2], and its clinical efficacy inspired a new class of antipsychotic drugs that are characterized by a mix of partial agonistic and antagonistic properties. The effect of aripiprazole’s unique binding properties has been suggested to have positive cognitive benefits, e.g. on working memory [3]. While aripiprazole has been reported to be a partial agonist for inhibitory cAMP production at D2 receptors, it has also been shown to have partially activating β-arrestin pathways and trigger receptor internalization [1,4-6]. While receptor internalization can be visualized in vitro, relatively little is known about in vivo internalization, yet, has been shown to modulate PET and fMRI temporal profiles [7,8]. Our goal was to characterize the in vivo functional response and signaling properties of aripiprazole in comparison to the full D2 antagonist prochlorperazine using simultaneous PET/fMRI.

Methods: [11C]raclopride-PET and fMRI were acquired in three anesthetized non-human primates (rhesus macaque) with simultaneous PET/fMRI. Aripiprazole was injected intravenously at four different doses: 0.01, 0.02, 0.05 and 0.1 mg/kg (n = 2), and the full D2 antagonist prochlorperazine was injected at 0.1 and 0.3 mg/kg (n = 1) for comparison purposes. The drug injection occurred as a within-scan challenge at ~35 min after the start of a bolus-plus-infusion of [11C]raclopride dynamic scan that lasted for 100 min, during which fMRI was continuously acquired. Before each scan, iron oxide was injected to improve fMRI contrast and detection power [9]. PET data were analyzed with a GLM and cerebral blood volume (CBV) changes were derived. PET data were quantified and binding potentials were derived with a simplified reference tissue model that included a term for dynamic binding changes and used the cerebellum as the reference [10].

Results: Binding potentials from [11C]raclopride PET showed dose-dependent D2/D3 receptor occupancies due to aripiprazole in the range of ~20–90% for the doses. Due to the potency of the drug, extremely small doses very quickly produced high receptor occupancies more than 50%. fMRI signals due to aripiprazole injection produced an overall functional activation (positive CBV changes). The lowest 0.01 mg/kg dose produced 20–30% receptor occupancy and a maximum 2.3% CBV change in the putamen. All other dose injections produced a dose-dependent receptor occupancy in the range of 50–89% that exhibited similar %CBV magnitudes with an average of 4.8% ± 1.0% (std. dev.) in the putamen. While the occupancy data is clearly dose-dependent, the CBV response stayed small in magnitude and was not dose-dependent once 50% receptor occupancy was reached. PET and CBV timecourses showed similar dynamics for all doses. PET dynamic changes in binding potential were described best with a sigmoidal regressor, which meant that the signal stayed depressed for the duration of the experiment. In comparison, the full D2 antagonist showed ~90% receptor occupancy for both 0.1 and 0.3 mg/kg doses with a 13.6% ± 0.8% (std. dev.) positive % CBV in the putamen, i.e. a markedly larger functional signal magnitude compared to ariprazol. CBV timecourses were found to be long-lasting with both drugs, matching the PET occupancy timecourses.

Conclusions: Our findings demonstrate that the atypical antipsychotic aripiprazole displays characteristics that are more consistent with D2/D3 antagonist properties in vivo, based on our functional imaging characterization. Aripiprazole displays a positive CBV response with a substantially lower magnitude compared to full D2 antagonists (as opposed to a negative magnitude as would be expected for an agonist [7]) at similar receptor occupancies. Therefore, we would propose to characterize the in vivo imaging signature as a partial antagonist at D2/D3 receptors. The matched timecourses between PET and fMRI further support that aripiprazole acts like a partial antagonist. We did not observe diverging timecourses that would suggest aripiprazole induces receptor desensitization and internalization in vivo. Further experiments with repeated injections or other partial agonists may reveal insight on in vivo drug function that may be invaluable for predicting therapeutic effects of drugs.

References:

Keywords: Dopamine (D2, D3) Receptors, Aripiprazole, Functional MRI (fMRI), Hybrid PET/MR, Raclopride

Disclosure: Nothing to disclose.

T209

Evidence for Intact Neural Responses of the Social Approach and Avoidance Systems in People With Psychotic Disorders and Their Unaffected Siblings: An Event-Related Potential Study

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Background: Disturbances in social motivation (e.g., asociality) are debilitating and poorly understood in people with psychotic disorders (PSY). Social motivation includes two separate systems: social approach, which promotes social engagement and attachment, and social avoidance, which promotes avoidance of social rejection and discord. We used electroencephalography to examine neural responses of the early stages of processing (i.e., within the first 1000 ms of processing) involved in these two systems, and self-report measures of social approach and avoidance. We examined these systems in a broad sample of individuals with PSY, unaffected siblings of PSY (SIB) and healthy controls (HCS) to determine: 1) if there are different patterns of responding of either system in PSY and SIB compared to HCS by comparing threatening versus affiliative social stimuli; and 2) if these systems are generally intact or impaired in PSY and SIB compared to HCS. Inclusion of the SIB group allows us to explore if disturbances in social motivation are present in those with genetic vulnerability to psychosis.

Methods: We assessed 76 PSY (21 female), 27 SIB (15 female), and 38 HCS (11 female) after they provided written informed consent. Two tasks were administered: a facial affect identification task and a social scene viewing task. For the facial affect task, participants viewed happy (affiliative), angry (threatening), or neutral faces, and pictures of buildings. In separate blocks participants identified the emotion of the face, the gender of the face, or how many stories the building had. We examined two event-related potentials (ERPs): the face-sensitive N170
over the parieto-occipital region, and the emotion-sensitive N250 over the frontal midline region. For the social scene viewing task, participants passively viewed images of scenes depicting social affiliation, social threat, or neutral content. We examined the late positive potential (LPP) over the centro-parietal region. To examine if the pattern of neural responses of the social approach and avoidance systems differed between the groups, we tested for the interaction between group and valence (affiliative vs. threatening stimuli) for the ERP components assessed in the two tasks. To examine if there were general deficits, we examined N170 and N250 responses to faces vs. buildings across groups, and LPP responses to affiliative and threatening vs. neutral scenes across groups. We assessed self-report levels of social approach with the Need for Belonging Scale and social avoidance with the Fear of Rejection Scale. For effect sizes (ES), we present partial $\eta^2_p$.

Results: For the facial affect identification task, there was no significant group X valence interaction for the N170 when comparing responses to affiliative and threatening faces in the emotion identification block, $F(2, 138) = 0.24, p = 0.778, ES = 0.00$. For the N250, there was also no significant group X valence interaction, $F(2, 138) = 1.64, p = 0.199, ES = 0.02$. Examining for general face processing deficits, there was a significant group X task interaction, with PSY having significantly reduced N170 amplitudes to faces but not to buildings, compared to SIBS and HCS, $F(4, 276) = 6.64, p = 0.001, ES = 0.09$. PSY had significantly reduced N250 amplitudes to all stimuli compared to SIB and HCS, $F(1, 138) = 6.31, p = 0.002, ES = 0.08$. N250 amplitudes were significantly larger for faces compared to buildings, $F(2, 276) = 61.95, p < 0.001, ES = 0.31$, in all three groups. Regarding the social interaction task, there was no significant group X valence interaction for the LPP when comparing responses to affiliative and threatening scenes, $F(2, 138) = 0.51, p = 0.603, ES = 0.01$. Examining for general deficits, PSY had significantly reduced LPP amplitudes to all stimuli compared to SIBS and HCS, $F(2, 276) = 37.66, p < 0.001, ES = 0.21$. For the self-report measures, there were no significant differences between groups on the Need for Belonging Scale, $F(2, 138) = 0.90, p = 0.41, ES = 0.01$. However, PSY and SIB reported significantly elevated scores on the Fear of Rejection Scale compared to HCS, $F(2, 138) = 16.68, p < 0.001, ES = 0.20$. None of the ERP measures from either task were significantly correlated with the self-report measures.

Conclusions: First, we did not find any differences in the pattern of responding to affiliative versus threatening stimuli among the three groups for any ERP measure in either task, suggesting that all three groups processed the valence of faces and more complex social scenes in the same manner. Second, we replicated N170 and N250 deficits in PSY compared to HCS and found no ERP deficits to faces in SIB compared to HCS. Third, our results indicate that PSY and SIB have elevated levels of self-reported social avoidance (i.e., Fear of Rejection) compared to HCS, but not for social approach (i.e., Need for Belonging), suggesting that those with a psychotic disorder and their siblings have an overactive social avoidance system. Finally, none of the ERP indices correlated with self-report measures of social approach and avoidance. Collectively, our findings suggest that those with a psychotic disorder and those sharing genetic risk for psychotic disorders have relatively intact neural responses of the social approach and avoidance systems during the early stages of processing of socially relevant stimuli.

Keywords: Dimensions of Psychosis, EEG/ERP Electrophysiology, Social Motivation, Siblings

Disclosure: Nothing to disclose.

T210

The Effects of Age and Sex on Cognitive Impairment in Schizophrenia: Findings From Cognitive From the Consortium on the Genetics of Schizophrenia (COGS) Study

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Background: Recently emerging evidence indicates accelerated age-related changes in the structure and function of the brain in schizophrenia, raising a question about its potential consequences on cognitive function. This study examined whether schizophrenia patients show accelerated age-related decline in cognition using a battery of tasks across multiple cognitive domains and whether an age-related effect differs between females and males.

Methods: We utilized data of 1,415 schizophrenia patients and 1,062 healthy community from a large case-control study of endophenotypes collected by the second phase of the Consortium on the Genetics of Schizophrenia (COGS-2). A battery of cognitive tasks included the Letter-Number Span Tsk, two forms of the Continuous Performance Test, the California Verbal Learning Test, the PENN Emotion Identification Test and the PENN Facial Memory Test. The effect of age and gender on cognitive performance was examined with a general linear model.

Results: We observed age-related changes on most of cognitive measures, which was similar between males and females. Compared to controls schizophrenia patients showed greater deteriorated performance on attention/vigilance and greater slowing of processing social information with increasing age. However, controls showed greater age-related changes in working memory and verbal memory compared to patients. Age-related changes ($\eta^2_p$ of 0.001 to 0.008) were much smaller than between-group differences.

Conclusions: Using a large sample of schizophrenia patients and controls and a battery of tasks that tap into multiple cognitive domains, we found that patients showed continued decline of cognition on some domains but stable impairment or even less decline on other domains with increasing age. The current findings indicate that age-related changes in cognition in schizophrenia is subtle and not uniform across multiple cognitive domains.

Keywords: Schizophrenia, Cognitive Aging, Sex

Disclosure: Nothing to disclose.

T211

Investigating the Association Between Plasma Clozapine to N-Demethylclozapine Ratio in Patients With Obesity and Ultra-Treatment Refractory Schizophrenia

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Background: Antipsychotic (AP) medications are the cornerstone of treatment for schizophrenia. Clozapine (CLZ), remains the sole AP with superior efficacy for treatment refractory schizophrenia. However, it carries a number of adverse effects, including cardiometabolic adversities, which contribute to exceedingly high rates of metabolic co-morbidities like obesity and type 2 diabetes. The main metabolite of CLZ, N-desmethylclozapine (NDMC), has shown potent 5-HT2C antagonist properties, which may in part explain the weight gain, insulin resistance, and hyperlipidemia observed with CLZ treatment. Conversely, a possible benefit of NDMC may lie in its partial agonistic activity at dopamine D2, D3, and muscarinic M1 receptors, supporting decreases in cognitive impairment previously reported in association with a lower CLZ:NDMC ratio. The aim of this study therefore, was to investigate the association between metabolic parameters (body mass index (BMI) and insulin resistance) or cognition, in relation to the CLZ:NDMC ratio.

Methods: A sample of 28 clinically stable patients with obesity, a history of ultra-treatment refractory schizophrenia or schizoaffective disorder (i.e. Clinical Global Impression Severity score ≥4 and/or score of <50 on the Global Assessment of Functioning scale), receiving ≥350 mg daily of CLZ or with plasma CLZ levels >300 ng/mL, was studied. Patients are participants in an ongoing weight-loss intervention clinical trial (clinicaltrials.gov: NCT02808533). All 28 patients have completed baseline anthropometric measures, fasting bloodwork, and cognitive assessments which were used in this analysis (before intervention). Multivariate regressions were performed using SPSS version 25, to assess the association of CLZ:NDMC ratios with the homeostatic model assessment of insulin resistance (HOMA-IR), BMI, Brief Assessment of Cognition in Schizophrenia (BACS) composite t-scores, and BACS digit sequencing t-scores. All regressions were adjusted for factors known to influence CLZ:NDMC ratios (i.e. age, gender, and smoking status).

Results: A statistically significant inverse association was found between HOMA-IR and CLZ:NDMC ratios (B = −1.406 SE B = 0.630, β = −0.415 p < 0.05), but not for BMI (B = −0.010 SE B = 2.545, β = −0.001 p > 0.05), BACS composite t-scores (B = 2.151 B SE = 4.398, β = 0.093 p > 0.05), or BACS digit sequencing t-scores (B = 1.810 B SE = 5.177, β = 0.071 p > 0.05), and CLZ:NDMC ratios.

Conclusions: Staggering rates of new onset type 2 diabetes have been reported in patients with refractory schizophrenia (37% over 5 years of starting CLZ), illustrating the clinical relevance of identifying those most at risk for developing this serious cardiovascular risk factor. Based on our results, the CLZ:NDMC ratio may be a useful marker in predicting the development of insulin resistance. Our findings also suggest that one mechanism underlying high rates of glucose dysregulation in patients receiving CLZ may be ascribed to a direct effect of NDMC on metabolic processes. The absence of association between the CLZ:NDMC ratio and BMI may be explained by limited spread of BMI in our sample, attributable to obesity as a study inclusion criterion. Failure to show an association between cognition and CLZ:NDMC ratio could be explained by the illness severity of our population, who were ultra-treatment refractory and receiving a higher range of CLZ doses. This raises the possibility that illness severity may override pro-cognitive effects driven by NDMC or, alternatively, that high CLZ levels may saturate the N-demethylation process. Going forward, there is a need for more studies to understand factors influencing the association between the CLZ:NDMC ratio, cognitive performance, and metabolic functioning.

Keywords: Treatment-Resistant Schizophrenia, Clozapine, Drug Metabolism, Cognition, Insulin Resistance

Disclosure: Nothing to disclose.
Effects of SEP-363856 on Negative Symptoms in Schizophrenia: Analysis of an Acute, Placebo-controlled Trial of a Novel Psychotropic Agent With No Dopamine-D2/5-HT2A Antagonist Activity

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Background: SEP-363856 is a novel trace amine associated receptor-1 (TAAR1)/5-HT1A agonist with no dopamine-D2/5-HT2A antagonist activity. In a recent placebo-controlled trial, SEP-363856 showed significant antipsychotic efficacy in patients with schizophrenia, and a safety and tolerability profile distinct from currently available antipsychotics. Here we evaluate the effects of SEP-363856 on negative symptoms in schizophrenia, and the specificity of its effects in patients with more-prominent severity of negative symptoms at pre-treatment baseline. We report an exploratory analysis of the specific effect on negative symptoms of treatment with SEP-363856 compared to pooled results of 5 placebo-controlled clinical trials of a D2-based antipsychotic agent (lurasidone; N = 1710). In order to ensure that improvement in the severity of negative symptoms was a domain-specific treatment effect, and not a nonspecific effect secondary to improvement in correlated PANSS items (ie, "pseudospecificity"), we utilized an Uncorrelated PANSS Score Matrix (UPSM) transformation of the PANSS scale that has been previously validated (Hopkins et al., Schizophr Bull. 2018;44:593-602), and has been shown to reduce the between-PANSS-factor correlations across a wide variety of clinical trials in schizophrenia.

Methods: Patients aged 18-40 years with an acute exacerbation of schizophrenia (NCT01969382) were randomized, double-blind, to 4-weeks of flexible-dose treatment with SEP-363856 (N = 120) given once daily (50 or 75 mg) or placebo (N = 125). Prespecified efficacy measures included the PANSS total score, the UPSM-PANSS negative-apathy/avolition (UPSM-NAA) and negative-deficit of expression (UPSM-DE) factors, and the Brief Negative Symptom Scale (BNSS) total score. In addition, the effect of SEP-363856 was studied in the patient type defined by having specific severity of UPSM-PANSS negative symptom factors at baseline.

Results: The primary analysis showed significant week 4 improvement in LS mean (SE) PANSS total score for SEP-363856 vs. placebo at week 4 (−17.2 [1.7] vs. −9.7 [1.6]; P = 0.001; effect size [ES] = 0.45). Domain-specific effects on negative symptoms were evident for SEP-363856 (vs. placebo) at week 4 on the UPSM negative symptom factors UPSM-DE (ES, 0.32) and UPSM-AA (ES, 0.32). Confirming that SEP-363856 treatment effects were specific to negative symptoms, week 4 change score correlations between the UPSM-PANSS positive symptom factor and the UPSM-DE and UPSM-NAA factors were low (−0.050 and 0.143, respectively). In contrast to the efficacy of SEP-363856 in treating negative symptoms, analysis of pooled data found treatment with lurasidone to have lower effect sizes on both the UPSM-DE (ES, 0.04) and the UPSM-AA (ES, 0.22). In separate trials, patients with UPSM-defined prominent negative symptoms at baseline, the subset (N = 51) treated with SEP-363856 demonstrated greater improvement in negative symptoms (as measured by UPSM-PANSS-AA, UPSM-PANSS-DE, and BNSS total scores) compared to the improvement observed in the prominent negative symptom subset (N = 286) treated with lurasidone. In an additional secondary analysis, treatment with SEP-363856 (vs. placebo) showed week 4 improvement in negative symptoms as assessed by the BNSS total score (ES, 0.48), and subtotal scores for blunted affect (ES, 0.51), avolition (ES, 0.42), anhedonia (ES, 0.39), social withdrawal (ES, 0.47), alogia (ES, 0.32), and distress (ES, 0.23). However, the week 4 change score correlation between the BNSS total score and the UPSM-PANSS positive symptom factor score (0.319) was higher than the change score correlation with UPSM-DE and UPSM-NAA factors (−0.050 and 0.143, respectively). This suggests that the BNSS total score is a less specific measure of negative symptoms than the UPSM-DE or UPSM-NAA.

Conclusions: SEP-363856 demonstrated improvements relative to placebo on BNSS total and UPSM-transformed negative symptoms and in patients with UPSM-prominent negative symptoms, consistent with a specific effect on negative symptoms. Improvements in negative symptoms appeared to be specific, i.e., over and above correlated improvements in PANSS observed in prior trials with standard atypical agents. The effects of SEP-363856 remained apparent in the subset of patients who were defined at baseline as having UPSM-prominent negative symptoms, in contrast to a known D2-based antipsychotic. Together these results highlight the potential for a distinct efficacy profile of a non-D2 mechanism of action in schizophrenia, with particular relevance for negative symptomatology.

Reduced Glutamate in the Left Superior Temporal Gyrus in First Episode Schizophrenia: A Whole Brain 1H-MRS Study

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Background: Proton magnetic resonance spectroscopy (1H-MRS) studies have examined glutamatergic abnormalities in schizophrenia, 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, schizophrenia 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), in early schizophrenia.

Methods: Whole-brain proton spectroscopic imaging was acquired at 3T using echo-planar acquisition with spin-echo excitation EPI sequence (TE = 17.6 ms, TR = 1551 ms, TR = 511 ms, non-selective lipid inversion nulling with TI = 198 ms, FOV = 280 × 280 × 180 mm, voxel size of 5.6 × 5.6 × 10 mm, echo train length of 1000 points, bandwidth of 2500 Hz, reduced k-space sampling (acceleration factor = 0.7), and a nominal voxel volume of 0.31 cm³. Young schizophrenia subjects (n = 36, mean age = 23; 19 antipsychotic-naive and 17 antipsychotic-treated) and healthy controls (HC, mean age = 23; n = 29) were studied. Glx (glutamate +glutamine), N-acetylaspartate (NAA), choline, creatine and myoinositol were fitted with MIDAS, referenced to water and partial volume corrected for CSF. Volumes 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. Subsequently, cluster-corrected family-wise error (FWE, p < 0.05) was implemented.

**Results:** Schizophrenia subjects had lower Glx in the left superior (STG) and middle temporal gyri (16 voxels, FWE p = 0.04) and increased creatine in two clusters involving left temporal, parietal and occipital regions (32, and 18 voxels, p = 0.02 and 0.04, respectively). Antipsychotic-treated and naïve patients (vs HC) had similar Glx reductions (8/16 vs 10/16 voxels respectively, but FWE p’s > 0.05). However, creatine was higher in antipsychotic-treated vs HC’s in a larger left hemisphere cluster (100 voxels, FWE p = 0.01). Also, in treated patients, choline was increased in left middle frontal gyrus (18 voxels, FWE p = 0.04). Finally, in antipsychotic-naïve patients, NAA was reduced in right frontal gyri (19 voxels, FWE p = 0.05) and myo-inositol was reduced in the left cerebellum (34 voxels, FWE p = 0.02). All these results persisted after controlling for significant group differences in history of cannabis use disorder and socioeconomic status in family of origin.

**Conclusions:** Unbiased spectroscopic brain examination supports that reductions in Glx in the left STG may be critical to the pathophysiology of schizophrenia. The only prior study that examined STG reported a trend glutamate reduction (p = 0.1) only on the left side in chronically-treated schizophrenia (Atagun et al., 2006). This supports a model of NMDA hypofunction in areas critical for sound discrimination and language, early in the illness, regardless of antipsychotic therapy. Postmortem and neuroimaging schizophrenia studies focusing on left STG, may provide critical mechanistic and therapeutic advancements, respectively.

**Keywords:** Glutamate, Antipsychotic-Naïve First-Episode Schizophrenia, Proton Magnetic Resonance Spectroscopy

**Disclosure:** UpToDate, Royalties

**T217**

**Sex Differences in Schizophrenia: A Longitudinal Methyome Analysis**

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**Background:** Methylation studies show that there are substantial sex differences in DNA methylation. On the other hand, in schizophrenia being male is strongly associated with early onset. The primary aims of the current study is 1) to identify differentially methylated regions between males and females with schizophrenia; and 2) to investigate sex differences in DNA methylation at CpG site, gene and CpG island level.

**Methods:** In this pilot analysis, we have collected detailed clinical information and DNA samples from 135 schizophrenia patients, allowing us to measure genome-wide methylation at single-base resolution in 97 males and 38 females. This cross-sectional DNA sample included subjects with a diagnosis of schizophrenia that were epigenotyped using the Illumina Infinium 450. We applied a novel epigenetic association strategy, repeating the analysis in few subjects over-time (longitudinal methylation analysis) assuming that epigenetically determined sex is a trait marker and it is stable over time.

CpG sites across the genome were analyzed using the software IMA, to detect sex differences.

**Results:** The sex analysis of the autosomal probes generated cg11955727 as the top hit on chromosome 2 (10-35) and male gender was associated with hypomethylation (the methylation difference was 22%). However, the CpG cg11955727 is distant from functional genes. We repeated the analysis in 13 subjects (nine females and four males) to confirm the sex difference and to reduce the effect of potential confounders. As expected, the number of differentially methylated CpG sites at significant level went down, mainly because the smaller sample size.

**Conclusions:** As expected, the overall results showed robust association between autosomal CpG sites and sex; however, the longitudinal methylation analysis can be used as internal replication to confirm epigenetic variants that are stable over-time.

**Keywords:** Schizophrenia, Sexual Dimorphism, DNA Methylation

**Disclosure:** Nothing to disclose.

**T218**

**Impaired Potentiation of Theta Oscillations During a Visual Cortical Plasticity Task in Patients With Schizophrenia**

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**Background:** Impaired neuroplasticity has been implicated in the core pathophysiology of schizophrenia (SZ), contributing to cognitive deficits. Long-term potentiation (LTP), a form of experience-dependent synaptic plasticity, has been demonstrated in humans by showing that repeated stimulation with a visual stimulus results in the persistent enhancement of EEG-based visual evoked potentials (VEPs). Using this paradigm, we previously showed that high-frequency stimulation produced sustained potentiation of VEPs in healthy controls (HC), but not in SZ. We extend this prior work by examining EEG oscillatory activity following visual high-frequency stimulation in SZ and HC participants.

**Methods:** EEG data were recorded from 19 SZ and 21 HC during a visual LTP paradigm. VEPs were elicited by a standard black and white checkerboard stimulus (~0.83 Hz, several 2 minute blocks) before and after exposure to visual high-frequency stimulation (~8.8 Hz, 2 minutes) designed to induce potentiation of VEPs. A cluster-based permutation testing approach was applied to time-frequency data to examine LTP-like effects (i.e., potentiation after visual stimulation).

**Results:** A mixed model of mean inter-trial coherence (ITC) of phase in the identified cluster (0-496 ms, dominated by theta activity at occipital electrodes) showed a Group x Time interaction (p < 0.0001). Post-hoc tests demonstrated that mean ITC was significantly enhanced in HC compared to SZ at early post-stimulation blocks relative to baseline (p < 0.005). Similarly, a mixed model of mean total power in its cluster (0-450 ms, theta activity only) revealed a Group x Time interaction (p < 0.0001); relative to baseline, total power was significantly enhanced in HC but not SZ across post-stimulation blocks (p < 0.0005). Changes in mean ITC and total power following visual high-frequency stimulation were not associated with positive or negative symptoms in schizophrenia patients (p > 0.05).

**Conclusions:** Visual high-frequency stimulation enhanced theta power and phase consistency in HC but not in SZ. Results demonstrate visual LTP-like plasticity alterations in SZ, consistent with hypothesized NMDA receptor dysfunction.

**Keywords:** Schizophrenia, Long-Term Potentiation, EEG

**Disclosure:** Nothing to disclose.
Imaging Synaptic Dopamine Availability in Individuals at Clinical High-Risk for Psychosis: A [11C](+)-PHNO PET Study

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Background: Abnormalities in intrasynaptic dopamine (DA) transmission in schizophrenia (SCZ) are present both at baseline and after pharmacological challenge, mainly within the associative striatum (AST). These findings are consistent with the observation, in patients at clinical high-risk (CHR) for psychosis, of elevations in [18F]DOPA uptake in the AST, which have been shown to predict transition to syndromal psychosis. Data in CHR, however, are primarily limited to the striatum and in large part to [18F]DOPA. A few [18F]DOPA studies have examined extra-striatal regions, finding increases in extra-striatal dopamine synthesis capacity, specifically in the midbrain both in SCZ and CHR subjects. Our findings with [11C]FLB457 and the amphetamine challenge paradigm instead indicate the presence of a generalized deficit in dopamine release in extra-striatal regions, especially the dorsolateral prefrontal cortex (DLPFC) and the midbrain, in SCZ. We do not know, however, whether the extra-striatal deficits, and striatal elevations, in synaptic DA transmission are also present in CHR patients. We used methylphenidate, a DA reuptake inhibitor, and [11C](+)-PHNO PET, to measure synaptic DA availability in CHR both in striatal and extra-striatal brain regions.

Methods: We recruited 14 CHR individuals (age: 22.36 ± 2.84; 9M, 5F; 2C, 6AA, 5Mixed, 1As, 5 Hispanic) and 14 matched control subjects (age: 22.71 ± 2.84; 8M 6F; 3C, 5AA, 5Mixed, 1As, 5 Hispanic). All CHR subjects met criteria for the Attenuated Positive Symptom Psychosis-Risk Syndrome (APSS), Progression subtype, as delineated by the Structured Interview for Psychosis-Risk Syndromes (SIPS). Subjects were non-substance using and free of all psychotropic medications. We selected the D2/3 radiotracer [11C](+)-PHNO given its ability to simultaneously detect D2/3 receptors in striatal and extra-striatal regions and the larger magnitude of change, compared to antagonist radiotracers, after a pharmacological challenge, related to its agonist properties. Subjects were administered a bolus injection of [11C](+)-PHNO and scanned for 120 minutes. [11C](+)-PHNO BPND, the binding potential relative to the nondisplaceable compartment, was measured at baseline and following administration of a single oral dose (60 mg) of methylphenidate. BPND was derived using the simplified reference tissue model with cerebellum as reference tissue. The percent change in BPND between conditions, ΔBPND, was computed as an index of synaptic DA availability.

Results: We observed significantly greater ΔBPND in the ventral striatum (VST) in CHR patients (34% ± 14% >12%; p = 0.009). There were no significant differences in AST, thalamus, or midbrain. There was a strong negative correlation between ΔBPND in VST and baseline total negative symptoms in the CHR group (r = −0.66, p < 0.01). We observed minimal change in total positive symptoms related to the methylphenidate challenge.

Conclusions: Our data do not show the predicted DA elevations in AST of CHR. Instead we show excess in VST and, for the first time, an inverse relationship between VST DA and negative symptoms, the latter consistent with our observations in SCZ. Furthermore, this study indicates that striatal DA alterations may precede extra-striatal alterations in the pathogenesis of psychotic disorders, although longitudinal follow-up is needed to ascertain what proportion of this sample will actually transition to psychosis or will show other pathology. Additional considerations related to the specifics of the radiotracer and the heterogeneity of the CHR sample may contribute to the findings we describe and will be discussed.

Keywords: Dopamine, Positron Emission Tomography (PET), Clinical High-Risk, Psychosis, Methylphenidate

Disclosure: Nothing to disclose.

Brain Metabolites and Microstructural Changes With Disease Progression in Psychosis: A Longitudinal Study by MRS of Relaxation and Diffusion Measurements

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Background: Diffusion tensor imaging (DTI) characterizes the nature of water molecular movement, to provide information on white matter (WM) integrity but DTI alone cannot discern exact features associated with WM such as axon vs. myelin problems because of the nonspecific nature of DTI measurements. Magnetic resonance spectroscopy (MRS) can provide an additional window to the brain’s cellular microenvironment through the measurement of transverse relaxation (T2) and metabolite diffusion. In addition, T2 could be a confounding factor for metabolites quantification, which should be considered especially during disease progression. Furthermore, some metabolites have a preferential cellular compartmentalization, e.g. NAA in neurons, myoinositol (mIns) primarily in glia, and this makes measurements of their dynamics (e.g. relaxation and diffusion) cell-specific probes to explore intracellular changes.

For psychotic disorders, it is well-known that its brain microstructure and metabolites are altered with disease progression. However, we still lack a clear understanding of which parameters are more sensitive to disease progression and associated with underlying mechanisms and we have no specific markers and targets for effective treatment and early intervention. Therefore, we used a combination of T2 and diffusion tensor spectroscopy (DTS) techniques to study first episode psychosis (FEP) schizophrenia (SCZ) patients with scans at baseline and one and two year follow-up. We compared our results from the FEP group to those from chronic SCZ and a group of matched healthy controls, all studied using the same experimental protocols as we have reported previously.

Methods: After approval by the McLean Hospital Institutional Review Board, we recruited 14 participants with FEP. MR scans were performed at baseline, 1st and 2nd year follow-up. 9 healthy subjects with matching demographic factors were also recruited as controls and scanned at baseline and 2 years. T2-MRS and DTS techniques (water and metabolites) were implemented on a 4T full body MR scanner. A 1×3×3 cm3 single MRS voxel was placed on the pure WM in the corona radiata, centered at the level of the genu of the corpus callosum but lateral and posterior to it.
Metabolite quantifications were performed with LCModel. T2 relaxation and diffusion ADC were determined by home-grown software. Statistical analyses were performed using SPSS.

**Results:** 3 major metabolites: NAA, total creatine (tCr) and total choline (tCho) were analyzed. A repeated measures ANOVA with a Greenhouse-Geisser correction determined that NAA T2 decreased statistically from baseline to 1 and 2 year follow-up in FEP. The same analysis on NAA ADC demonstrated a borderline statistically significant increase from baseline to subsequent time points. No significant was observed for T2 or ADC measurements of tCr and tCho from baseline to 1st and 2nd year follow-up. Because NAA is located intracellularly and almost exclusively localized to neurons, the shortened NAA T2 suggests increased interactions with intracellular macromolecules with disease progression. The increased NAA ADC may reflect an increase in intra-axonal space available for diffusion. The axon-selectivity of the NAA ADC findings is further supported by the absence of similar changes in tCr and tCho ADC. The control group didn’t show significant changes in either NAA T2 or ADC. Regarding the water measurements, the FEP group showed slight increases in both T2 and ADC, which could be caused by chronic macro-structure alterations such as atrophy of brain with increased CSF percentage.

All these findings in our longitudinal study in FEP (elevated water and NAA ADC, elevated water T2 and reduced NAA T2) are similar to what we have previously observed in cross-sectional studies in FEP and chronic SCZ patients. So here we may be finding evidence that these abnormalities are not pronounced at baseline and are the result of an active process early on in disease which eventually settles down to the patterns we see in chronic patients. Interestingly, as a marker of neuron intracellular molecule dynamics, NAA T2 and ADC seem to be more sensitive to early disease progression compared to water indices, which are more of a reflection of the combination of both micro- and macro-structural alterations and show more significant changes in the long term.

It is notable that reduction of NAA concentrations with disease duration has been commonly observed in the past but it is possible this could be caused by T2 relaxation effects. However, after correction of T2 effects in our current large sample of chronic SCZ patients (N = 150), we still observed NAA declines slightly with disease duration. This finding indicates that there is a genuine reduction in NAA concentration that cannot be accounted for by T2 relaxation effects.

**Conclusions:** Our enriched data-set acquired by multimodal neuroimaging indicates that alterations of WM integrity, microscopic environment of the extra-/intra-cellular space, as well as metabolite concentrations, are associated with disease progression in SCZ. Multi-parameter MR measures would provide more precise tools for deeper insight into biological mechanisms in disease progression and exploring effective treatments for SCZ patients.

**Keywords:** Neuropsychiatric Disorders [Schizophrenia, Parkinson’s Disease, Major Depressive Disorder], Disease Progression, Brain Microstructure

**Disclosure:** Nothing to disclose.

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**T222**

**Imputation of Brain Gene Expression and Chromatin Accessibility 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 chromatin accessibility and transcriptomes for 30 neuropsychiatric traits to study the effects of risk loci on epigenetic states and gene expression.

**Methods:** We generated brain-specific predictive models for (1) transcription at the gene and isoform level from $n = 924$ brains of the PsychENCODE and (2) chromatin accessibility from $n = 272$ brains of the CommonMind Consortium. Then, we imputed the transcriptomes and chromatin accessibility for 30 neuropsychiatric traits. Finally, we studied the relationship of imputed changes in chromatin accessibility within proximal and distal (by leveraging brain-specific Hi-C data) cis-regulatory elements (CRE) such as promoters and enhancers with imputed changes in transcription.

**Results:** The imputed quantitative traits suggest that a subset of risk loci within CREs affect chromatin accessibility and are associated with either imputed gene expression changes or differential expression of gene isoforms. We identify unique, shared and converging mechanisms of transcriptional regulation across neuropsychiatric diseases.

**Conclusions:** Imputing disease-specific transcriptomes and chromatin states can help us formulate hypotheses for the functional roles of risk loci and enables the study of trans-disease processes.

**Keywords:** Transcription Imputation, Epigenomics, Comparative Studies

**Disclosure:** Nothing to disclose.

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**T223**

**Digital Markers of Motor Activity Captured Over Smartphone is Associated With Negative Symptoms of Schizophrenia: Results From a Pilot Observational Study**

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**Background:** Motor activity is a key feature of negative symptoms of schizophrenia, is treatable, and has well characterized neurobiological targets that are relevant across multiple neuropsychiatric disorders. However, clinician assessments motor activity have low inter-rater reliability and typically require laboratory based procedures to accurately quantify.

**Methods:** We piloted the assessment of motor activity based on changes in x, y, and z coordinates in a computer vision generated model using data from individuals with schizophrenia (SCZ, $n = 21$) and healthy controls (HC, $n = 9$) who were recruited as part of an observational study conducted at the Mount Sinai Hospital in New York City. Severity of negative symptoms was assessed using the positive and negative syndrome scale (PANSS) along with a brief smartphone-based passive assessment where subjects answered brief questions on daily activities, and viewed visual stimuli and reported on it. Head motion was assessed as changes in x, y, and z coordinates at a rate of 33 estimates per second. Total head motion was compared between HC and SCZ and in association with specific negative symptoms of schizophrenia.

**Results:** Individuals with schizophrenia demonstrated significantly less head motion when compared to healthy controls ($\mu_{HC} = 2.49$ mm/frame, $\mu_{SCZ} = 1.52$ mm/frame; t-test $p = 0.04$).
Measure of head movement correlated strongly with PANSS items P-total (r = −0.31; p = 0.1), G-total (r = −0.47; p = 0.01), N-total (r = −0.44; p = 0.01), P4 excitement item score (r = 0.16; p = 0.4), N1 blunted affect item score (r = −0.50; p = 0.007), G5 mannerisms and posturing item score (r = −0.41; p = 0.03), and G7 motor retardation item score (r = −0.53; p = 0.004).

Conclusions: Digital markers of movement, captured through a brief remote cell-phone based assessment, differentiate patients with schizophrenia from their healthy counterparts. Further, results show that head motion only specifically correlates with negative symptom severity along with multiple specific symptoms related to motor functioning (excitement; mannerisms and posturing; motor retardation; blunted affect).

Keywords: Artificial Intelligence, Machine Learning, Motor Function, Schizophrenia; Technology, Digital Phenotyping

Disclosure: AiCure; Employee

T224

FKBP5 Gene Expression and DNA Methylation in Human Brain Over the Lifetime: Impact of Severe Psychopathology

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Background: FKBP5, encoding FK501 Binding Protein 51, is a critical regulator of stress hormone signalling, impacting multiple downstream pathways relevant for disease. There are four annotated FKBP5 splice variants, (V1-4), with V4 generating a truncated protein. Heightened FKBP5 expression (pan) has been implicated in psychiatric disease by gene-by-environment studies, animal models, and postmortem brain studies from individuals with severe psychopathology. Given that FKBP5 expression 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. We used a large collection of postmortem human brain samples to determine the following: (1) Cell-type specificity of FKBP5 expression in the human brain and age-related changes; (2) The FKBP5 alternative transcript variants and DNA methylation (DNAm) changes contributing to age-related effects in FKBP5; (3) If age-related changes in FKBP5 are moderated by FKBP5 genotype or psychiatric disease status.

Methods: The dorsolateral prefrontal cortex (DLPFC; Brodmann Area 9, BA9), which is highly implicated in severe psychopathology, was assessed in 681 individuals from two 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 (BDP; 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 neuropsychological illness. Methods used included gene 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 HumanHap650Y_V3 or Human 1M-Duo_V3 BeadChips). Statistics were performed in R, with linear regression modelling used to assess case-control differences in gene expression, DNAm, and effects of genotype. Spearman correlations, loess fit curves and sm.ancova analyses were used to determine correlations with age, and differences between age trajectories in cases vs controls. FDR corrected P values are reported unless otherwise noted.

Results: (1) Our characterisation studies indicated that FKBP5 pan protein is exclusively expressed in the cytoplasm and nucleus of neurons in the DLPFC of humans, and expression was not observed in astrocytes, microglia nor oligodendrocytes. RNA-scope® experiments showed that FKBP5 long mRNA isoforms (V1-3) were significantly increased with age only in the upper region of the BA9 cortex (layers I, II, III); this is an area that consists predominately of pyramidal neurons. The lower layers of BA9 are characterised by smaller pyramidal cells and a high density of glia in layers V and VI; expression here was low and did not significantly increase with age.

(2) Age-specific analyses indicated that both 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. FKBP5 mRNA variants (all) were more strongly increased with age in SZ relative to CTRLs (sm.ancova analyses comparing non-parametric regression curves: h [i.e. smoothing parameter] = 3.718, P = 0.0046).

Accordingly, the majority of CpGs across regulatory regions of the FKBP5 gene were significantly demethylated in cases (especially in S2) vs CTRLs in older subjects. Interestingly, CpGs previously shown to be moderated by age in the periphery (cg20813374, cg00130530; Zannas et al., 2019, PNAS) were not moderated by age in the brain.

(3) The aging trajectory of FKBP5 mRNA variants (all) and DNAm for 3 CpGs were significantly affected by an interaction of FKBP variant rs9470080 (proxy for the FKBP5 risk haplotype) with diagnosis, with cases carrying the high-induction T allele having significantly higher mRNA expression at older ages (P < 0.048), and generally lower DNAm (P < 0.037) in CpGs in the CTCF binding site (e.g. cg26868354), although this was CpG dependent.

Conclusions: These results indicate that in general, FKBP5 gene and protein expression are heightened with age, while DNA demethylation occurs in FKBP5 regulatory gene regions with age. This aging effect is exacerbated by the FKBP5 risk genotype and severe psychopathology. We also provide novel evidence that FKBP5 gene expression in the human brain and in aging is exclusive to neuronal subtypes and not glia. This work highlights that peripheral evidence of FKBP5 involvement in severe psychiatric disorders and immune signalling likely differs to brain pathology. 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, Neuropsychiatric Disorders, Early Life Stress

Disclosure: Nothing to disclose.

T225

Behavioural Phenotypes of Intrinsic Motivation in Schizophrenia

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Background: Intrinsic motivation (IM) deficits are a prominent feature of negative symptoms seen in schizophrenia that have been linked to functional disability experienced by affected individuals. Previous investigations have primarily assessed IM as a general construct or towards specific activities, typically utilizing
clinch-rated or self-report measures. While these approaches have yielded consistent findings indicative of a general impairment in IM, there have been limited objective investigations that can disentangle underlying components of IM. The aim of this study was to conduct a multifaceted examination of IM using objective and ecologically valid tasks to uncover distinct behavioural phenotypes that contribute to clinical motivation deficits seen in schizophrenia.

**Methods:** Using wireless motion capture, we quantified the real-world behaviour of 45 stable adult outpatients with schizophrenia (SZ) and 47 demographically-matched healthy controls (HC). Specifically, this comprised of quantification of exploratory behaviour with the Novelty Exploration Task (NET) and uncentitized effortful engagement with the Activity Preference Task (APT), aligning with distinct aspects of IM. We applied cluster analysis to identify subgroups with behaviourally differentiable phenotypes of IM. Analysis of variance was then used to investigate cluster-based differences in clinical motivation deficits, cognitive functioning, and community functioning.

**Results:** Cluster analysis revealed three distinct clusters comprised of: high performance on both tasks (HiAPT/HiNET; nSZ = 10, nHC = 20), medium APT performance with low NET performance (MdAPT/LoNET; nSZ = 20, nHC = 19), and low APT performance with medium NET performance (LoAPT/MdNET; nSZ = 15, nHC = 8). Cluster assignment did not significantly predict diagnostic group. Analysis of variance revealed a significant effect of cluster on clinical motivation scores (F(2,89) = 5.493, p = 0.006), with post-hoc analyses revealing that low performance on either task corresponded to clinical motivation deficits compared to the high performance group. Analyses also revealed a significant cluster by diagnostic group interaction effect (F(2,86) = 3.148, p = 0.048), with impairment in either aspect of IM being related to deficits in motivation particularly for individuals with SZ compared to within-cluster HC counterparts. Similarly, a significant cluster by diagnostic group effect emerged for clinician-rated community functioning (F(2, 86) = 5.493, p = 0.006), with post-hoc analyses revealing progressively lower community functioning in SZ participants across cluster (HiAPT/HiNET > LoAPT/MdNET > MdAPT/LoNET). There were no significant cluster or cluster by diagnosis effects for cognition.

**Conclusions:** Multidimensional characterization of IM using objective tasks revealed distinct behavioural phenotypes across SZ and HC individuals with differential performance across facets of IM. Further, impairment in either facet of IM appeared detrimental to general motivation and functioning specifically for subgroups of individuals with SZ. These distinct profiles of impairment in IM processes may represent differential treatment targets for the amelioration of motivation deficits in SZ. Moreover, the use of objective tasks such as those utilized in this study may serve to rapidly assess and characterize these behavioural deficits, guide personalized treatment planning, and subsequently monitor progress and treatment response.

**Keywords:** Intrinsic Motivation, Negative Symptoms, Behavioral Phenotyping

**Disclosure:** Nothing to disclose.

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**T226**

**NMDA Receptor Positive Allosteric Modulator CAD-9303 Reverses Mismatch Negativity and Behavioral Impairments in Pharmacological Models of NMDA Receptor Hypofunction**

T226

**Timothy Piser**, David Anderson, Ezio Bettini, Marie Bureau, Clizia Capuani, Edward Christian, Michael Curtis, Christopher Fanger, Earl Gordon, Steven Leiser, Gregg Keaney, Sam Malekiani, Frank Menniti, Robert Volkmann

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**Background:** Hypofunction of the glutamate-gated, N-methyl-D-aspartate-selective ion channel receptor (NMDAR) is implicated as a cause of schizophrenia by converging lines of clinical evidence including reduced amplitude of the NMDAr-dependent auditory novelty detection EEG (electroencephalography) biomarker, Mismatch Negativity (MMN). In patients with schizophrenia, MMN amplitude correlates negatively with cognitive and negative symptoms and global function, and deficits in MMN have predicted psychosis onset in high-risk subjects.

**Methods:** Novel NMDAr positive allosteric modulators (PAMs) with CNS drug-like properties were discovered by high-throughput screening, and optimized for potency and pharmacokinetic properties. Electrophysiological measurement of NMDAr potentiation in Xenopus oocytes injected with recombinant human cDNAs were confirmed in cultured rat cortical neurons, and rat hippocampal brain slice. MMN was measured using the auditory-oddball procedure in awake-behaving rats with surgically-implemented frontal EEG electrodes. Object memory was measured as the difference in exploration time between novel and familiar objects after a brief delay between object exploration periods. Drug versus vehicle effects were evaluated in rats on both MMN acutely impaired by a single injection of MK-801 (N = 16), and object memory persistently-impaired after one week of washout from seven days of twice-daily phencyclidine (PCP) injections (N = 12).

**Results:** CAD-9303 exhibited positive allosteric modulation of human recombinant and rat neuronal NMDA receptors, potentiation of NMDAr-mediated EPSCs in rat hippocampal slices, oral bioavailability, brain levels, and brain-to-plasma ratio supporting rat pharmacological studies and IND-enabling toxicology currently in progress. Oral CAD-9303 prevented MK-801-induced MMN deficits and reversed persistent object memory deficits caused by sub-chronic PCP administration in rats (p < 0.05, 1-way ANOVA, Dunnet’s Post-Hoc).

**Conclusions:** Normalization of MMN and behavior in pharmacological models of NMDAr hypofunction by the potent, drug-like NMDAr PAM, CAD-9303, provides evidence of therapeutic potential in the treatment of schizophrenia in clinical trials currently being planned.

**Keywords:** NMDA Receptor, Schizophrenia Novel Treatment, Mismatch Negativity, Positive Allosteric Modulators

**Disclosure:** Cadent Therapeutics, Employee

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**T227**

**Plasma Exosomal Amyloid and Tau Biomarkers in Schizophrenia and Non-Psychiatric Comparison Subjects: Relationships With Cognitive, Mental, and Physical Functioning**

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**Background:** Cognitive deficits in schizophrenia (SZ) are a major predictor of disability and functioning, yet there is limited understanding of the underlying pathophysiology of these impairments. The role of amyloid and tau biomarkers (considered hallmarks of Alzheimer’s disease) has not been identified in SZ. Exosomes or extracellular vesicles, involved with cell-to-cell communication and waste removal, provide a novel source to assess brain-based proteins using peripheral blood samples. Elevated exosomal amyloid and tau levels have been shown to predict conversion to Alzheimer’s Disease. We did not find any published study of exosome-derived amyloid and tau protein
levels in relationship to cognitive, mental, and physical functioning in a sample of younger and older adults with schizophrenia.

We present cross-sectional data from ongoing longitudinal studies of SZ and non-psychiatric comparison (NC) subjects. Our hypotheses were: 1) Plasma Exosomal amyloid and tau levels will be higher in SZ than in NC subjects. 2) Exosomal amyloid and tau will be related to cognitive functioning in both SZ and NC groups. We also explored the relationship of exosomal amyloid and tau levels with psychopathology, physical health measures, as well as inflammatory and oxidative stress biomarkers.

**Methods:** The sample included 51 subjects with SZ (DSM-IV-TR criteria) and 49 NCs (mean age 46.4 years, SD 10.0, range 26 to 65 years). People with dementia or other major neuropsychiatric disorders were excluded. Cognitive measures included executive functioning (Delis-Kaplan Executive Function System) and global cognition (Telephone Interview for Cognitive Status - modified). Psychopathology measures included Scales for the Assessment of Positive and Negative Symptoms (SAPS and SANS, respectively). We examined physical well-being (Medical Outcomes Survey – Short form 36 item), body mass index (BMI), and self-reported sleep duration (hours).

Exosomes were extracted and precipitated from fasting plasma samples. The exosomes were identified as being neuronal-derived exosomes (NDEs) or astrocyte-derived exosomes (AEs). Human-specific ELISAs were used to quantify levels of Aβ42, and phosphorylated tau (ptau).

Other blood-based biomarkers assessed inflammation (high-sensitivity C-reactive protein or hs-CRP, interferon gamma or IFN-γ), and oxidative stress (F2-isoprostanes).

Independent sample t-tests, Chi-square tests, and Mann-Whitney U tests were used to assess differences between SZ and NC groups. Spearman’s correlations were performed to assess the relationships of exosomal Aβ42, and ptau with demographic and clinical variables as well as other biomarker levels. Non-parametric partial correlations were performed to control for age. Fisher’s Z transformations were performed to compare correlation coefficients.

**Results:** The SZ and NC groups were comparable on age, sex, and race. SZ group had fewer years of education and worse executive functioning and global cognition than the NC group (Cohen’s d = 0.53, 1.0 and d = 0.88, respectively). SZ group had similar levels of total protein, Aβ42, and ptau from NDEs and AEs to the NC group. NDE ptau levels in the NC group were associated with age (r(47) = −0.40). The other NDE and AE amyloid and ptau levels did not correlate with age.

NDE Aβ42 levels were significantly correlated with executive functioning in both diagnostic groups (NC: r(35) = −0.51, SZ: r(36) = −0.34.) On controlling for age, the significant associations between NDE Aβ42 levels and executive functioning were only observed in NCS (r(32) = −0.52). Exosomal amyloid and ptau levels were not associated with global cognition.

In SZ group, NDE ptau and AE ptau levels were associated with negative symptoms (r(48) = −0.29 and r(46) = −0.40, respectively). On controlling for age, AE ptau levels remained significantly related to negative symptoms (r(43) = −0.39, p = 0.009.)

Physical well-being was associated with AE Aβ42 levels only in NC group (r(19) = 0.50). BMI was differentially related to NDE Aβ42 levels in the two groups (NC: r(32) = 0.42, SZ: r(37) = −0.36, z = 3.26, p < 0.001.) Sleep duration was correlated with NDE Aβ42 only in SZ group (NC: r(33) = 0.05, SZ: r(33) = −0.39, z = 1.79, p = 0.04). The sleep duration-NDE Aβ42 relationship in SZ remained significant on controlling for age (r(30) = −0.36).

IFN-γ levels were significantly associated with NDE ptau only in SZ group (r(31) = −0.47); this correlation persisted on controlling for age (r(28) = −0.47.) Hs-CRP was not associated with any amyloid or tau measures.

F2 isoprostane levels were associated with NDE ptau and AE ptau only in SZ group (r(45) = 0.30 and r(43) = 0.48, respectively.)

Controlling for age, the oxidative stress measure was associated with both NDE ptau and AE ptau in SZ group.

**Conclusions:** Plasma exosomal amyloid and tau provide novel insights into cognitive, mental, and physical functioning in SZ. While exosomal Aβ42 levels were related to cognitive and physical functioning, exosomal ptau levels were related to inflammatory and oxidative stress biomarkers. The relationships of exosomal amyloid and tau with these health factors differed between SZ and NC participants. While these cross-sectional data support our hypotheses partially, longitudinal examination of the amyloid and ptau relationships to cognition and health measures is warranted. This study is continuing and updated results will be presented at the meeting.

**Keywords:** Neurodegeneration, Psychosis, Aging

**Disclosure:** Nothing to disclose.

**T228**

**Cortical Region- and Layer-Specific Alterations in a Subclass of Chandelier Cells in Schizophrenia**

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**Background:** The axons of chandelier cells (ChCs) target the axon initial segment of pyramidal neurons, forming an array of boutons termed a cartridge. We recently showed that in schizophrenia the density of cartridges detectable by vesicular GABA transporter (vGAT) immunoreactivity is higher in layer 2 of the prefrontal cortex. In addition, we showed that in schizophrenia ChC cartridge boutons contain normal levels of the 67 kDa isofrom of glutamic acid decarboxylase (GAD67) protein, the enzyme responsible for GABA synthesis in these boutons. To assess the regional specificity of these findings, we quantified the densities of vGAT+ ChC cartridges in cortical layers 2 and deep 3 of the following four brain regions involved in visuospatial working memory (vsWM); primary visual (V1), association visual (V2), posterior parietal (PPC), and dorsolateral prefrontal (DLPFC) cortices.

**Methods:** For these studies, tissue sections from 20 matched pairs of schizophrenia and unaffected comparison subjects were immunolabeled for vGAT, parvalbumin, GAD67, and GAD65, and assessed using quantitative immunofluorescence microscopy methods.

**Results:** The density of ChC vGAT+ cartridges was 31% higher in DLPFC selectively in layer 2 in schizophrenia subjects. In contrast, cartridge density did not differ between subject groups in layers 2 or deep 3 of V1, V2, or PPC. In support of our previous findings, ChC terminal GAD67 protein levels did not differ between groups in the DLPFC. However, GAD67 protein levels were 12%–28% lower in schizophrenia in the other brain regions studied.

**Conclusions:** Our findings suggest that in schizophrenia region-specific pathological mechanisms give rise to different neural circuit alterations across the vsWM network.

**Keywords:** GABA Neuron, Gabaergic Boutons, Cortical Layers, Visuospatial Working Memory

**Disclosure:** Nothing to disclose.

**T229**

**High-Throughput Approaches to Uncover the Molecular Underpinnings of Neuropsychiatric and Neurodevelopmental Disorders Using Zebrafish**

Abstract not included.
Dexmedetomidine – Highly Favorable Pharmacokinetic and Pharmacological Features for a CNS Therapeutic Drug

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Background: BioXcel Therapeutics, using proprietary search algorithms, identified agitation in patients with neuropsychiatric diseases as a significant unmet therapeutic need. A systematic search of drug databases identified dexmedetomidine, an alpha2-adrenergic agonist, as a promising drug for treatment of agitation. Alpha2-adrenergic receptor agonists activate receptors on neurons within the locus coeruleus (LC). The neurons within the LC release norepinephrine in response to stressful stimuli. Excessive norepinephrine is responsible for many symptoms of agitation. By reducing norepinephrine release, alpha2-adrenergic agonists may prevent emergence of agitation in stressed patients. Drugs currently used to treat agitation include antipsychotics (haloperidol, e.g.) and benzodiazepines (lorazepam, e.g.); these classes of drugs, unlike alpha2-adrenergic agonists, do not treat the LC neurons directly but rather mask symptoms by sedating patients. The use of antipsychotic and benzodiazepine drugs is not optimal because they may be overly sedating, cognitively impairing, cause respiratory depression when combined with alcohol use (common in this patient population), or associated with motor or cardiovascular safety concerns. Alpha2-adrenergic agonists like dexmedetomidine at low doses do not have these liabilities.

Alpha2-adrenergic agonists include dexmedetomidine, clonidine, lofexidine and guanfacine. These drugs have been used for many indications including blood pressure control, attention deficit disorders, and opioid withdrawal. Dexmedetomidine administered by the intravenous route is used for procedural sedation and anesthesia. Dexmedetomidine cannot be administered orally because of poor oral bioavailability. BioXcel Therapeutics has formulated dexmedetomidine on a sublingual film and early results in clinical studies indicates that this formulation achieves higher bioavailability. In the poster we will show data demonstrating high free brain levels after sublingual dosing in rats. We also compare the pharmacological properties of dexmedetomidine to clonidine, lofexidine and guanfacine.

Methods: Structural attributes: BioXcel Therapeutics compiles a database of compounds that have been through Phase 1 of clinical development. Structural attributes are assigned to these compounds and scores generated to determine if a drug has high probability to achieve high free brain levels after dosing and therefore be useful for CNS indications.

Potency and intrinsic activity: Human Chem-1 cells (alpha2A) or human CHO-K1 cells (alpha2B and alpha2C) were transfected with corresponding plasmids encoding the receptor of interest. Cells were incubated with [35S]GTPyS for 30 minutes at 30 degrees centigrade with the indicated concentration of agonist compound. Cells were harvested and radioactivity counted. A concentration response curve was determined using GraphPad Prism.

Microdialysis: Free brain levels were determined by using microdialysis probes placed in the prefrontal cortex of Sprague Dawley rats. After sublingual dosing, the microdialysate was collected, analyzed and concentrations calculated. These concentrations were plotted versus time and compared to free plasma concentrations from the same animals.

Results: Dexmedetomidine is predicted to have high brain penetrability based on its physiochemical attributes. The predictive model was validated by measuring free brain levels using microdialysis after dosing. As predicted, free brain levels were high compared to free plasma levels. Dexmedetomidine exhibited persistent brain levels that peaked approximately 1 hour after peak plasma levels. The peak brain concentrations approximated those achieved in plasma.

Dexmedetomidine is more potent and has higher intrinsic activity than other alpha2 agonists tested. Potency as indicated by EC50 values of dexmedetomidine was approximately 3 nM (600 pg/ml) at both alpha 2A and 2B adrenergic receptors. To achieve 10% occupancy, a plasma level of 66 pg/ml would be required.

Dexmedetomidine is also a full agonist compared to other agonists tested and would be expected to achieve a greater reduction in norepinephrine release based on its relative activity. Unlike other alpha2A-adrenergic agonists, dexmedetomidine would be equally effective in activating alpha2B-adrenergic receptors. Because both alpha2A and 2B-adrenergic agonists contribute to reducing norepinephrine release, this could be an added benefit of dexmedetomidine.

Conclusions: Microdialysis reveals enduring level of free dexmedetomidine in brain after sublingual dosing. Dexmedetomidine is highly CNS penetrant after dosing as shown by near 1:1 ratios between free brain and free plasma levels.

Dexmedetomidine is more potent than other agonists tested at all 3 alpha adrenergic receptors, alpha2-A, B and C as measured by potency in increasing GTPyS binding.

Dexmedetomidine has higher intrinsic activity than all other agonists tested as measured by an increase in GTPyS binding. These findings indicate that a plasma concentration of 66 pg/ml would be sufficient to minimally activate alpha2A and alpha2B adrenergic receptors.

The implications of these findings with respect to clinical development of BXCL501 will be discussed.

Keywords: Dexmedetomidine, Agitation, Agitation, Alpha-Adrenergic

Disclosure: CSO of BioXcel Therapeutics, Employee

Gut Microbiome Alterations in Patients With Chronic Schizophrenia and Association With Clinical Characteristics

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Background: Recent years have seen a rapid growth of interest in the intestinal microbiome and gut-brain axis in the pathophysiology of human health and disease. Specifically, there is an emerging body of empirical support for the view that the gut microbiome can impact brain and behavior. Schizophrenia is associated with debilitating psychiatric and cognitive dysfunction, worse health outcomes, and shorter life expectancies. The pathophysiological understanding and therapeutic resources of these neuropsychiatric disorders are still limited. Evidence suggests that schizophrenia is associated with accelerated biological aging, driven by altered inflammatory processes and metabolic dysfunction. Microbial dysbiosis may underlie the pro-inflammatory milieu and other physiological abnormalities that have been implicated in schizophrenia.

Methods: This study investigated gut microbiome composition in 86 individuals, including 43 persons with chronic

T231

Gut Microbiome Alterations in Patients With Chronic Schizophrenia and Association With Clinical Characteristics

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Methods: This study investigated gut microbiome composition in 86 individuals, including 43 persons with chronic
schizophrenia and 43 non-psychiatric comparison subjects (NCs) between ages 27-76 years. Given that technical differences (e.g., DNA extraction methods, sequencing platforms, taxonomy databases) can produce systematic biases that may obscure biologically meaningful compositional differences, NCs were matched to schizophrenia subjects by sequencing plate. We selected the nearest matching neighbors on the sequencing plate based on age, sex, race, BMI category (obese vs. not obese), and antibiotic use (in the past year) to control for known major drivers of microbiome changes that could confound results. Stool samples were collected and assayed using 16S rRNA amplicon sequencing of the V4 region. Sequence data were processed and analyzed in QIME2. PICRUSt was performed to predict functional potential of microbial communities, based on metagenomes inferred from 16S data. Primary measures of the gut microbiome included (1) microbial taxonomic composition and (2) functional pathways. For each, we examined within-sample differences (alpha-diversity) using Shannon Index and Faith’s Phylogenetic Diversity (PD) metrics, and between-sample differences (beta-diversity) using UniFrac and Bray-Curtis distance matrices. Spearman’s correlations were calculated between alpha- and beta-diversity and clinical characteristics.

**Results:** (1) No group differences were observed in alpha-diversity of taxonomic composition. For the combined sample, alpha-diversity was negatively correlated with BMI (Shannon, Faith PD, ps < 0.03). Within the schizophrenia group, greater alpha-diversity (Faith PD) was associated with fewer self-reported cognitive failure (rho = −0.37, p = 0.02), better physical wellbeing (rho = 0.37, p = 0.02), and higher daily antipsychotic doses (rho = 0.31, p = 0.04). Examination of beta-diversity indices revealed significant community-level separation in taxonomic composition between schizophrenia and NC groups using unweighted UniFrac (pseudo-F = 2.02, p = 0.005) and Bray-Curtis (pseudo-F = 2.48, p = 0.001). Beta-diversity of microbes was positively correlated with daily antipsychotic dose (rho = −0.19, p = 0.01). (2) Analysis of functional pathways did not reveal alpha- or beta-diversity differences between groups or beta-diversity correlations. However, within the schizophrenia group, greater alpha-diversity in functional pathways was associated with longer illness duration (rho = 0.34, p = 0.03), greater severity of negative symptoms (rho = 0.26, p = 0.05), and higher antipsychotic dose (rho = 0.37, p = 0.005).

**Conclusions:** Our study is the largest known study of the gut microbiome in chronically ill patients with schizophrenia living in the US. It builds upon and expands our previously published results (Nguyen et al., 2019, Schizophr Res). Patients with schizophrenia exhibited global microbial community differences compared to NCs. Notably, these differences were observed after controlling for demographics, sequencing plate, and other clinical factors known to have major influences on the gut microbiome. Greater evenness/richness of microbes was associated with improved self-reported cognition and physical wellbeing as well as higher daily doses of antipsychotic medication in the schizophrenia group. Although the data are cross-sectional and we cannot determine directionality or causality relationships, these findings lay the groundwork for future investigations to elucidate the role of the gut microbiome in the development, presentation, and progression of schizophrenia. Further longitudinal studies are needed. Although in its early stages, microbiome research holds promise for predicting clinical prognosis, assessing risk for morbidity and mortality, and informing intervention development to improve patients’ quality of life.

**Keywords:** Gut Microbiome, Neuropsychiatric Disorders [Schizophrenia, Parkinson’s Disease, Major Depressive Disorder], Gut-Brain Axis

**Disclosure:** Nothing to disclose.
reward, and arousal networks. These results provide novel insight into rsFC predictors of worsening rhythmicity and highlight the potential utility of neuroimaging predictors in the identification of individuals at-risk for rhythmicity disturbances. Ongoing longitudinal data collection in this sample, with biannual clinical assessments, will promote more detailed future investigations.

Keywords: Bipolar Disorder, Resting State Functional Connectivity, Circadian Rhythms, Seasonality

Disclosure: Nothing to disclose.

T234

Sex Differences in Stress Inoculation of Addiction-Like Phenotypes

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Background: Opioid use disorder is on the rise and the economic and human cost is staggering. It remains unclear why only a subset of people who take opioids develop substance use disorder (SUD), prompting efforts to understand factors that promote vulnerability to opioid misuse. However, it is also critical to identify factors that promote resilience to SUD. Experiences early in life can alter risk/resilience to the later development of disorders. For example, early life stress that is not overwhelming can have an “inoculating” effect that promotes the development of resilience in adulthood. Here we use a rat model of early life adversity, the limited bedding and nestling (LBN) model, to assess how this manipulation affects addiction-like phenotypes in adulthood.

Methods: In LBN, rat dams and their pups were exposed to a low resource environment during the pups’ first week of life, which induces stress in the pups. When male and female offspring reach adulthood, they were tested on behaviors related to substance abuse: impulsivity and drug self-administration. Impulsive choice was examined using the delayed discounting task where rats can choose between a smaller/immediate vs. a larger/delayed reward. Drug taking behavior was measured using morphine self-administration on a fixed ratio 1 (FR1) reinforcement schedule, and motivation for morphine was assessed with a progressive ratio (PR) reinforcement schedule. To understand how LBN could affect later behavior, we are assessing changes to neuroepigenetic processes in the nucleus accumbens (NAc), which mediates both impulsivity and drug-seeking. We focused on genome-wide changes in histone modifications, which are known to regulate the conformation of chromatin and expression of neighboring genes. We measured single histone post-translational modifications (PTMs) using a HPLC-MS mass-spectrometry-based assay and combined this approach with RNA sequencing (RNA-seq) to delineate LBN-derived changes in gene expression and accompanying alterations in histone modifications in the NAC.

Results: We first tested whether adult male and female rats with a history of LBN exposure had altered impulsive choice in the delayed discounting task. LBN-exposed males more often chose the larger/delayed reward, indicating a reduction in impulsivity condition (interaction [F1, 33] = 6.83, p = 0.013). LBN did not alter choice in delayed discounting in female rats. High impulsivity is associated with drug taking, but here we found LBN reduced impulsivity in males. Similarly, intake of morphine (0.25mg/kg/infusion on and FR1 schedule) and breakpoint for PR were reduced in LBN vs. control males ([F(1, 10) = 6.8, p = 0.026], U = 2, p = 0.009). LBN exposure had no effect on these endpoints in females ([F(1, 10) = 1.922, p = 0.196]; U = 9, p = 0.329). Given that only LBN males appeared protected from addiction-related behavioral phenotypes, sex-specific changes in histone modifications in the NAc were assessed. LBN increased histone deacetylase 10 (Hdac10) expression in the NAc of males (q value = 0.025), but not females (q value = 0.13). Analysis comparing male controls to male LBN samples identified HDAC1 as the top upstream regulator (p = 2.84 x 10^-5). This result suggests that although HDAC1 expression is not altered by LBN, this enzyme may contribute to LBN-derived changes in gene expression. Using a mass spectrometry-based genome-wide screen of histone PTMs, we found that LBN significantly altered 1 modification in females as compared to 3 modifications in males. One histone modification increase in LBN vs. control males was acetylation of lysine 8 on Histone 4 (H4K8ac), which is often found in active promotors. In summary, LBN produced divergent patterns of gene expression in males and females and elicited sex-specific signatures of histone modifications in the NAc. Ongoing and future studies will attempt to link which epigenetic modifiers and histone modifications critically contribute to regulating LBN-derived changes in gene expression and behavior.

Conclusions: Taken together, these data indicate that LBN can inoculate males against addiction-like behavioral phenotypes. These alterations in behavior are likely driven by sex-specific epigenetic modifications in the NAc induced by LBN. Given that the addiction-related behavior appears unaffected by the LBN manipulation in females, comparing LBN-induced epigenetic changes in males vs. females can reveal novel mechanisms that can promote resilience to SUD, which may lead to the development of better therapies to reduce opioid misuse.

Keywords: Early Life Stress, Substance Abuse, Impulsivity, Epigenetics

Disclosure: Nothing to disclose.

T235

Sex Differences in Chronic Ethanol Effects on Nucleus Accumbens Astrocyte Function During Reward Seeking in Mice

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Background: The ability to flexibly regulate behavior is impaired in many neuropsychiatric illnesses, including alcohol use disorders. This may involve insensitivity to negative consequences, failure to update behavior when contingencies change, or altered sensitivity to stress or cue-induced relapse like behaviors. Substantial sex and gender differences are observed in both humans and preclinical models of alcohol dependence. In a mouse model in which ethanol dependence is induced by chronic intermittent ethanol exposure, we previously observed that females exhibited increased sensitivity to aversive experiences, suggesting that sex may interact with alcohol dependence to influence sensitivity to stressors. Chronic alcohol exposure is known to dysregulate accumbens glutamate signaling and may promote the loss of behavioral flexibility in a sex-specific manner. Glutamate levels are in part regulated by astrocyte function, which may be altered following chronic ethanol exposure in a sex-specific manner. Based on this, we investigate whether differences in accumbens astrocyte function may underlie sex differences in dependence-induced changes in reward seeking behaviors in a mouse model.

Methods: Sex differences in astrocyte function and reward seeking behavior were assessed in adult male and female C57BL/
males or Air controls. They further point to accumbens astrocyte extinction and exhibit increased reinstatement compared to seeking, such that CIE exposed females are relatively resistant to the consequences of CIE on extinction and stress-induced reinstatement. Our findings indicate that CIE did not impact basal expression of food CPP, but had sex specific consequences on extinction. In particular, we observed that CIE exposed females were resistant to extinction compared to males or Air control females. We further observed increased sensitivity to stress-induced reinstatement of food seeking in CIE exposed females (p < 0.05). Consistent with sex differences in astrocytic contributions to reward seeking, we observed a trend toward increased numbers of calcium transients during reward seeking in a CPP expression test in females relative to males. We also observed increased calcium transients during reward seeking behavior in a stress-induced reinstatement paradigm in both males and females (p < 0.01). Finally, our chemogenetics data indicate that activation of Gq-signaling in accumbens astrocytes can attenuate both the expression of CPP and resistance to extinction observed in CIE exposed females, without impacting stress-induced reinstatement (p’s < 0.05).

Conclusions: Together these data identify sex specific effects of CIE on extinction and stress-induced reinstatement of reward seeking, such that CIE exposed females are relatively resistant to extinction and exhibit increased reinstatement compared to males or Air controls. They further point to accumbens astrocyte function as a contributor to failure to extinguish reward seeking behavior observed in alcoholic dependent females. These findings highlight the need to use multiple behavioral models to identify the consequences of chronic alcohol exposure, and the importance of including both males and females in studies. Further, they implicate chronic alcohol-induced dysregulation of accumbens astrocyte function in altered reward seeking in females.

Keywords: Sex Differences, Astrocytes, Alcohol, Extinction, Stress-Induced Reinstatement

Disclosure: Nothing to disclose.
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Background: Cannabis is the most commonly used illicit drug in the world. Cannabinoids have been shown to modulate immune responses; however, the effects of cannabis on neuroimmune function have never been investigated in vivo in human brain. Microglia are key players in the immune surveillance system of the central nervous system, where they act as resident macrophages and first responders to brain insults. Activated microglia increase the expression of a mitochondrial protein, translocator protein 18KDa (TSPO), making it an important marker of immune activation in the brain.

Methods: Twenty-four chronic cannabis users and 27 non-cannabis using controls underwent a positron emission tomography scan with [18F]FEPPA. Cannabis users were included if they had a positive urine drug screen for only cannabis, and if they used cannabis at least 4 times weekly for the last 12 months. Total distribution volume (VT) across prioritized brain regions. Stress and anxiety, and peripheral measures of inflammatory cytokines and CRP were also measured.

Results: Compared with 27 non-cannabis using controls (mean [SD] age, 23.6[4.2]; 18 women[67%]), cannabis users (n = 24; mean [SD] age, 23.1[3.8]; 9 women[38%]) showed higher [18F]FEPPA VT (main group effect: F(1, 48) = 6.5, p = 0.01; ROI effect: F(1, 200) = 28.4, p < 0.001; ~23% higher; Cohen’s d = 0.6), with an even more prominent effect in the CUD subgroup (n = 15, main group effect: F(1, 39) = 8.5, p = 0.006; ROI effect: F(1, 164) = 19.3, p < 0.001; ~31% higher; Cohen’s d = 0.8). Greater brain TSPO levels were associated with stress and anxiety in cannabis users, and higher circulating CRP levels.

Conclusions: Our findings suggest higher TSPO levels in cannabis users, with a more prominent effect in CUD. Greater brain TSPO levels were associated with increased blood CRP levels, stress and anxiety. This study is important given cannabis legalization trends across the world.

Keywords: Cannabis Dependence, Marijuana, Immune Biomarkers, TSPO, CRP

Disclosure: Nothing to disclose.

T239

A Pilot Study Examining Working Memory Cognitive Training During Treatment: Feasibility and Preliminary Outcomes Contrasting Emotional and Neutral Multi-Modal Stimuli

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Background: A large literature demonstrates alcohol-related neurobehavioral compromise persisting beyond detoxification. Although compromise may be observed across diverse neurocognitive domains and brain systems, those abilities most dependent on the integrity of the prefrontal cortex (PFC) and its interconnections appear to be most vulnerable. Neurobehavioral compromise has been associated with poorer treatment engagement and less successful outcomes. Therefore, it is not surprising that there is recurring interest in the utility of cognitive training to improve outcomes. Recently there has been a resurgence of interest in this topic, fueled by advances in brain/cognitive training protocols. These facts led us to initiate a pilot study through which we could, a) determine the feasibility of conducting a computerized cognitive training protocol in treatment settings, and, b) leveraging data showing emotion processing deficits in treatment-seekers, determine if stimulus valence (neutral (NEU) vs. emotional (EMO)) differentially impacted training gains and transfer of training using a multimodal WM (n-back) training task in which emotional valence was irrelevant to task demands. Based on extant work, our preliminary hypothesis was that WM maintenance would be compromised in the EMO condition, resulting in lower training gains relative to the NEU condition. To the contrary, in assessing transfer of training, we anticipated that repeated training where emotion is irrelevant would benefit performance on other tasks where emotional content of stimuli was irrelevant. Thus, in this preliminary analysis, we focus on transfer of training to the Emotional Stroop Task (EST).

Methods: Fifty-eight youth (mean age: 18.5 ± 0.6) were recruited from a cohort that has been followed since birth (N = 2692). All participants were characterized as having high (high EXT; N = 27; 16F/11M) or low externalizing traits (low EXT; N = 31; 20F/11M); all underwent 90-min positron emission tomography [18F]fallypride scans, and completed the Barratt Impulsiveness Scale (BIS-11), Alcohol Use Disorders Inventory Test (AUDIT) and Sensitivity to Punishment (SP) & Sensitivity to Reward (SR) Questionnaire.

Results: Compared to those in the low EXT group, high EXT participants exhibited elevated BIS-11, AUDIT, and SR scores, greater prevalence of psychiatric disorders, and higher [18F]fallypride binding potential values in the frontal cortex, insula and amygdala. Group differences were not evident in the midbrain; however, across all participants, there was a positive correlation between midbrain BPND values and SP scores as well as a negative correlation with SR to SP ratios.

Conclusions: Altered DA2/3R availability in the dopamine cell body and extra-striatal terminal regions might constitute biological vulnerability traits for addictions and addiction susceptibility-related behaviors.

Keywords: Dopamine, Alcohol and Substance Use Disorders, Youth, Positron Emission Tomography Imaging, Externalizing Disorders

Disclosure: Nothing to disclose.
Methods: Methods were approved by the UF IRB-01 and registered with ClinicalTrials.gov (NCT031376554). Participants provided written consent prior to study. Men and women seeking inpatient/residential treatment for a substance use disorder (SUD), meeting criteria for a DSM 5 moderate to severe alcohol use disorder, and reporting 21 to 90 days of sobriety are eligible for consideration. Persons with significant medical or psychiatric disorders (excluding SUDs) are ineligible. Participants are assigned to: treatment as usual (TAU) or training (EMO or NEU). All groups complete a cognitive battery including the EST at two points in time, baseline and ~3 weeks later. Training sessions (~30 min.) occur in the interim period for 2-4 times/week depending on facility/participant availability. The EMO and NEU groups train on a dual n-back task using visual (faces) and auditory (words) stimuli and requiring them to indicate if the current stimuli match that in the appropriate n-back condition. Visual stimuli were judged as to whether they occurred in the same position in a 4 x 4 grid. In the NEU condition, stimuli in both modalities were neutral. In the EMO condition, the faces and words communicated negative affect. Training progression in both conditions is determined by participants’ success, i.e., adaptive training, difficulty ranges from n-1 to n-6. Here, we analyzed training gain differences between the NEU (n = 7) and EMO (10) participants who have currently completed at least 8 training sessions. We evaluated transfer effects by comparing pre- and post-training performance on the EST for the combined training groups vs. the TAU group. At the time of analysis, sufficient post-training data for a 3 group comparison were not available. The dependent variable is the highest n-back condition achieved during each training session.

Results: Training session 1 performance did not differ between the EMO and NEU groups (average “n” = 1.68 (SD 0.89) and training gains were significant across time (p = 0.001, β = 0.512). The trajectories for the two conditions were equivalent across sessions 1-3, with averages in each group ranging from 1.58 to 2.6, after which the groups diverged. For trials 4-8, the average “n” for the EMO group was 3.45 (1.4), for the NEU group, 2.25 (1.5). At session 8, the mean for the EMO group was 4.8 (1.1), for the NEU, 2.25 (1.5) (p = 0.02, Cohen’s d = 1.94).

The analysis of transfer of training to the EST showed no significant benefit with training. However, the TAU group showed a reduction of 63 ms in average RT at post-test, whereas the training groups’ average reduction was 134 ms. The effect sizes for improved efficiency (ACC/RT) were substantially larger for the training groups relative to TAU (βtraining = 0.484 vs. BTAU = 0.007). Unfortunately, sex is not yet sufficiently distributed for analysis in training or transfer.

Conclusions: On-going recruitment and minimal attrition indicate that complex computerized cognitive training interventions can be accomplished in residential/inpatient treatment settings. Furthermore, post-training surveys indicate that 90% of those in the training conditions would recommend the program to others. The directional divergence in training trajectories was not anticipated. We speculate that the EMO group’s performance may be attributable to greater engagement, reflecting the import of stimulus characteristics in training. This issue awaits further analysis as training is completed. The transfer data are encouraging, although falling short of significance. RT and efficiency benefits were greater for the trained groups vs TAU. Given effect sizes, we anticipate that statistical significance will be obtained with a larger n. Finally, we look forward to addressing potential sex effects and conducting the 3 group analyses to more fully address our 2nd hypothesis.

Keywords: Emotion Processing, Alcohol and Substance Use Disorders, Cognitive Training, Computerized Cognitive Training

Disclosure: Nothing to disclose.

T240

Alcohol Use and Misuse Show a Distinct Genetic Architecture: A Genome-Wide and Polygenic Risk Scoring Approach

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Background: Alcohol use disorders (AUD) can be dissected into two core components – the extent to which an individual consumes alcohol and the potential problems that they experience related to their intake.

Methods: We obtained measures from the Alcohol Use Disorder Identification Test (AUDIT), which is a 10-item screening questionnaire that measures both aspects of alcohol consumption (items 1-3, AUDIT-C) and problematic use (items 4-10, AUDIT-C), from a population-based cohort, UK Biobank (UKB, N = 121,630), and performed two genome-wide association (GWAS) analyses. Next, we calculated polygenic risk scores (PRS) of AUDIT-C and AUDIT-P and we estimated their correlations with various alcohol-related outcomes in four independent samples with differing age and ascertainment characteristics. Data on alcohol-related phenotypes were drawn from a cohort ascertained for family history of alcoholism, the Collaborative Study on the Genetics of Alcoholism (COGA; N = 6,850); and three population-based cohorts, the Avon Longitudinal Study of Parents and Children (ALSPAC; N = 5,911), Generation Scotland (GS; N = 17,461), and a subset of the UKB (N = 245,947).

Results: We identified that genetic liability to AUDIT-C was positively correlated with educational achievement and unrelated to psychopathology, whereas liability to AUDIT-P was negatively correlated with educational achievement and positively correlated with psychopathology. In general, AUDIT-P PRS was associated with a range of alcohol-related phenotypes, including DSM-IV alcohol dependence (COGA, R2 = 0.70%, p = 1.9e-9; ALSPAC, R2 = 0.50%, p = 5.75e-4) and ICD AUD-related disorders (UKB, R2 = 0.20%, p = 2e-16), DSM-5 symptom count (COGA, R2 = 0.70%, p = 9.76e-11), maximum drinks (COGA, R2 = 0.50%, p = 2.53e-8, ALSPAC, R2 = 3.3%, p = 1.59e-3), CAGE (a screener for problem drinking) scores (GS, R2 = 0.40%, p = 9e-7), and increased risk of onset of alcohol dependence (COGA, HR = 1.15, p = 1.64e-08), in both population-based and high-risk clinically ascertained cohorts, while AUDIT-C PRS showed less utility in the ascertained cohort.

Conclusions: These findings suggest that alcohol consumption and AUD have an overlapping yet distinct genetic architecture, as well as demonstrate the influence of ascertainment schemes on polygenic analyses.

Keywords: Alcohol Abuse, Alcohol Consumption, GWAS, Alcohol Use Disorders, Polygenic Risk Scores

Disclosure: Nothing to disclose.

T241

Evidence for Abnormal Amygdala Reactivity

Agrawal, Toni Clarke, Alexis Edwards

Background: Alcoh...
Background: Fear conditioning and extinction are vital processes in adaptive emotion regulation and have been shown to be disrupted in anxiety disorders and posttraumatic stress disorder. Despite substantial comorbidity between alcohol dependence (ALC) and anxiety disorders and reports of altered negative emotion processing in ALC, neural correlates of fear conditioning and extinction have not yet been investigated in an alcohol-dependent population.

Methods: This cross-sectional, multimodal, case-control study used a two-day fear conditioning and extinction paradigm in 43 healthy participants (24 women) and 43 recently detoxified individuals with ALC (14 women) at the National Institutes of Health. Main outcomes included functional brain magnetic resonance imaging (fMRI), clinical measures (Spielberger State-Trait Anxiety Inventory, Perceived Stress Scale, Alcohol Dependence Scale, Montgomery-Asberg Depression Scale), as well as skin conductance responses (SCRs) to confirm differential conditioning. Analyses were performed using mixed model analyses of covariance (ANCOVAs) for SCR data and two-stage mixed model ANCOVAs for fMRI data. All analyses controlled for age, gender, and exposure to early life stress.

Results: Successful fear conditioning and extinction were demonstrated across participants by differential SCRs in the fear conditioning phase and no difference in SCRs to the conditioned stimuli in the extinction phase. The ALC group showed significantly reduced blood oxygenation level-dependent (BOLD) responses in the right amygdala during fear conditioning (Cohen’s d = 0.89, P(FWE) = 0.037), and in the left amygdala during fear renewal (Cohen’s d = 0.68, P(FWE) = 0.039). Right amygdala activation during fear conditioning was significantly correlated with ALC severity (r = 0.39, P(Bonferroni) = 0.009), depressive symptoms (r = 0.37, P(Bonferroni) = 0.015), trait anxiety (r = 0.41, P(Bonferroni) = 0.006), and perceived stress (r = 0.45, P(Bonferroni) = 0.002).

Conclusions: These results revealed significantly blunted amygdala BOLD responses during fear conditioning and fear renewal in individuals with ALC compared to controls, suggesting dysregulated fear learning in ALC. Furthermore, amygdala activation during fear conditioning was associated with negative emotion- and alcohol dependence-related clinical measures. Given the importance of amygdala activation to threat for normal aversive learning, individuals with ALC might have abnormal fear learning and subsequently diminished sensitivity to negative consequences of alcohol use, which may contribute to disease progression and relapse risk. These findings indicate that the fear conditioning and extinction paradigm may be a promising tool to investigate structures involved in negative affect regulation during early abstinence, which might inform the development of novel treatment approaches for ALC.

Keywords: Alcohol Use Disorder, Amygdala, Fear Conditioning, Addiction, Anxiety

Disclosure: Nothing to disclose.

T242

Cannabis Abstinence in Adolescents Associated With Increased Alcohol Use That may be Transient

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Background: Cannabis (CB) use is common among adolescents, and rates are likely to increase as states adopt more permissive stances on CB legality. While most are concerned about the high rates of youth use, many are hesitant about intervening for fear that this may inadvertently result in an increase in alcohol consumption (i.e., substitution hypothesis). The current study examined the effects of one month of monitored CB abstinence on patterns of alcohol use among adolescents.

Methods: Participants were adolescents (n = 215), aged 13-25, with (n = 147) and without (NU; n = 68) current, regular (≥ 1x/week) CB use. At baseline, CB users were randomized to one month of biochemically-verified CB abstinence (CB-Abst; n = 89) or monitoring (CB-Mon; n = 58). Data were drawn from baseline and six follow-up assessments during the one-month monitoring period. Participants completed a Timeline Followback interview at baseline to quantify frequency and amount of past 30-day alcohol and CB use, and at subsequent visits to approximate use during the period between study visits. Analyses controlled for the following potential confounds: age, sex, race, current symptoms of anxiety and depression (Mood and Anxiety Symptom Questionnaire; MASQ), impulsivity (UPPS Impulsive Behavior Scale; UPPS-P); alcohol dependence (Alcohol Use Disorder Identification Test; AUDIT); cannabis dependence (Cannabis Use Disorder Identification Test; CUDIT), and cannabis use motives (Marijuana Motives Measure; MMM).

To assess change over time in the proportion of days spent drinking in-between visits for the three groups, we fit a logistic regression to the raw count data using a penalized regression approach implemented within a Bayesian framework. To control for repeated measures and individual differences, we fit a hierarchical model with subject-level intercepts and slopes. We used a piecewise regression approach to model change over time, fitting two separate linear trends, one between baseline and week 1, and the second between week 1 and the final time point, week 4. We assessed the difference at baseline and over the linear trends between the abstinent group and the two control groups (non-abstinent CB users and non-users).

Results: At baseline, CB users endorsed more frequent (M = 2.8 days; SD = 3.4) past 30-day alcohol use compared to non-users (frequency: M = 0.3 days; SD = 0.7; p < 0.001). Older age (β = 0.82, p < 0.001), higher levels of alcohol dependence (β = 0.49, p < 0.001), and greater motives to use cannabis for purposes of social enhancement (β = 0.81, p < 0.001) were all associated with more frequent alcohol use regardless of group status.

In the abstinent group, there was an increase in the proportion of drinking days from baseline to week 1 (β = 0.09, p = 0.047), with abstinent CB users increasing from approximately 21% drinking days prior to initiation of abstinence to 34% drinking days in the first week following CB discontinuation. Drinking days did not significantly decline in the abstinent group between week 1 and 4 of abstinence (β = −0.02, p = 0.83); however, there was a trend for drinking frequency to begin to return to baseline levels at this final time point (β = −0.44, p = 0.06). Proportion of drinking days did not change over time for CB users who did not abstain from use (β = −0.02, p = 0.82) or non-users (β = −0.02, p = 0.94).

Conclusions: This study replicated prior reports that CB use is dose-dependently linked with level and severity of alcohol involvement in adolescents. CB abstinence appears to be associated with an initial increase in frequency of alcohol use. Although there is some signal that levels may return to baseline with extended periods of abstinence, clinicians should be aware that treating adolescent CB use may inadvertently result in a slight
uptick in alcohol consumption for some, and therefore both behaviors may warrant consideration as simultaneous treatment targets.

**Keywords:** Alcohol, Cannabis, Contingency Management, Adolescence

**Disclosure:** Nothing to disclose.

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**T243**

**The Effects of Systemic Endocannabinoid Manipulation on Social and Exploratory Behavior in Prairie Voles**

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**Background:** Anandamide is an endocannabinoid that contributes to certain aspects of social behavior, like play and reward, by binding to cannabinoid receptor type 1 (CB1). Most interesting is the recent discovery that anandamide may be mobilized by oxytocin receptor activation under certain contexts, particularly in the nucleus accumbens. Given the established role of oxytocin and the nucleus accumbens in the neurobiology of pair bonding, we investigated whether systemic administration of brain-permeable modulators of the endocannabinoid system could alter preferential partner contact in both male and female prairie voles.

**Methods:** We tested whether intraperitoneal administration of the neutral CB1 antagonist AM4113 (4.0, 8.0, and 16.0 mg/kg) or the anandamide hydrolysis inhibitor URB597 (5.0, 10.0, and 20.0 mg/kg) could prevent or facilitate partner preference formation in prairie voles (Microtus ochrogaster). We also administered an elevated plus-maze test and light/dark test to examine whether or not social effects were mediated by changes in anxiety-like behavior.

**Results:** AM4113 administration had no effect on preferential or indiscriminate contact when subjects were given a choice to interact with a familiar or novel mate. While URB597 also had no effect on preferential mate contact, females that received the low dose (5.0 mg/kg) increased social contact with both males and females. Moreover, URB597 increased social contact with novel mates of both sexes but had no effect on social contact with familiar mates. We also observed that URB597 increased social contact with novel mates in both male and female subjects.

**Conclusions:** Our results reveal that anandamide administration can modulate social behaviors in prairie voles.

**Keywords:** Tobacco Smoking, Human Genetics, Molecular Genetics

**Disclosure:** Nothing to disclose.

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**T244**

**Convergent Findings From Genetic and Functional Studies Implicate a Critical Role of rs148582811 in ARVCF for Nicotine Dependence**

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**Background:** Nicotine dependence (ND) is a chronic disease with substantial heritability. Various genes and variants have been implicated for ND in both European-American and African-American populations, but their involvement with smoking in Chinese population is largely unknown. Furthermore, molecular mechanisms underlying those identified susceptibility genes and variants for ND are rarely investigated.

**Methods:** In this study, we conducted a whole-genome sequencing study in 1,329 Chinese unrelated subjects with 805 heavy smokers and 524 age-matched non-smokers, which identified multiple loci associated significantly with smoking status and CPD at a genome-wide significance level ($P < 5 \times 10^{-8}$).

**Results:** Of these identified variants, seven were replicated in an independent sample with 3,744 subjects, among which SNP rs148582811 of Armadillo Repeat gene deleted in Velo-Cardio-Facial syndrome (ARVCF) gene was also overlapped with the peaks of enhancer associated markers in SK-N-SH cells based on the chromatin immunoprecipitation sequencing (ChIP-seq) data from the ENCODE Consortium. Furthermore, luciferase reporter assay demonstrated that the DNA region containing rs148582811 acted as an enhancer and the activity of which was significantly affected by rs148582811 genotype. Considering that rs148582811 is located in a potential enhancer region, we generated rs148582811-knockin (KI) and knockout (KO) HEK239T cell lines by using CRISPR/Cas9 genome editing technique and found that rs148582811 significantly regulated ARVCF expression in these edited cells. We also evaluated the mechanism underlying the rs148582811 and found that a transcription factor XRC5 binds to rs148582811 based on DNA pull-down and electromobility shift assays. Finally, we demonstrated that XRC5-knockdown (KD) decreased the expression of ARVCF in SH-SY5Y cells.

**Conclusions:** In sum, these findings from genetic and functional studies strongly suggest the involvement of rs148582811 of ARVCF in the etiology of ND and likely other addictions.

**Keywords:** Alcohol, Cannabis, Contingency Management, Adolescence

**Disclosure:** Nothing to disclose.

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**T245**

**Activation of ACCumbens SHELL Versus Core Dopamine Transmission Under Responding for IV Heroin Depends on Modus Operandi (Nose Poking Versus Lever Pressing)**

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**Background:** It has been shown by brain microdialysis and by a variety of in vivo techniques including PET and fast cyclic voltammetry, that drugs of abuse and palatable food stimulate dopamine (DA) transmission in the nucleus accumbens/ventral striatum of rats and humans. Our laboratory has reported that non-contingent administration of different drugs of abuse to drug naive rats, preferentially increases dialysate DA in the accumbens shell as compared to the core. Palatable food as well as sucrose given non-contingently to naive rats also preferentially increase dialysate DA in the shell of naive rats. A fortiori, in rats fully trained to respond for sucrose by nose poking, responding for sucrose is associated to a selective and robust increase of DA in the shell (Bassareo et al., 2015a). In contrast however, and quite unexpectedly, responding for sucrose by lever pressing is asso multiple loci associated significantly with smoking status and CPD at a genome-wide significance level ($P < 5 \times 10^{-8}$).

**Results:** Of these identified variants, seven were replicated in an independent sample with 3,744 subjects, among which SNP rs148582811 of Armadillo Repeat gene deleted in Velo-Cardio-Facial syndrome (ARVCF) gene was also overlapped with the peaks of enhancer associated markers in SK-N-SH cells based on the chromatin immunoprecipitation sequencing (ChIP-seq) data from the ENCODE Consortium. Furthermore, luciferase reporter assay demonstrated that the DNA region containing rs148582811 acted as an enhancer and the activity of which was significantly affected by rs148582811 genotype. Considering that rs148582811 is located in a potential enhancer region, we generated rs148582811-knockin (KI) and knockout (KO) HEK239T cell lines by using CRISPR/Cas9 genome editing technique and found that rs148582811 significantly regulated ARVCF expression in these edited cells. We also evaluated the mechanism underlying the rs148582811 and found that a transcription factor XRC5 binds to rs148582811 based on DNA pull-down and electromobility shift assays. Finally, we demonstrated that XRC5-knockdown (KD) decreased the expression of ARVCF in SH-SY5Y cells.

**Conclusions:** In sum, these findings from genetic and functional studies strongly suggest the involvement of rs148582811 of ARVCF in the etiology of ND and likely other addictions.

**Keywords:** Tobacco Smoking, Human Genetics, Molecular Genetics

**Disclosure:** Nothing to disclose.
Methods: Male Sprague-Dawley rats of 9 weeks were implanted with a catheter in the jugular vein and a guide cannula aimed at the NAc shell or core. After 10 days recovery separate groups of rats were trained to acquire heroin self-administration (0.05 mg/kg) under NP and LP respectively as modus operandi, for 10 days under FR1 schedule, with additional 5 days under FR5 schedule for FR5 experiments. At the end of acquisition, DA was monitored by microdialysis in the shell and core of LP and NP groups during 3 consecutive daily sessions: first day under responding for heroin (0.05 mg/kg) on FR1 and FR5; second day under extinction, by substituting the reinforcer with saline while maintaining all the other cues; on the third day, a set of LP and NP animals DA was monitored in shell and core under responding for 0.025 mg/kg of heroin while in another set of rats, DA was monitored under non-contingent administration of 0.05 mg/kg heroin, in both FR1 and FR5 trained animals. After completion of microdialysis sessions rats were euthanized for histological assessment of microdialysis probe location. Behavioural data were analyzed as cumulative responses over sessions by tree-way ANOVA with modus operandi (NP vs LP) and response (active vs inactive) as between subject factors and sessions as repeated measure. Microdialysis data were analyzed as percent changes of dialysate DA compared to baseline by tree-way ANOVA with modus operandi (NP vs LP) and area (shell vs core) as between subject factors and time as repeated measure. Tukey’s post hoc test with p < 0.05 as significant statistical level was applied when interaction of factors was statistically significant.

Results: The number of responses (FR1: 7 ± 0.4 active NP/h vs 6.9 ± 0.3 active LP/h; p > 0.05; FR5: 31.38 ± 1.56 active NP/h vs 31.24 ± 2.31 active LP/h; p > 0.05) and the intake (FR1: 0.35 ± 0.02 mg/kg for NP group vs 0.35 ± 0.01 mg/kg for LP group; FR5: 0.31 ± 0.02 mg/kg for NP group vs 0.31 ± 0.02 mg/kg for LP group, p > 0.05) did not differ between the LP and NP groups. However, in the NP group, responding for heroin increased DA over basal by 80% in the shell and by 25% in the core (Tukey’s test: p < 0.05), while in the LP group DA similarly increased in the shell and core (about 60% over basal), both on FR1 and FR5 schedules. During extinction session DA increased in the core of LP group under FR5 schedule (Tukey’s test: p < 0.01) by 75% over basal, while no changes were observed on FR1 schedule in the shell nor in the core of NP and LP groups. When heroin dose was halved (0.025 mg/kg), the NP group showed a larger increase of DA in the shell (max 130%) compared to the core (max 50%; p < 0.05) while in the LP group DA increased by a similar extent (about 70%) in the NAc shell and core. Finally, under non-contingent presentation of heroin DA levels increased to a similar extent in the shell and in the core of both LP (by about 75%) and NP groups (by about 100%).

Conclusions: The main finding of the present study is that the pattern of changes in dialysate DA in the accumbens shell and core during responding for iv heroin is related to the response modality utilized to obtain the drug. Namely, if response is NP, DA is activated preferentially in the shell while if response is LP, shell and core DA are activated to a similar extent. Moreover, the increase in core DA under LP is larger than under NP while the reverse applies to shell DA. It appears that under LP core DA is recruited, consistent with a role of core DA in performing complex, unnatural, learned motor responses that do not belong to the species-specific repertoire, as is instead the case of NP. Activation of shell DA under NP is consistent with encoding by shell DA of the rewarding properties of the drug stimulus. The present results extend to a drug of abuse like heroin the observations made in rats responding for sucrose (Bassareo et al., 2015b) and suggest that the dependence of the pattern of DA responsiveness from modus operandi reflect basic differences in the function of DA in the shell versus core.

Keywords: Heroin Self-Administration, Nucleus Accumbens Core, Nucleus Accumbens Shell

Disclosure: Nothing to disclose.

T246

Can Gene Expression Data Identify Treatments for Alcohol Use Disorders?

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Background: Alcohol Use Disorders (AUD) are chronic, relapsing conditions and are a major public health problem. Though recovery is possible, there are few pharmaceutical treatments available to aid in the recovery process. Systems-based computational strategies that integrate gene expression signatures of pharmaceuticals and disease states have shown promise for identifying compounds that treat disease symptoms (termed in silico gene mapping or connectivity mapping). In silico gene mapping compares the gene expression signatures of disease and pharmaceuticals to find compounds (or combination of compounds) that oppose the disease-state's molecular disruption. The basis of the compound selection algorithm is that the compounds with signatures that oppose signatures of the disease state will `correct' the biological functions of the system and ameliorate disease phenotype. We used this approach to identify novel compounds that reduced ethanol drinking in continuous and intermittent alcohol behavioral tests in rodents.

Methods: We constructed gene network signatures using brain gene expression data from human patients and matched controls (N = 6-73 per condition) as well as brain gene expression data from a genetic rodent model of high ethanol drinking, High Alcohol Drinking (HAD1) and Low Alcohol Drinking (LAD) rats (N = 9-10 per condition), and C57BL/6J mice after alcohol exposure (N = 9-12 per condition). We compared the AUD, genetic selection for high ethanol drinking, and alcohol-induced gene expression signatures to those of compounds in the L1000 database to find compounds with opposing gene expression signatures. We also reasoned that it may be sufficient for a treatment to target genes in the same biological pathway perturbed by AUD (but not necessarily the exact same genes). Thus, we identified the biological pathways disrupted in AUD brain using gene set enrichment analysis of KEGG pathways, and selected compounds in the L1000 database that targeted those pathways in an opposing manner. We tested some of the top compounds in HAD-1 rats and C57BL/6J mice to assess their effects on alcohol and water intake in two bottle choice drinking tests (N = 5-10 per condition).

Results: Preliminary data suggest that our drug selection algorithm successfully identified compounds that modulated ethanol intake in rodents in two bottle choice drinking tests. These included trichostatin-A (p < 0.001), ticiclibine (p < 0.01), berberine (p < 0.001), and SN-38 (p < 0.01). Our analysis pointed to pathways by which these compounds could be reducing ethanol intake, including complement and coagulation cascade, MAPK, and area (shell vs core) as between subject factors and time as repeated measure. Tukey’s post hoc test with p < 0.05 as significant statistical level was applied when interaction of factors was statistically significant.

Results: The number of responses (FR1: 7 ± 0.4 active NP/h vs 6.9 ± 0.3 active LP/h; p > 0.05; FR5: 31.38 ± 1.56 active NP/h vs 31.24 ± 2.31 active LP/h; p > 0.05) and the intake (FR1: 0.35 ± 0.02 mg/kg for NP group vs 0.35 ± 0.01 mg/kg for LP group; FR5: 0.31 ± 0.02 mg/kg for NP group vs 0.31 ± 0.02 mg/kg for LP group, p > 0.05) did not differ between the LP and NP groups. However, in the NP group, responding for heroin increased DA over basal by 80% in the shell and by 25% in the core (Tukey’s test: p < 0.05), while in the LP group DA similarly increased in the shell and core (about 60% over basal), both on FR1 and FR5 schedules. During extinction session DA increased in the core of LP group under FR5 schedule (Tukey’s test: p < 0.01) by 75% over basal, while no changes were observed on FR1 schedule in the shell nor in the core of NP and LP groups. When heroin dose was halved (0.025 mg/kg), the NP group showed a larger increase of DA in the shell (max 130%) compared to the core (max 50%; p < 0.05) while in the LP group DA increased by a similar extent (about 70%) in the NAc shell and core. Finally, under non-contingent presentation of heroin DA levels increased to a similar extent in the shell and in the core of both LP (by about 75%) and NP groups (by about 100%).

Conclusions: The main finding of the present study is that the pattern of changes in dialysate DA in the accumbens shell and core during responding for iv heroin is related to the response modality utilized to obtain the drug. Namely, if response is NP, DA is activated preferentially in the shell while if response is LP, shell and core DA are activated to a similar extent. Moreover, the increase in core DA under LP is larger than under NP while the reverse applies to shell DA. It appears that under LP core DA is recruited, consistent with a role of core DA in performing complex, unnatural, learned motor responses that do not belong to the species-specific repertoire, as is instead the case of NP. Activation of shell DA under NP is consistent with encoding by shell DA of the rewarding properties of the drug stimulus. The present results extend to a drug of abuse like heroin the observations made in rats responding for sucrose (Bassareo et al., 2015b) and suggest that the dependence of the pattern of DA responsiveness from modus operandi reflect basic differences in the function of DA in the shell versus core.

Keywords: Heroin Self-Administration, Nucleus Accumbens Core, Nucleus Accumbens Shell

Disclosure: Nothing to disclose.
systemic lupus erythematosus, and oxidative phosphorylation pathways.

Conclusions: Overall, our findings suggest that, although still in its infancy, in silico gene mapping can successfully guide drug discovery and repurposing efforts for AUD, and possibly other CNS diseases.

Keywords: Gene Expression, Systems Pharmacology, Alcoholism

Disclosure: Nothing to disclose.

T247
Midbrain Functional Connectivity and Striatal Dopamine D2-type Receptor Availability: Associations With Impulsivity and Craving in Stimulant Users

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Background: Stimulant use is associated with higher self-reported impulsivity and risk-taking, striatal dopamine D2-type receptor (DRD2/3) deficits, and atypical mesocorticimbic resting-state functional connectivity (RSFC, Kohno et al., 2014). Interrelationships between these measures have been observed in other studies, with a negative correlation between striatal DRD2/3 availability and impulsivity found in methamphetamine (MA) users (Lee et al., 2009) and dysregulation of striatal-insula/cortical connectivity observed in impulsive cocaine users (McHugh et al., 2013; Hu et al., 2015). Self-reported drug craving, which persists in abstinent stimulant users, is also positively correlated with neural activity in the nucleus accumbens (NAc), anterior cingulate, putamen, and inferior frontal-orbital region (Risinger et al., 2005). However, one study of MA users failed to observe an association between striatal DRD2/3 availability and craving (Morales et al., 2013). Additionally, associations between DRD2/3 availability, midbrain functional connectivity, and self-reported drug craving, impulsivity, and risk-taking have not been assessed in the same sample of subjects. Assessment of these variables in the same individuals may clarify inconsistent findings in the literature.

Methods: Nineteen stimulant users (17 men and 2 women) underwent resting-state fMRI and completed self-report measures of impulsivity, risk-taking, and craving for their stimulant of choice (cocaine, n = 9; MA, n = 10). Fourteen participants also underwent Positron Emission Tomography (PET) scans. All participants met criteria for Stimulant Use Disorder (DSM-5 MINI), were in an inpatient treatment program, and were abstinent from substances for less than 2 months.

The Barratt Impulsiveness Scale (BIS; Patton et al., 1995) and the Domain-Specific Risk-Taking Scale (Blais & Weber, 2006) were administered to assess total self-reported impulsivity and risk-taking, respectively. The MA Craving Questionnaire – Brief (MCQ-Brief) and Cocaine Craving Questionnaire – Brief (CCQ-Brief; Sussner et al., 2006) were used to assess craving for the participants’ stimulant of choice.

To measure resting-state functional connectivity (RSFC), resting-state fMRI was acquired on a Siemens Prisma 3-T scanner using multiband imaging (MB = 8, TR = 0.8). Images were acquired for 10 minutes while participants viewed a black screen. Motion corrected data were cleaned for motion-related artifacts by using “scrubbing” procedures and regressing out 24 motion parameters and eigenvalues of the white matter and cerebrospinal fluid time-series (via aCompCor50). Data were bandpass filtered (0.009–0.08 Hz). T1-weighted structural scans were also acquired.

DRD2/3 availability (binding potential, BPND) was measured with [18F]-fallypride PET (5 mCi bolus injection). Reconstructed PET data acquired on a Siemens Biograph mCT were combined into 16 images containing data averaged across 10 minutes and corrected for motion with FSL MCFLIRT. The images were co-registered with FSL FLIRT to T1-weighted structural scans. Bilateral VOIs in the caudate nucleus and NAc were derived from MPRAGE images with FSL FIRST. Time-activity curves were imported into PMOD Kinetic Modeling to calculate BPND with the simplified reference tissue model using the cerebellum as a reference region.

Seed-based analyses using the left and right ventral temporal area (VTA) as seeds tested associations between RSFC, DRD2/3 BPND, and self-report measures. The time series from each seed was entered as a covariate in two whole-brain, voxel-wise generalized linear models, resulting in a contrast (connectivity) map for each seed. Each of these maps was then entered into a whole-brain, voxel-wise group analysis with covariates of interest (BIS, DOSPERT, and MCQ/CCQ-Brief). In separate models, we included NAc DRD2/3 BPND and the total BIS score as covariates. Resulting statistical maps were corrected for multiple comparisons using cluster-based statistics (height threshold of Z > 2.3; cluster threshold of p < 0.05). Correlational analyses controlling for sex and age tested for associations between the self-report measures and striatal DRD2/3 BPND.

Results: Whole-brain voxel-wise analyses showed that RSFC between the right VTA and medial prefrontal cortex (mPFC) was positively correlated with right NAc BPND. Left VTA – mPFC RSFC was positively correlated with self-reported total impulsivity on the BIS. Left VTA – insula/precuneus RSFC and right VTA – mPFC RSFC was positively correlated with self-reported craving. Partial correlations showed that NAc DRD2/3 BPND was negatively correlated with total impulsivity measured by the BIS (r = −0.73, p = 0.012) and stimulant craving on the MCQ/CCQ-brief (r = −0.69, p = 0.019).

Conclusions: Preliminary evidence from this small sample of stimulant users indicates that functional connectivity between components of the mesocorticimbic dopamine pathway (VTA and mPFC) is positively correlated with striatal D2-type receptor availability, impulsivity, and craving. Together, with our observations that striatal DRD2/3 BPND is also correlated with impulsivity and craving, these findings suggest that the heightened impulsivity and craving observed in chronic stimulant users is linked to system-level activity within the mesocorticimbic circuit – and that deficits in DRD2/3 availability may contribute to dysregulated signaling between the striatum and prefrontal cortex.

Keywords: Positron Emission Tomography Imaging, Resting State Functional Connectivity, Impulsivity, Craving, Stimulants

Disclosure: Nothing to disclose.

T248
Cocaine Withdrawal Symptoms Predict Response to Topiramate in Patients With Cocaine Use Disorder
Kyle Kampman*, Mila Tamminga, Kevin Lynch

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Background: Topiramate is a novel antiepileptic drug that increases GABAergic activity in the brain, as well as antagonizes the AMPA / kainate subtype of glutamate receptors. Topiramate has been shown to reduce anxiety, including alcohol withdrawal...
induced anxiety in animal models. In clinical trials, topiramate has been shown to reduce cocaine use in cocaine dependent patients. In a clinical trial of topiramate involving 170 subjects with comorbid cocaine and alcohol dependence, topiramate was found to be more effective in subjects with more severe symptoms of cocaine withdrawal measured by the Cocaine Selective Severity Assessment (CSSA). The CSSA is an 18-item scale measuring cocaine withdrawal symptoms. This secondary analysis is intended to identify which particular withdrawal symptoms were associated with topiramate effect and to explore the response of individual withdrawal symptoms to topiramate over the course of the trial.

Methods: The study was a double-blind, placebo-controlled, 14-week trial involving 170 DSM-IV alcohol and cocaine dependent, treatment-seeking subjects. After achieving a period of abstinence from both alcohol and cocaine, subjects were randomized to topiramate, titrated over 8 weeks to 300 mg daily, or identical placebo capsules. Medications were continued at full dose for 5 weeks then tapered during the last week of the trial. Primary outcome measures included alcohol use, measured by the timeline follow back, and cocaine use, measured by self-report and thrice weekly urine drug screens. CSSA scores were measured weekly.

Results: Topiramate-treated subjects, compared to placebo-treated subjects, were more likely to be abstinent from cocaine (20%vs. 6%) during the last three weeks of the trial. There was no difference in drinking between the two groups. Subjects with CSSA scores in the highest tertile (corresponding to patients with CSSA score above 18) on the day of randomization appeared to exhibit larger topiramate effects than subjects with lower scores. Individual items from the CSSA that predicted a better response to topiramate included: hypersomnia, inactivity and lack of energy.

Conclusions: Patients with more severe cocaine withdrawal symptoms, particularly patients with more severe anergia, inactivity and hypersomnia appeared to have responded better to topiramate. Further exploration of the effects of cocaine withdrawal symptoms on response to topiramate in subjects with cocaine and alcohol dependence will be presented at the meeting.

Keywords: Cocaine, Medication Assisted Treatment, Alcohol Withdrawal Syndrome, GLT-1, xCT, Glutamate, Withdrawal

Disclosure: Nothing to disclose.

T249

Neural Correlates of Ethanol-Induced Changes in Aggressive Behavior in Healthy Social Drinkers

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Background: The correlation between alcohol use and aggression has been well-documented. Evidence to date suggests that the link between impulsivity and alcohol use disorder is reciprocal, with impulsivity contributing to alcohol use, and heavy alcohol use increasing impulsive behavior. The positive relationship between alcohol consumption and impulsivity, and how it interacts with aggression requires further study. Previous fMRI studies indicate that the DLPFC, OFC, and limbic brain regions correlate with violent behavior and alcohol addiction when studied separately.

Methods: We recruited N = 12 healthy social drinkers (that did not heavy drink), six male and six female, who gave informed consent to participate. Subjects each participated in three fMRI sessions in which they were administered (in single blind fashion) oral alcohol at three doses; none (placebo), moderate (intended to bring them to a BrAC of 0.02 g/210L), and high dose (intended to bring them to a BrAC of 0.06 g/210L). After being dosed with alcohol, subjects participated in the computerized Point Subtraction Aggression Protocol (PSAP) task while fMRI scans were being acquired. Whole brain voxel analysis (SPM12) was used to compare the difference in post-provation BOLD activation between the high dose alcohol and placebo alcohol conditions. The a priori hypothesis was that amygdala activation would positively correlate with the Aggressive Press Ratio (i.e., the number of aggressive button presses divided by the number of monetary reinforced button presses) in the setting of alcohol exposure.

Results: After High-dose alcohol (relative to placebo), there was a statistically significant (FWE corrected two-tailed cluster p = 0.026) positive regression of the change in Post-Provocation activation on Aggressive Press Ratio in portions of right (R) rectus gyrus, R superior orbital frontal g, R amygdala, R inferior orbital frontal g, R putamen (including portions of ventral striatum), R olfactory gyrus, and R globus pallidus. In addition, there was a trend significant (FWE corrected two-tailed cluster p = 0.074) positive regression of the change in Post-Provocation activation on Aggressive Press Ratio in portions of L inferior frontal g pars opercularus, L inferior frontal g pars triangularus, and L insula.

Conclusions: After high dose alcohol, increased aggressive responding in social drinkers was associated with greater post-provocation activation in areas of the brain associated previously with aggression. Other areas in which activation correlated with increased aggressive behavior observed in the study correspond to circuitry thought to be involved with the cortical-basal ganglia reward circuit which integrates reward-based executive function with limbic centers and their related functions. These findings suggest that alcohol causes disinhibition of that circuitry which can result in increased aggressive behavior in response to provocative environmental stimuli.

Keywords: Alcohol, Aggression, Impulsivity, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

T250

Acute Effects of Alcohol on Local Functional Brain Connectivity in Healthy Social Drinkers

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Background: Individual variation in the subjective response to alcohol can predict subsequent use. However, the mechanisms underlying this variation in humans are largely unknown. Here, we used a pharmacological fMRI approach to determine how alcohol influences local and long-distance functional brain connectivity in healthy humans, and how this variation relates to alcohol’s acute subjective effects.

Methods: Healthy social drinkers (N = 32; 50% women) completed two sessions consisting of alcohol or placebo administration under blinded conditions on separate days. Thirty minutes after oral alcohol consumption (0.06g/kg), participants completed an fMRI session consisting of resting state, reward (e.g. alcohol cue) processing, and threat processing. Breath-alcohol concentrations and self-reported subjective mood and drug effects were repeatedly assessed throughout the sessions.
Results: We used regional homogeneity (ReHo) to assess local coherence of brain activity during rest. We found that alcohol induced increases in functional connectivity in certain areas (i.e., bilateral visual areas, left posterior insula) while it reduced local coherence in other areas (i.e., right putamen, right dorsomedial prefrontal cortex, bilateral dorsolateral prefrontal cortex). An exploratory factor analysis yielded 3 factors of ReHo clusters, two of which were related to negative effects of alcohol, such as sedation ($r = 0.54$, $p = 0.003$) and anxiety ($r = 0.41$, $p = 0.030$). Current analyses are underway to determine how alcohol influences local connectivity during threat and reward processing, as well as how local changes in connectivity influence long-distance connectivity.

Conclusions: We provide novel data showing that individual variation in sensitivity to the negative effects of alcohol may be driven by changes in local functional brain connectivity in healthy, non-dependent humans. This could represent a previously unknown protective factor against the development of problematic alcohol use. Future work will assess how changes induced by acute alcohol exposure relate to those induced by repeated exposure in patients with an alcohol use disorder.

Keywords: Alcohol Sensitivity, Functional MRI (fMRI), Subjective Effects, Resting State Intrinsic Connectivity, Alcohol Drinking

Disclosure: Nothing to disclose.

T251

Examination of Traits, Consumption Patterns and Pharmacodynamic Responses to Alcohol in High-Intensity Binge Drinkers

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Background: High intensity drinking, i.e., drinking alcohol at multiple levels of the binge threshold (5+ drinks for males (M), 4+ drinks for females (F)) has been identified as a significant risk factor for developing alcohol problems. High-intensity bingers have been shown to suffer greater negative consequences related to excessive drinking, and have higher rates of alcohol use disorder and associated comorbidities. However, little is known about potential traits and personality characteristics of this high-risk group. Also, differences in pharmacodynamic sensitivity to alcohol in this group have not been investigated. This study aims to evaluate personality traits in high-intensity binge drinkers as well as consumption patterns and subjective response in a human laboratory intravenous alcohol self-administration (IV-ASA) study.

Methods: This study included a sample of non-treatment-seeking alcohol drinkers ($n = 1180$), classified into 4 groups: Level 0 (no binges), Level 1 (F: 4-7, M: 5-9 drinks) Level 2 (F: 8-11, M: 10-14 drinks) and Level 3 (F: 12+, M: 15+ drinks), based on the highest drinking session in the 90-day Timeline Followback assessment. Several trait measures (UPPS-P Impulsivity Scale, Barratt's Impulsiveness Scale, Buss Perry Aggression Questionnaire) were examined as predictors of binge drinking behavior. A subset of participants ($n = 200$) completed a free-access intravenous self-administration (IV-ASA) study to assess alcohol consumption and subjective responses on measures of stimulation, sedation, intoxication and craving in a controlled laboratory setting.

Results: Alcohol consumption patterns showed significant differences between groups, with number of drinking days and average drinks/drinking day all increasing with binge-intensity level. There were also significant differences for impulsivity and aggression measures among binge-intensity groups ($0 = 1 < 2 = 3$). These effects were consistent in both males and females. During IV-ASA, greater consumption (number of alcohol rewards, peak level) was proportional to the binge-intensity level ($0 < 1 < 2 = 3$). Results indicated that high-intensity bingers were more likely to consume alcohol to binge level (0.08 g%) more rapidly in the laboratory session. Greater urge for alcohol following priming and lower sensitivity (level of response) was also seen in high-intensity binge drinkers.

Conclusions: High-intensity binge drinkers reported higher impulsiveness and aggression compared to non-bingers. They also showed greater alcohol consumption overall, and achieved binge-level exposures more rapidly during the IV-ASA session. These findings suggest a behavioral profile and underlying pharmacodynamic mechanism associated with greater risk for developing alcohol use disorder in high-intensity binge drinkers.

Keywords: Binge Drinking, Alcohol Sensitivity, Impulsivity, Aggression, Alcohol Use Disorder

Disclosure: Nothing to disclose.

T252

Selective Attention Processing is Both Helped and Hindered Across Subgroups of Nicotine Smokers

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Background: During acute nicotine abstinence, smokers experience a constellation of symptoms that together constitute the nicotine withdrawal syndrome (NWS). These symptoms include affective and cognitive disruptions that often lead to resumption of smoking via negative reinforcement. A better understanding of the neurobiological basis of the NWS is an important step towards more effective cessation treatments. While the affective symptoms of withdrawal are well characterized, the cognitive disruptions are more poorly understood. Recent reviews of the literature on cognitive disruptions during acute nicotine abstinence report small effect sizes and low test-retest reliability across the cognitive paradigms tested. To the extent the literature converges, a consistent finding appears to be increases in lapses of attention, as characterized by reaction time (RT) variability, errors of omission, and decreased working memory performance. Concurrent neuroimaging studies of abstinence-related cognitive disruption are relatively rare and report similar inconsistencies. The current study interrogates cognitive disruptions via behavioral performance and task-evoked fMRI activation in a longitudinal sample of cigarette smokers.

Methods: Data were collected from N = 44 smokers on two occasions—during baseline smoking satiety and again following ~48-hours of full, biochemically assessed nicotine abstinence. All participants completed a 25-minute version of the parametric flanker task (PFT) during MRI scanning (3T Siemens Trio/Prisma, 2s TR). The PFT is a modified version of the classic Eriksen flanker task designed to represent varying levels of demand for cognitive control on a trial-by-trial basis. Further, the PFT is a 2-choice forced alternative task, requiring a button press on every trial. Thus, RT and counts of errors of commission and omission are characterized at a single trial level. As compared to other tasks of cognitive processing during abstinence (i.e. N-back, Go/No-Go, RVIP), PFT allows for characterization of behavioral performance at higher temporal resolution.
Results: Behavior: As demand for control increased, an expected slowing in RT and a reduction of adjusted accuracy (correct responses/total commission + correct responses) was observed. However, no SESSION (sated/abstinent) effects were observed. That said, in the most difficult, high demand for control condition, a bimodal distribution of adjusted accuracy was observed: n = 19 participants performed the task at >70% accuracy (avg 88.93%, sd = 6.28) and n = 25 participants performed at <70% accuracy (avg 51.03%, sd = 6.09). Behavioral analysis from this high/low performer dichotomization found a GROUP (high/low performer) * SESSION interaction for errors of omission such that high performers show increased errors of omission following 48-hour abstinence across all demand conditions.

fMRI: Whole brain neuroimaging results (p-voxel < 0.001; p < 0.05 corrected) showed a GROUP effect in the highest demand condition such that high performers showed increased activation in the left superior parietal lobule (SPL, MNI xyz -24, -70, 50) as compared to low performers on correct trials in both the sated and abstinent states.

Conclusions: Behavioral analyses of task accuracy in a high cognitive demand selective attention task (the PFT) identified two subgroups of smokers: one able to do the task at a high level and the other unable to perform the task at an above chance level. Importantly, this behavioral difference in task performance was observed independent of the nicotine smoking condition, suggesting a trait-level facilitatory effect in a large sample of smokers. The observed behavioral improvement in a subgroup of smokers coincided with increased activation in the left SPL, which has been previously implicated in selective attention during flanker task performance. Moreover, this region is a node in the canonical fronto-parietal control network as well as in the rich club network architecture of the brain. Thus, the SPL is uniquely situated to play an outsized role in coordinating attentional response in selective attention tasks like the PFT. Importantly, these GROUP effects were observed in an ROI distinct from prefrontal cortical regions more traditionally associated with cognitive control (i.e. salience or cingulo-opercular control network nodes), suggesting the observed effects are distinct from deficits in prefrontal processing.

Paradoxically, the observed facilitatory task effects are separable from the attentional disruptions associated with the NWS. The high-performance subgroup displaying enhanced SPL task activation across nicotine states was more susceptible to disruptions of sustained attention during abstinence while the low performers appear to be protected from disruptions in attentional processing during abstinence. Thus, the current results may help explain previous evidence of inconsistent effects of withdrawal on cognitive processes. Further, a focus on selective attention and SPL dysfunction may suggest new avenues on which to fractionate smokers to study and mitigate the cognitive disruptions associated with the NWS.

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Keywords: Withdrawal, Nicotine, Attention, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

Elevated Glutamate Levels in Thalamus are Associated With Poor Diffusion and Impulsivity in Alcohol Use Disorder

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Background: Alcohol use disorder (AUD) is associated with impaired cognitive abilities and enhanced impulsivity, but its underlying neural pathways remain largely unexplored. While elevated glutamate in the anterior cingulate cortex (ACC) in patients with cocaine use disorder has been linked to impulsivity in previous research, this has not been investigated in AUD.

Methods: Here, we used proton magnetic resonance spectroscopy (1H-MRS) to investigate the correlation between glutamate levels in the ACC and thalamus with impulsivity in n = 15 patients with AUD and n = 14 age-, gender-, and BMI-matched controls. Moreover, we used a multimodal imaging approach to associate glutamate, myo-inositol (mI) and N-acetylaspartate (NAA) concentrations in ACC and thalamus with fractional anisotropy (FA) in these regions using diffusion-weighted imaging (DWI), which has been described as marker of neuroinflammation.

Results: AUD compared to controls had higher scores on the CAARS impulsivity scale (t = 2.2, p = 0.03), and higher levels of glutamate in thalamus (t = 2.2, p = 0.04), but not in ACC (p = 0.3). Glutamate levels in both thalamus and ACC correlated positively with CAARS impulsivity (r = 0.58 p = 0.03, and r = 0.55 p = 0.036, respectively). Moreover, FA was lower in AUD than controls in thalamus (t = 2.1, p = 0.04) but not in ACC (p = 0.3). Within the AUD group only, glutamate levels and FA correlated at trend level in thalamus (r = −0.52, p = 0.058) but not in ACC (r = −0.43, p = 0.11). There were no group differences for mI or NAA, and no associations with FA in thalamus or ACC.

Conclusions: In sum, in AUD participants we show that in the thalamus, but not in ACC, glutamate was elevated and associated with increased impulsivity whereas FA was reduced and negatively associated with increased glutamate (trend level). These findings provide further evidence for the vulnerability of the thalamus to the neurotoxic effect of chronic alcohol and implicate these effects in impulsivity.

Keywords: Alcoholism, Glutamate, Impulsivity

Disclosure: Nothing to disclose.

Identifying Neural Correlates of Nicotine Withdrawal in Mice: The Neuregulin Signaling Pathway

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Background: Addiction to nicotine and the ability to quit smoking are influenced by genetic factors. Therefore, it is important to understand how genes and drugs of abuse mechanistically impact each other. Previous work from our lab has shown that cAMP response element-binding protein (CREB) activity in the ventral hippocampus (Vhipp) mediates anxiety-like responses in mice during nicotine withdrawal (WD) (Fisher et al., 2017). High throughput chromatin immunoprecipitation sequencing (ChIP-seq) studies determined that WD from nicotine differentially modulates CREB binding to a gene called Neuregulin-3 (nrg3). Interestingly, the Neuregulin Signaling Pathway, which has been widely implicated in schizophrenia, has also been recently linked to nicotine dependence in humans. For example, work from our lab (Turner et al., 2014) and that of our colleagues (Loukola et al., 2014) has shown that polymorphisms within two genes in the
Neuregulin Signaling Pathway, NRG3 and its cognate receptor ERBB4, have been linked to failed smoking cessation. Nrg3, a neural-enriched epidermal growth factor-like protein located on pyramidal cells binds to and activates ErbB4 receptors located on GABAergic interneurons. With high hippocampal expression, the interaction between these two synaptic proteins plays an important role in synaptogenesis and overall plasticity at these excitatory/inhibitory synapses within the Vhipp. This work aims to evaluate how Neuregulin signaling in the VH may impact nicotine WD-induced affective phenotypes in mice and the underlying physiological and biochemical changes that occur.

Methods: Quantitative PCR (qPCR). Western blotting and fluorescent in situ hybridization experiments were conducted on Vhipp tissue from male and female 8–10 week old B6129-F1 mice implanted with pulsatile osmotic minipumps and separated into treatment groups (saline, nicotine (12 mg/kg/day), 24h WD) (N = 10-12). ErbB4-floxed animals were generated for behavioral testing and functional experimentation using whole cell patch clamp electrophysiology and Ca2+ imaging techniques. Both male and female animals were stereotactically injected with AAV-CRE or AAV-GFP (control) into the Vhipp at 6 weeks of age to induce temporal and spatial knockout of erbB4 (N = 12-15).

Results: qPCR and Western blotting experiments established that Nrg3 and ErbB4 are upregulated at the 24h WD time point in the Vhipp, with expression returning to baseline by 1-week post WD. Furthermore, conditional Vhipp deletion of ErbB4 blocks WD-induced anxiety-like behaviors as measured by the Novelty-induced Hypophagia test and the Open Field Exploratory test, two well-validated behavioral models of anxiety-like behaviors in mice. This phenotype is accompanied by decreased levels of inhibitory GABAergic release and altered network clustering of excitatory pyramidal cells within the ventral CA1, an area enriched in Nrg3 and ErbB4 mRNAs sensitive to nicotine WD.

Conclusions: These data support a model of aberrant Nrg3-ErbB4-induced plasticity underlying nicotine withdrawal-induced behaviors. We found that disruption of Vhipp Nrg3-ErbB4 signaling attenuates WD-induced anxiety-like phenotypes through altering GABAergic modulation of CA1 pyramidal cell activity. This suggests blocking Nrg3 activation of ErbB4 at GABAergic synapses weakens inhibitory inputs and regulation of excitatory pyramidal cell activity and prevents WD-induced synaptic remodeling. Further examination of downstream signals of ErbB4 activation may lead to the identification of potential targets for treating nicotine withdrawal symptomology.

Keywords: Nicotine Withdrawal, Neuregulin-3, Nicotine Addiction
Disclosure: Nothing to disclose.

T255

Alcohol Dependence Alters Noradrenergic Influence Over the Medial Prefrontal Cortex

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Background: Alcohol abuse and dependence represents a major clinical burden in the US. Alcoholics have reduced prefrontal cortex volumes and deficits in medial prefrontal cortex (mPFC)-related tasks (e.g. risk-taking, emotional processing) that are linked to a loss of “top-down” control over subcortical regions. Within the mPFC, the infralimbic cortex (IfL) is implicated in anxiety-like behaviors and excessive drinking, suggesting that dependence-induced dysregulation of specific IfL-subcortical projections may contribute to the negative affective state of addiction. Thus, the challenge of current and future studies is to identify key mPFC-subcortical neuroadaptations that drive specific aspects of dependence.

The IfL projects to the central amygdala (CeA), and we propose that chronic ethanol-induced neuroadaptation of this circuit may lead to CeA overactivation, a hallmark of dependence. Moreover, the noradrenergic system has been implicated in the excessive drinking of alcoholics and it tightly regulates cognitive function. Therefore, we hypothesize that alcohol dependence increases noradrenergic influence over the IfL to dysregulate its output to the CeA and drive withdrawal-induced cognitive dysfunction and anxiety-like behavior.

Methods: We induced alcohol dependence by exposing male and female C57BL/6J mice to the chronic intermittent ethanol vapor-2 bottle choice paradigm (CIE-2BC). Slice electrophysiology recordings were performed in IfL layer V pyramidal neurons that project to the CeA, using an intra-CeA injection of a retrograde fluorescent tracer, and analyzed using Mini Analysis software (Synaptosoft Inc, Fort Lee, NJ). Cognitive function and anxiety-like behavior were assessed using the Barnes maze, digging and novelty-induced suppression of feeding tasks, and data were analyzed with EthoVision software (Noldus Information Technology, Wageningen, The Netherlands). A minimum of 6 cells from 4 animals were used per group for the electrophysiology studies, and 9 animals per group for the behavioral studies. Final values were analyzed for independent significance using one-sample t-tests and compared using unpaired t-tests or one-way ANOVA with post hoc analyses as appropriate with Prism software (GraphPad, San Diego, CA).

Results: We found that norepinephrine (NE) regulates GABAergic input onto IfL layer 5 pyramidal neurons that project to the CeA, and its modulatory effects are significantly affected by alcohol dependence. Specifically, 1 μM NE increases GABA release in naïve/non-dependent mice (p < 0.05), but decreases it in dependent mice (p < 0.05). Dependent mice also express heightened anxiety-like behavior (increased number of digging bouts and increased time spent digging, as well as greater latency to feed in the novel arena during the novelty-induced suppression of feeding task) and cognitive deficits (increased latency to escape and increased number of errors in the Barnes maze retention test) compared to naïve/non-dependent mice (all p < 0.05). Ongoing work is assessing which specific adrenergic receptors mediate NE’s synaptic effects in naïve/non-dependent vs. dependent mice, as well as probing the potential role of the noradrenergic system in modulating dependence-induced cognitive dysfunction and anxiety-like behavior.

Conclusions: Collectively, our results indicate that noradrenergic influence over the IfL-CeA circuit is dysregulated by alcohol dependence, potentially contributing to CeA overactivation (a hallmark of the transition to the alcohol-dependent state) and several alcohol withdrawal-associated behaviors. There are currently multiple clinical trials exploring the efficacy of noradrenergic treatments for alcohol use disorder, and this work may help identify specific subpopulations that can most benefit from these treatments.

Keywords: Alcohol, Noradrenergic System, Medial Prefrontal Cortex
Disclosure: Nothing to disclose.

T256

The Neural Correlates of Altruistic Giving in Individuals With and Without Alcohol Use Disorder

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Background: The capacity to act altruistically has been shown to be reduced in a subset of alcohol dependent individuals. This capacity may be modulated by the noradrenergic system given that noradrenergic activity is positively associated with the capacity to act altruistically. Consequently, we conducted whole-body positron emission tomography scanning in a subset of healthy controls and alcohol dependent individuals to assess potential differences in noradrenergic activity as a potential mediator of differences in the capacity to act altruistically.

Methods: We included patients with alcohol dependence (n = 17) and healthy controls (n = 17). Participants were scanned during a period of abstinence and, following an initial familiarization phase with the experimental setup, given a financial incentive to allocate monetary rewards to two fictitious individuals. Altruism was quantified using a decisional index, the proportion of rewards given to the fictitious individual in need, and was compared across groups.

Results: Healthy controls allocate a significantly higher proportion of monetary rewards to the fictitious individual in need compared to alcohol dependent patients (p = 0.001). This difference in altruism was associated with a significant decrease in noradrenergic activity in the alcoholic group as compared to controls (p = 0.024). This decrease was not present in healthy controls (p = 0.305).

Conclusions: The results support the hypothesis that noradrenergic activity plays a role in the development of differences in altruistic behavior in healthy controls and alcohol dependent individuals.

Keywords: Alcohol, Noradrenergic System, Altruism
Disclosure: Nothing to disclose.
Background: Prosocial or altruistic decision making is thought to involve the integration of reward evaluation and social/cognitive processing, engaging areas including the ventral striatum and insula respectively. Individuals with alcohol use disorder (AUD) have demonstrated impairments in both of these systems, but no work has been done to examine how chronic alcohol use may influence the brain processes associated with altruism.

Methods: Preliminary analysis was conducted in abstinent male and female adult individuals receiving treatment for alcohol use disorder (n = 9) and healthy control volunteers (n = 8). Participants completed two runs of a hypothetical charitable donation task while undergoing an fMRI scan. During this task, participants were given a scenario in which they donated money from their personal account or from a 3rd-party fund to a charitable cause; participants could then decide to accept that scenario and donate or reject it and not donate.

Results: Individuals with AUD were more likely than controls to donate to charity during the task (X2(1) = 5.7501, p = 0.016; freqAUD = 69% of trials; freqcontrols = 62% of trials); individuals in both groups were more willing to donate when there was no cost to themselves (i.e., if the funds to donate were from a 3rd-party). When deciding to donate to charity at cost to themselves, individuals with AUD had less engagement compared to controls in parahippocampal gyrus, primary sensory, post-central, frontal eye fields, lingual gyrus, superior temporal gyrus, and inferior parietal lobule. On the other hand, individuals with AUD had greater engagement than controls in the inferior frontal gyrus. These effects were attenuated if there was no cost to themselves.

Conclusions: Individuals with AUD engage less neural resources associated with perception, memory, and context than controls when deciding to donate to charity. Put together with our finding that individuals with AUD were more likely to endorse decisions to donate to charity, this pattern of neural response may suggest decreased consideration of context and other external features by individuals with AUD when deciding whether or not to help a cause. Future work should explore this further by focusing on how context influences neural processing in altruism across diagnoses.

Keywords: Brain Imaging, fMRI, Alcohol Use Disorder, Human Neuroimaging, Altruism, Adult

Disclosure: Nothing to disclose.

T257

Age and Sex Differences in the Brain’s Structural and Metabolite Responses to Binge Ethanol Exposure

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Background: In vivo magnetic resonance imaging (MRI) suggests a greater vulnerability of the aging brain to alcohol. We modeled this age-alcohol interaction and examined the role of sex in groups of young and old, male and female rats in a longitudinally controlled study using in vivo structural MRI and MR spectroscopy (MRS).

Methods: To model the interaction of alcohol and aging on the brain, 28 old (~17 months) and 28 young (~4 months) Fisher 344 rats were acquired from the National Institute of Aging. Animals [14 young (FY: 178.1 ± 10.9 g), 14 old (FO: 221.8 ± 14.8 g) females; 14 young (MY: 312.4 ± 25.8 g), 14 old (MO: 427.7 ± 20.2 g) males] were triaged into ethanol (E, intragastric gavage of up to 3 g/kg EtOH every 8hr for 4 days) or control (C) groups. Two old female and 3 old male rats did not survive binge EtOH exposure; final groups were as follows: FYC n = 6, FYE n = 8, FOC n = 6, FOE n = 6, MYC n = 6, MYE n = 8, MOC n = 6, MOE n = 5. MRI and MRS with a voxel localized to the striatum were conducted at baseline, following binge EtOH exposure, and after 1 week of recovery. Separate MANOVAs were conducted for each dependent variable including metabolites with average Cramer-Rao bounds below 15%.

Results: (i.e., FY, FO, MY, MO) that showed a follow-up time-by-diagnosis effect separately are described in terms of percent change from baseline. Time-by-diagnosis effects were significant for weight, cerebral spinal fluid (CSF) and gray matter volume; striatal N-acetyl-aspartate (NAA), total creatine (tCr), glutamate (Glu), glutathione (GSH), inositol (Ins), and tau (Tau).

Between baseline and the binge time point, EtOH-exposed groups lost weight (FYE −7%, FOE −8%, MYE −14%, MOE −12%), showed an increase in CSF volume (FYE 7%, MYE 14%, MOE 10%), and a decrease in gray matter volume (FOE −0.7%, MYE −2%); all 3 variables showed recovery with 1 week of abstinence. Between baseline and the binge time point, EtOH-exposed animals also showed reduced striatal NAA (MYE −8%, MOE −0.3%), tCr (FYE −8%, MYE −10%, MOE −5%), GSH (MYE −15%), Ins (FOE −13%, MOE −8%), and Tau (MYE −13%, MOE −11%); and higher Glu (FYE 3%, FOE 10%).

Comparing with our previous studies performed in wild-type Wistar rats, binge EtOH exposure caused transient ventricular enlargement and reductions in NAA and tCr. The current study suggests that these reproducible, EtOH-sensitive measures are more affected in young male rats than in older male or female rats. Following binge-EtOH treatment, young male rats were the only group to show significantly reduced GSH; Tau was reduced only in the male (young and old) groups, Ins only in the older (male and female) groups; and higher levels of Glu were only observed in the female (young and old) groups.

Relationships between percent change in CSF levels (between baseline and binge) and percent change in striatal metabolites, cumulative EtOH dose, peak blood alcohol levels (BALs), and blood markers at euthanasia including complete blood count measures and liver enzymes were explored. Percent change in CSF volume correlated with percent change in gray matter volume (p = −0.64, p = 0.0003), peak BALs (p = 0.33, p = 0.10), and alkaline phosphatase levels (p = −0.56, p = 0.002). Although our previous work and that of others has shown sensitivity of alkaline phosphatase to EtOH treatment in rats, the relevance of this liver enzyme measure to the human condition has not been forthcoming.

Conclusions: The current results raise new questions about the role of sex and brain metabolites in identifying mechanisms of alcohol’s action. Specifically, are the changes to CSF volume related to brain energetics (i.e., because tCr is an index of the substrates available for the brain’s high energy phosphate metabolism - is reduced)? Are female animals protected from more profound changes to CSF volume because of Glu reactivity? Are young male rats more sensitive to CSF volume changes because of reduced levels of the antioxidant GSH?

In summary, the current study shows that young male rats are more sensitive than old male rats or young or old female rats to CSF enlargement and reductions in NAA and tCr resulting from exposure to high doses of alcohol. Rat strain differences (e.g., Wistar vs. Fisher 344) also need to be considered when exploring the interaction between aging and alcoholism.

Keywords: Alcohol Sensitivity, Sexual Dimorphism, Aging

Disclosure: Nothing to disclose.
Persistent Behavioral Dysregulation and Neuroimmune Activation in the Dorsal Raphe Following Adolescent Alcohol Exposure

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Background: Early adolescence represents a critical period for the initiation of alcohol drinking that can predispose individuals to behavioral disturbances later in life. The adolescent brain is especially sensitive to the effects of immune activation, which can have long-lasting effects on neurodevelopment and behavior. Converging lines of evidence suggest that microglia may be critically involved in alcohol-induced neurotoxicity (Henriques et al., 2018), although how these neuroimmune interactions impact behavior has not been fully elucidated. Neuroinflammation in the DRN was found to have profound effects on emotional behavior (Howerton et al., 2014), so we asked whether voluntary ethanol consumption in early adolescence could induce persistent alterations in microglia and 5-HT neurons in the DRN, and whether this was associated with heightened anxiety-like behavior and pain sensitivity in the adult.

Methods: Adolescent mice were exposed to a 4-week two-bottle choice intermittent access ethanol drinking paradigm followed by a 4-week abstinence. Anxiety-like behaviors were measured in the open field and the social interaction tests, and pain sensitivity was measured in the Von Frey test. Mice were then perfused for immunohistochemical analysis of S-HT, p2Y12 and CD11b expression in the DRN and median raphe nucleus (MRN).

Results: Our results indicate that adolescent ethanol induces a persistent anxiety-like phenotype in the open field test and an increase in pain sensitivity in the Von Frey test. This was accompanied by a substantial reduction in the number of 5-HT-expressing neurons and an increase in the area of P2Y12+ cell bodies, which is indicative of microglial activation. These effects were especially pronounced in the rostral portion of the DRN. Interestingly, there was also an increase in the number of activated microglia in the MRN, but this was associated with an increase in 5-HT expression.

Conclusions: These results indicate that microglial activation may reduce 5-HT expression in the rostral DRN and cause anxiety-like behaviors. Increased 5-HT in the MRN may also contribute to these behaviors. Previous studies suggest that 5-HT in the DRN has an inhibitory effect on pain (Wang and Nakai, 1994), so the observed reduction in DRN 5-HT may also contribute to alcohol-induced hyperalgesia. Studies are ongoing to establish a role for observed reduction in DRN 5-HT may also contribute to alcohol-an inhibitory effect on pain (Wang and Nakai, 1994), so the effects of apremilast on excitability of dopamine receptor D1 or D2 expressing MSNs in the NAc with binge-and dependence-like alcohol drinking paradigms in male and female mice of 3 different strains of mice. Our behavioral pharmacology studies are complemented by gene expression and electrophysiology studies.

Methods: Using qPCR, we measured NAc expression of different Pde genes at 8 time points after chronic binge-like alcohol drinking (vs water controls; n = 5-7/time point/group) in mice selectively bred for binge-like drinking in the drinking in the dark paradigm (High Drinking in the Dark, HDID mice). Rolipram, a PDE4 inhibitor, was tested using a binge-drinking paradigm (0, 3, 5, 7.5, 10 mg/kg; n = 10-12/dose/sex). We then rigorously tested the effects of the FDA approved PDE4 inhibitor, apremilast (0, 20, 40 mg/kg; n = 11-12/sex/dose/study), on binge-like drinking in two lines of mice independently selectively bred to achieve high blood alcohol levels after the drinking in the dark (DID) paradigm which models binge-like drinking. To determine whether inhibition of PDE4 in the NAc was sufficient to reduce DID, we administered apremilast intra-accumbens (0 or 2 ug, via bilateral cannulae; n = 15-17/group). We also measured the effects of apremilast on intake of water and saccharin. To test whether PDE4 inhibitors could alter relapse-like drinking in alcohol dependent mice, we tested the effects of apremilast (0 or 20 mg/kg) on drinking using a chronic intermittent ethanol vapor exposure paradigm in high drinking C57BL/J mice (vs air control; n = 9-13/sex/vapor group). Lastly, we measured the effects of apremilast on excitability of dopamine receptor D1 or D2 expressing MSNs in the NAc using patch clamp slice electrophysiology (using Drd1a-tTomato mice; n = 10-24 cells/condition/experiment).

Results: Rolipram (5, 7.5, 10 mg/kg, but not 3 mg/kg) reduced binge-like drinking in HDID-1 mice. Apremilast reduced binge-like drinking and blood alcohol levels in male and female HDID-1 and HDID-2 mice (all p’s < 0.01). Apremilast produced modest reductions in water, but not saccharin, intake. We found that intra-NAc administration of apremilast robustly reduced binge-like alcohol intake without altering intake of other fluids. Further, apremilast significantly reduced relapse-like drinking in both ethanol dependent and non-dependent male and female C57BL6/J mice, suggesting apremilast may be beneficial for reducing harmful drinking in individuals at different stages of alcohol use disorder. Lastly, we found that apremilast uniformly enhanced excitatory input to MSNs, and differentially regulated the membrane excitability of NAc D1 versus D2 MSNs.

Conclusions: These results show that the effects of PDE4 inhibition on alcohol intake are relatively specific to alcohol, and generalizable to more than one drug, as well as other alcohol drinking paradigms and strains. Further, PDE4 inhibition specifically in the accumbens is sufficient to reduce binge drinking and results in enhanced excitability of D1 MSNs.

Keywords: Behavioral Pharmacology, Gene Expression, Electrophysiology, D1/D2, Genetic Animal Model

Disclosure: Nothing to disclose.
The Rostromedial Tegmental Nucleus Integrates Diverse Punishment Signals to Modulate Reward Seeking

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Background: While the reinforcing and motivational properties of natural rewards and abused drugs has been the focus of a large body of research, relatively little is known regarding how negative experiences modify the subjective value of rewards to influence future decision-making. The rostromedial tegmental nucleus (RMTg) provides dense inhibitory projections to midbrain dopamine neurons and receives input from a diverse array of brain regions involved in decision-making and aversive signaling. As such, the RMTg is well-positioned to influence encoding and expression of reward seeking in response to negative experiences. Recently, we found that inhibition of RMTg projections to dopamine neurons in the ventral tegmental area (VTA) increased the intensity of footshock rats were willing to endure to receive food reward, but it remains unclear whether seeking of drug rewards is similarly modulated by the RMTg, and which of the many RMTg inputs drive encoding of aversive stimuli.

Methods: Adult male Sprague Dawley rats (n = 6-10 per group) were bilaterally injected with virus encoding either excitatory (AAV2-hSyn-hChR2-(H134R)-mCherry) or inhibitory (AAV2-hSyn-eArch3.0-eYFP) opsins, or excitatory (pAAV2-hSyn-HA-hM3D(Gq)-mCherry)/ inhibitory (rAAV2/hSyn-HA-hM4D(Gi)-IRE6-mCitrine) designer receptors exclusively activated by designer drugs, into either the RMTg or cell bodies of select RMTg inputs. Chronic indwelling optical fibers or cannulae were then implanted to target terminal regions of RMTg inputs, or RMTg projections to the VTA. After surgery, rats were trained to either lever press for food reward or intravenous infusions of cocaine.

Results: Chemogenetic silencing of RMTg terminals in the VTA profoundly impaired the suppressive effect of footshock on cocaine self-administration, while optogenetic stimulation of this pathway during self-administration dramatically reduced cue-induced cocaine seeking when tested several weeks later, indicating effects greatly outlasting the duration of the stimulation itself. Using optogenetic inhibition of various RMTg afferents, we found that distinct acquisition and expression phases of a punished food seeking task are driven by separable inputs to the RMTg encoding discrete learning and motivational signals, and that the prelimbic prefrontal cortex (PL) is particularly necessary for RMTg responses to cues. Ongoing work suggests that the RMTg role in cue-induced drug seeking may at least partially depend upon input from the PL, as chemogenetic stimulation of PL terminals in the RMTg selectively reduced cue-induced reinstatement of cocaine seeking.

Conclusions: Together, these findings suggest an important and previously unrecognized role for prefrontal-midbrain interactions in cue-induced reinstatement, and identify the RMTg as an important target for suppressing reward seeking, particularly through its projections to midbrain dopamine.

Keywords: Reward and Aversion, Cocaine Reinstatement and Taking, Risky Decision-Making

Disclosure: Nothing to disclose.

Fetal Alcohol Effects on Stress and Behavioral Responses are Transmitted for Multiple Generations via Germline in Rat Offspring

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Background: Alcohol use during pregnancy often causes development of fetal alcohol spectrum disorders (FASDs), which are developmental disorders characterized by diverse symptoms, including delayed growth, craniofacial abnormalities, intellectual impairment, anxiety, depression, and social impairment. Recently, data have emerged demonstrating the heritable effects of prenatal alcohol from maternal drinking in animal models which passes for multiple generations. Previous work from our laboratory has demonstrated propagation of stress axis hyperresponsiveness in the offspring of fetal alcohol exposed animals for three generation via germline. We evaluate whether alcohol exposure in utero produces epigenetic modification of genes critically involved in controlling neuroendocrine and behavioral functions.

Methods: We employed salivary samples from children 7-16 years old with prenatal ethanol exposure (PAE) or non-alcohol exposed controls or maternal blood samples collected from mothers who reported frequent moderate to heavy drinking and had a child with or without evidence of fetal alcohol spectrum disorders (PAE), and women who reported low or no alcohol consumption in pregnancy (control) (Study 1, n = 144). We also used tissue and sperm samples of fetal alcohol exposed or control isogenic Fischer 344 (F344) rats (study 2, n = 208) bred three generations using male and female germiles. Using pyrosequencing and methylation specific PCR analysis (MSPR) we measured gene methylation, real-time-PCR for gene expression, ELISA for hormone level changes in tissue and biological fluid samples. Genome wide gene methylation was measured employing Bisulfite sequencing (RRBS) analysis.

Results: In human studies, we found significant increase in methylation of POMC gene in mothers who gave birth PAE children as compared to controls. Additionally, we found higher DNA methylation of POMC an elevated level of cortisol and ACTH levels in salivary samples of PAE children as compared to control children. In animal studies using Fischer 344 rats, our data revealed that PAE increased DNA methylation of proopiomelanocortin (POMC) gene and decreased mRNA expressions of POMC genes. These methylation and gene expression defects in PAE offspring persisted in the F2 and F3 male offspring from male germline. Determination of hormonal responses to stress revealed that adult PAE offspring had elevated stress hormones (corticosterone) responses. PAE animals also showed higher anxiety like behaviors. Determination of the hippocampal-dependent spatial learning and memory, using Morris Water Maze revealed a significant increase in latencies in PAE rats in multiple generations. Determination of differentially methylated CpG sites in germ cells of F1, F2 and F3 male offspring from male germline revealed that a substantial overlap of differentially methylated regions (DMRs) between three generations. The analysis resulted in 266 probes which were differentially methylated and common in all three generations. Many of these common pathways are connected with the neuronal development.

Conclusions: Overall, these findings provide the evidence that fetal alcohol effects on stress response and neurobehavioral abnormalities persisted throughout adulthood and perpetuated into subsequent generations through the male germline.
The Relationship Between Endogenous Cannabinoids and the Acute Effects of Smoked Cannabis

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Background: Endocannabinoids (eCBs), anandamide (AEA) and 2-arachidonoylglycerol (2-AG), play an integral role in physiology, stress response, mood, and behavior. Cannabis is the most widely used illicit substance, and with expanding legalization of medicinal and recreational cannabis, its use is increasing worldwide. However, the impact of cannabis use on eCBs is not well understood. The purpose of this human laboratory study was to assess plasma levels of eCBs and tetrahydrocannabinol (THC) and ratings of cannabis intoxication (“high,” “good drug effect”) and cardiovascular endpoints before and for up to 3 hours after smoking cannabis in daily cannabis smokers.

Methods: Twenty-six medically healthy, non-treatment-seeking, normal-weight, daily cannabis smokers were recruited. The study used illicit substance, and with expanding legalization of medicinal and recreational cannabis, its use is increasing worldwide. However, the impact of cannabis use on eCBs is not well understood. The purpose of this human laboratory study was to assess plasma levels of eCBs and tetrahydrocannabinol (THC) and ratings of cannabis intoxication (“high,” “good drug effect”) and cardiovascular endpoints before and for up to 3 hours after smoking cannabis in daily cannabis smokers.

Results: Smoked cannabis significantly increased ratings of intoxication (change from baseline to peak for “high”: 54 mm; t = 9.68, p < 0.00001; change from baseline to peak for “good drug effect”: 65 mm; t = 10.58, p < 0.00001), heart rate (17 bpm; t = 6.80, p < 0.00001) and plasma THC levels (change from baseline to peak: 43.16 ng/ml; t = 7.15, p < 0.00001). There was a significant positive correlation between baseline AEA levels and peak ratings of “high” (r = 0.56, p = 0.004) and “good drug effect” (r = 0.50, p = 0.01) following cannabis administration. In addition, baseline 2-AG levels were negatively correlated with frequency of cannabis use (average days/week: r = −0.56, p = 0.003), and showed trending relationships with other measures of current cannabis use (average amount smoked in grams/day: r = −0.38, p = 0.059; amount used in last week in grams/day: r = −0.34, p = 0.09).

Conclusions: These data show that baseline eCB levels are related to the acute subjective effects of smoked cannabis. Higher baseline plasma AEA predicted greater ratings of “high” and “good effect” after smoking. In addition, heavier cannabis use was associated with lower 2-AG levels. Although not directly assessed herein, this finding is consistent with the observation that baseline eCB levels were markedly lower in this sample of daily cannabis smokers as compared to data published on non-cannabis smokers. One possibility is that baseline eCBs are a biomarker of cannabis tolerance, i.e., heavier cannabis smokers have lower eCBs and are less sensitive to the intoxication effects of cannabis. Future research will further explore the impact of cannabis use on eCB levels in the plasma and the brain at baseline and following acute cannabis administration in individuals with varying levels of cannabis use. This research will allow us to test the hypothesis that baseline AEA is a biomarker for tolerance, and may lead to a clearer understanding of how basal and cannabis-induced changes in eCB levels impact physiology, stress response, mood, and behavior, including effects that may promote continued cannabis use, and/or the development of cannabis use disorder.

Keywords: Endocannabinoids, Cannabis Use, Subjective Effects, Behavioral Pharmacology

Disclosure: Nothing to disclose.

Reactivation of Nucleus Accumbens Shell Ensembles During Cocaine Conditioned Place Preference

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Background: Cocaine addiction involves the encoding and retrieval of drug-related experiences that shape future behavior. For instance, memories of environmental cues during initial drug taking can have a large influence on future drug seeking and taking behavior. Understanding these memory processes that drive addictive behavior is critical to developing new treatments. Associative memories rely on the reactivation of distinct neural ensembles to drive behavior, but the circuits critical for drug-context associations have not be well characterized. Using genetic tagging strategies (TetTag) we have previously found evidence that the population of neurons in the NAc shell that is activated by initial cocaine exposure becomes reactivated when experiencing cocaine again. This reactivation indicates that NAc shell contains a stable neural correlate of the drug experience. However, it remains unclear how this ensemble of NAc neurons contributes to drug-context associative memories when off the drug.

Methods: To characterize neural coding during drug-context association learning and retrieval, we performed in vivo calcium imaging using wireless miniature microscopes (Miniscope) in the NAc shell during cocaine conditioned preference (CPP). We designed a 4-context cocaine CPP paradigm, in which each mouse (N = 7) was trained and tested in a paired box (cocaine given during training on one side of the box) and an unpaired box (cocaine NOT given during training on either side of the box). Calcium imaging was performed during pre-test, training, and post-test. We then compared overall activity level in NAc shell during each phase between paired and unpaired groups, as well as ensembles reactivation rate between training and testing.

Results: Behaviorally, animals showed a significant preference (higher CPP score) for the drug side versus the saline side in the paired box during the test phase, but no preference was seen in the unpaired box. In addition, using calcium imaging in NAc shell, we found a significant reactivation of the drug-active neurons during the CPP test off drug. This indicates that cocaine CPP retrieval engaged a similar neural representation to drug training in NAc shell. Moreover, in the drug-paired group, we found a high correlation between ensemble reactivation rate from drug training to test and CPP score during test. In the unpaired group, we found a negative correlation (N = 6).

Conclusions: Our results indicate that neural ensembles in NAc shell that are activated during initial drug exposure are reactivated during drug-context memory retrieval. This indicates that the NAc circuit can produce a drug-like neural state that may be driving the decision to go to the drug-paired side. Therefore, NAc shell is a
strong candidate to be a target for future interventions to treat cocaine addiction and future studies will be aimed at testing the causal role of this NAc ensemble on CPP retrieval and drug seeking behavior.

Keywords: In Vivo Calcium Imaging, Conditioned Place Preference, Nucleus Accumbens Shell

Disclosure: Nothing to disclose.

T264

Vaping Electronic Cigarettes With Nicotine Produces Addiction-Like Behaviors and Prolonged Pulmonary Abnormalities in Rats

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Background: The debate about electronic cigarettes has divided healthcare professionals, politics, and communities. A central point of disagreement is whether vaping electronic cigarettes is addictive and whether it produces pulmonary abnormalities. Several epidemiological studies have attempted to address this issue, but the results have been confounded by the current and past use of tobacco products, thus making it difficult to establish causality.

Methods: We developed a novel model of nicotine vapor self-administration in rats using human-grade, commercially available electronic cigarette products. Rats were given access to a lever that was associated with the delivery of a puff of nicotine vapor (0.05-60 mg/ml) that was diluted in a 50/50 mixture of propylene glycol/vegetable glycerin. The rats were given access to the apparatus for a total of 6 weeks. They were also tested for somatic signs of withdrawal, mechanical pain sensitivity, anxiety-like behavior, and spontaneous relapse during acute and protracted abstinence. Peripheral lung structure integrity was evaluated using the mean linear intercept length, a measure that is sensitive to the increased in alveolar space.

Results: The rats voluntarily exposed themselves to nicotine vapor to the point of becoming dependent on nicotine and developed addiction-like behaviors and the smoking cessation drug varenicline reduced nicotine vapor self-administration. A history of ~6 weeks of nicotine vapor exposure was associated with prolonged increases in anxiety-like behavior, craving, and alveolar space, even after 3 weeks of abstinence. These results confirm the addictive properties and potential harmful effects of nicotine vapor on the lungs and identify varenicline as a potential medication for the treatment of electronic cigarette addiction.

Keywords: Nicotine Exposure, Self-Administration, Pulmonary, Lungs, Nicotine Vapor

Disclosure: Nothing to disclose.

T265

The Role of Microglia in Opioid Tolerance and Withdrawal

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Background: Recently it has become clear that microglia are perturbed by chronic opioid tolerance and are vigorously activated during acute opioid withdrawal. Opioid withdrawal is a serious medical problem and is a strong disincentive for addicts to achieving abstinence; therefore, we seek to identify novel strategies to modulate microglia for the treatment of opioid withdrawal.

Methods: We explored this issue using conditional expression of DREADD receptors or Ribotag in microglia in animals that received a 6 day, escalating schedule of noncontingent morphine sulphate (or vehicle) followed by treatment with naloxone precipitated withdrawal (or vehicle) in a 2X2 design. RNAseq results were analyzed for differential expression, gene set enrichment, and weighted gene co-expression network analyses (WGCNA), providing both function-guided and unbiased analyses of the data.

Results: Animals that received morphine then vehicle showed sensitized locomotor responses whereas animals that received morphine then naloxone displayed robust signs of withdrawal. Using CX3CR1-CreERT2/YFP mice, we conditionally expressed the hM4Di DREADD in microglia. This receptor is Gi/o coupled, similar to mu opioid receptors. Surprisingly, activation of hM4Di in microglia exacerbated, rather than mitigated, the signs of opioid withdrawal. We next used deep sequencing to examine striatal total RNA (“input”) and RNA actively undergoing translation in microglia (“IP”) using immunoprecipitation of Ribotag expressing ribosomes from microglia. As expected, naloxone had minimal effects on RNA expression in opioid-naïve control animals. Animals that were morphine tolerant showed a dramatic shift in RNA expression in both the input and IP fractions, and many of the RNAs that changed significantly (p values ranging to 1e-24) had previous been identified in past analyses of RNA transcriptome in striatal tissue. However, we also found that many of these changes are occurring in the microglial “translatome” as well, which is surprising. While many genes showed decreased translation in microglia in opioid tolerant mice, the reverse was observed after naloxone-precipitated withdrawal. Indeed, hundreds of ribosome-associated mRNAs were dramatically elevated in microglia after naloxone and they were enriched in several important gene sets; e.g. Camp signaling, potassium channels, and ATP signaling. We next performed WGCNA and one large module identified that was significantly decreased after tolerance and significantly increased after naloxone; this module contained many of the same microglia-expressed genes that were identified by DEseq2 and GSEA. Current experiments are testing whether key regulators predicted by our RNAseq analysis will blunt the signs of morphine and fentanyl withdrawal.

Conclusions: We conclude that microglia are dramatically impacted by chronic opioids and withdrawal and may be a novel target for mitigating the acute repercussions of opioid withdrawal and potentially impact the propensity to relapse to drug seeking. We are currently examining whether purinergic signaling is a critical mediator of microglial activation during opioid withdrawal.
Keywords: Morphine, Naloxone, RNA-Sequencing, Neuroinflammation, Microglia Priming

Disclosure: Nothing to disclose.

T266

Tinkering With THC-to-CBD Ratios in Marijuana: Comparative Responses in Adolescent Nonhuman Primates

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Background: The marijuana plant produces over 100 different cannabinoids, including the structurally distinct principals, ∆9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC concentrations in retail marijuana has risen dramatically, while CBD levels have declined, with THCCBD ratios currently at 104:1. High concentrations of THC and high ratios of THCCBD in marijuana are associated with more robust euphoria, anxiety, and psychotic symptoms in otherwise normal people. CBD attenuates THC-induced anxiety, cognitive deficits or psychosis and each cannabinoid engenders different molecular, pharmacological and neuropsychiatric effects. Recent evidence from our laboratory suggests that the combination of THC and CBD attenuates some of the adverse effects induced by prolonged THC treatment. These effects of CBD have not been thoroughly assessed on day-night activity, spontaneous behavior or blood and brain levels of THC. The current study sought to compare the behavioral and other effects of THC or THC combined with CBD in adolescent nonhuman primates, with a view to determining across a wide range of parameters, whether CBD modulates the effects of THC.

Methods: Twelve male adolescent squirrel monkeys (2.3-2.5yea) were divided into three treatment groups: vehicle control, THC, or THC+CBD (n=4 in each treatment group). Initially they were administered low doses of THC or THC+CBD weekly for four weeks. Thereafter, at the onset of the testing regimen, animals received THC (1 mg/kg) or THC+CBD (THC 1 mg/kg and CBD 3 mg/kg) daily for a period of 4 months. Animals had the following parameters measured: 1. Cognitive testing: Animals were trained to engage in touch-screen based cognitive tasks according to previously established methods from our laboratory. Testing was performed 7 days a week and are reported elsewhere (S. Withey et al. CPDD annual meeting 2019). 2. Day-night activity: activity was measured by fitbit units located in jackets fitted for the subjects. 3. Spontaneous behaviour: behaviors were video-taped weekly in plexiglass cages and rated by an observer blinded to the drug regimen. 4. Blood THC, CBD, and THC metabolites were measured four times over the course of the experiment. THC levels in two brain regions were measured following euthanasia. 5. Brain regions (29) were dissected after euthanasia and subjected to PCR and other analyses.

Results: 1. THC or THC+CBD (1:3 ratio) compromised initial learning of a cognitive task in primates, but tolerance developed to cannabinoids over time (S. Withey, in preparation). 2. Repeated THC for four months gradually increased sleep fragmentation over time, especially manifest in the final weeks of the experimental period. To some extent, CBD combined with THC attenuated THC-mediated sleep disturbances. 3. Spontaneous behaviors changed over time and the effects of THC alone on specific behaviors began to diverge from THC+CBD-treated animals over time, with one exception. In the first week of the study, THC induced emesis in all four subjects and emesis was attenuated if THC and CBD were co-administered. THC or THC+CBD suppressed locomotion and foraging, but after 100 days of daily administration, both effects were attenuated in subjects co-administered CBD along with the THC. 4. THC blood levels remained relatively stable over 100 and more days of treatment. If CBD was co-administered, blood THC averages were Formation of 11-hydroxy THC, a THC metabolite was suppressed by co-administered CBD but only after the first day of treatment. Brain levels of THC were approximately 4 times higher than blood level and THC levels were higher in occipital cortex than in cerebellum. The calculated % CB1 receptor occupancy in brain, based on brain levels was approximately 40% in the occipital cortex and 28% in the cerebellum 24 h after last dose. Brain (occipital cortex, cerebellum) levels of THC was not affected significantly if CBD was co-administered. In preliminary data, daily THC or THC+CBD administered to adolescent monkeys for four months altered mRNA expression of targeted genes, but not consistently, across five brain regions. 6. THC did not up-regulate D1-D2 heteromers in adolescent primate or rodent caudate, but did so in adult rat and adult monkey NAC and caudate. These results suggest a fundamental difference in adolescent and adult brain adaptation to THC, which may contribute to differences in drug reward in adolescents compared with adults (submitted by Dr. George to this meeting).

Conclusions: THC affects sleep quality and alters spontaneous behavior in monkey, some of which become manifest over time. CBD attenuates some but not all THC-induced behavioral and molecular adaptive changes in nonhuman primates. Attenuation is unlikely to be related to disrupted THC metabolism or brain entry. Results suggest that high THC:CBD ratios in current marijuana augment the adverse effects of marijuana. Our findings warrant further research into the pharmacological and pathological consequences of high/lows THC doses, highs/lows THCCBD ratios after long term use, and a comparison of adolescent and adult responses. Results add weight to concerns that THC:CBD ratios in retail marijuana are rising rapidly.

Keywords: High Potency THC, Marijuana Policy, Cannabidiol, Adolescents

Disclosure: Nothing to disclose.

T267

Ceftriaxone and mGlu2/3 Interactions in the Nucleus Accumbens Core Mediate the Reinstatement of Cocaine-Seeking in Male and Female Rats

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Background: There are currently no effective treatment options for cocaine use disorder. Several neuroadaptations have been observed in rodents after self-administration of cocaine, including decreased expression and function of the glutamate transporter GLT-1 in the nucleus accumbens core (NAc). Ceftriaxone, a β-lactam antibiotic, has been proposed as a potential treatment for cocaine relapse as it restores GLT-1 expression and function and attenuates the reinstatement of cocaine-seeking. We have previously demonstrated that GLT-1 upregulation in the NAc is necessary for ceftriaxone to attenuate reinstatement. However, we have also reported that AAV-mediated GLT-1 overexpression in the NAC is not sufficient to attenuate cue- or cocaine-primed reinstatement of cocaine-seeking. GLT-1 overexpression attenuates, but does not fully prevent, glutamate efflux during reinstatement. Here we test the alternative hypothesis that ceftriaxone also works to attenuate glutamate efflux through mGlu2 and mGlu3 receptors in the NAc, which are proposed to be release-regulating autoreceptors.
Methods: In Experiment 1, male and female rats self-administered cocaine for 12 days, followed by extinction training. Rats were killed after 2-3 weeks of extinction and a biotinylation assay was performed on fresh NAc tissue. Western blots for mGlu2 and mGlu3 total and surface expression were performed. In Experiment 2, we examined the role of mGlu2/3 in celptrixone's ability to attenuate reinstatement of cocaine-seeking. In this experiment, male and female rats self-administered cocaine and underwent 2-3 weeks of extinction training. During the final 6 days of extinction training, rats were treated with celptrixone (200 mg/kg) or vehicle. Immediately prior to a reinstatement test, rats received an infusion of the mGlu2/3 antagonist LY341495 (or vehicle) into the NAc immediately prior to a reinstatement test.

Results: We found that in both male and female rats, total mGlu2 expression was reduced by cocaine and restored by celptrixone. Surface mGlu2 expression was increased by celptrixone in both male and female rats. There were no effects of cocaine or celptrixone on total or surface expression of mGlu3 in male or female rats. We found no effects of estrous cycle phase on mGlu2 or mGlu3 expression. In Experiment 2, we found that antagonism of mGlu2/3 in the NAc during both cue- and cocaine-primed reinstatement tests prevents celptrixone from attenuating reinstatement of cocaine-seeking.

Conclusions: This work indicates that the ability of celptrixone to attenuate reinstatement depends on mGlu2/3 function and possibly upregulation of mGlu2 receptors. Future work will directly manipulate expression of mGlu2 along the pathways that project to the NAc to test this hypothesis.

Keywords: mGlu2/3 Receptor, Metabotropic Glutamate Receptor 2 (mGluR2), Glutamate, Relapse, Cocaine Addiction

Disclosure: Nothing to disclose.

T268

Evaluation of G Protein Signaling Biased MOR Agonist in the Mouse Locomotor Activity Assay

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Background: Mu-opioid receptor (MOR) agonists are currently indispensable for their pain-relieving effects however, they also carry a risk for unwanted side effects including respiratory suppression, tolerance, and addiction. Opioids act at mu opioid receptors (MORs) which are members of the G protein-coupled receptors (GPCRs) family that interact with G proteins and with the scaffolding protein, βarr2 (βarr2) to regulate and contribute to their activity. An ideal MOR agonist would preserve analgesic properties and at the same time avoid negative side effects. Previous work using βarr2 knockout (βarr2-KO) mice demonstrated enhanced morphine potency, less tolerance and less morphine-induced hyperactive ambulation relative to their wild type litter mates. We recently developed a series of Scripps Research (SR) MOR agonists that favor G protein coupling over βarr2 recruitment in cell-based assays. When evaluated in mice, the degree of G protein bias correlated with a widening of the therapeutic index between analgesia and respiratory suppression. Here we investigated the effects of the SR compounds alongside of oliceridine and morphine in the mouse locomotor activity assay.

Methods: In this study we compare opioid-induced psychomotor response from compounds with that have been reported to show biased agonist at different extents (oliceridine, SR-17018, SR-15099 and SR-11501). Doses were chosen based upon potencies observed in the mouse hot plate antinociception tests. We also test whether there is drug interaction at the MOR between biased agonists and morphine in the locomotor activity assay by co-treatment, pretreatment and post-treatment with morphine. Open field motor activity was recorded using a Versamex Animal Activity Monitoring System. Mice were individually placed into the activity monitoring boxes for 30 minutes to habituate to the new environment, removed, injected (i.p.) and immediately placed back into the boxes for monitoring. To assess for unconditioned behavioral sensitization, mice were treated daily in their home cage with the same dose for 6 days, then we compared locomotor activity on day 7 (chronic) relative to day 1 (acute). Experimenter was always blinded to treatments by another investigator.

Results: Our findings demonstrate that SR-17018 and SR-15099 produce less hyperactivity than morphine that does not appear until ~30 min after injection, while oliceridine produces an immediate onset of elevated hyperactivity at low doses. Daily dosing of morphine and oliceridine produce locomotor sensitization while daily dosing of SR-17018 or SR-15099 does not. When added together, SR-17018 reduces morphine-induced hyperactivity in a dose dependent manner while oliceridine does not. In the hot plate antinociception test, mid-potency doses of morphine and SR-17018 produce an additive effect. Oliceridine combined with morphine produces an additive effect on locomotor activity, without much gain in antinociception. SR-17018 did not produce off-target sedation, as it had no inhibitory effect on d-amphetamine-induced hyperactivity.

Conclusions: Our findings suggest that the SR series of G protein-biased agonists have a very different profile than the oliceridine compound, another MOR agonist that has shown G protein signaling bias in cell-based assays as the SR compounds to not promote hyperactivity. Further the interaction of SR-17018 with morphine appears to act in a competitive manner in the locomotor assay and in an additive manner in the antinociception studies. These results suggest that agonists may not play the same pharmacological role in different brain regions that control different behavioral responses.

Keywords: Mu-Opioid Receptors, Mu-Opioid Receptor Agonist, Biased Mu Agonists, Locomotor Activity, Morphine

Disclosure: Nothing to disclose.

T269

Comparing G-Protein Biased MOR Agonists Using Simultaneous PET/MRI

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Background: μ-Opioid receptor (MOR) agonists are the most effective analgesics for pain management. However, MOR agonists also elicit adverse effects such as respiratory depression. Upon agonist binding, MOR activates G-protein to induce downstream signaling [1]. In addition, beta-arrestins are recruited to regulate receptor activity by desensitizing and internalizing MORs [1]. Emerging evidence from pharmacological and genetic animal studies suggests that selective activation of the G-protein but not the beta-arrestin pathway may lead to effective analgesia without undesired side-effects [2, 3]. Therefore, biased agonism at the MORs has become a promising new direction for therapeutic development [3]. To date, several biased MOR agonists have been developed [3-5]. However, the in vivo action mechanisms of
biased MORs remains to be shown. The goal of this study is to measure and compare drug-receptor occupancy and drug-induced functional MRI responses of biased MORs using simultaneous PET/MRI in vivo.

**Methods:** PET/MRI images were acquired from three male macaques using a 3T Siemens BrainPET with a μ-opioid selective radiotracer, [11C]carfentanil (~5 μCi; specific activity: ~3 μCi/ nmol), given as bolus-infusion for 100 min. PET data were binned into 1-min frames. Cerebral blood volume (CBV-FMRI) was measured following an iron oxide (10 μg/kg, i.v.) injection (6). Biased MOR agonists, TRV130 (Oliceridine, 0.5 mg/kg) and SR-17018 (0.5 mg/kg), were given intravenously at 36 min post radiotracer administration. PET data were analyzed for binding potentials referenced to a non-displaceable compartment (BPND) using the simplified reference tissue model (7). A gamma-variant function was used to model the PET/FMRI temporal response to drug challenge.

**Results:** Under the baseline condition, PET BPND maps show a high-level of specific binding in the thalamus, caudate, putamen, amygdala, frontal cortex. Baseline time activity curves show that PET signal reached a steady-state with bolus/infusion of [11C]carfentanil. Both TRV130 and SR-17018 caused an apparent reduction in BPND (~22-40% for TRV130 and ~15-30% for SR-17018). We have previously shown that non-biased MOR agonists (like morphine) cause an increase in PET BPND, potentially reflects agonist-induced MOR trafficking mediated by beta-arrestins. This current study provides further evidence to support the notion that beta-arrestins are key for MOR desensitization and internalization.

Another significant result from this study is that TRV130 and SR-17018 induced opposite fMRI responses. Although activations were observed in similar brain regions, including the frontal and parietal cortex, thalamus, amygdala, and the brainstem, TRV130 caused a reduction in CBV (~10–20%) while SR-17018 caused an increase in CBV (8-15%). We were expecting to find a negative drug-induced CBV change because activation of MOR is inhibitory. Our result suggests that different molecular mechanisms (or signaling pathways) are involved in TRV130- and SR-17018-induced hemodynamic responses. Future studies are warranted to explore the underlying cause of this discrepancy.

**Conclusions:** In this study, we compared drug-receptor occupancy and drug-induced functional MRI responses of two biased MOR agonists, TRV130 and SR-17018, using simultaneous PET/MRI. We found that a dose of 0.5 mg/kg (i.v.) of TRV130 or SR-17018 reached comparable MOR occupancy although the equal-analgesic doses (morphine-equivalent dose) are estimated to be ~10x different based on rodent literature. TRV130 and SR-17018 may activate very different signaling pathways as we observed opposite fMRI responses (at comparable receptor occupancy). In vivo quantification of receptor occupancy and drug-induced fMRI response may be complementary to behavioral studies (e.g. analgesic response) to characterize novel compounds.

**Acknowledgments:** This research is support by NIH R00DA037928 and R21DA047133 to H.T.W.

**References:**

**Keywords:** Hybrid PET/MR, Mu-Opioid Receptors, Biased Mu Agonists

**Disclosure:** Nothing to disclose.
dramatic increase in latency to enter the light compartment in adult rat that was prevented by CBD cotreatment. In adolescent rat, there was no change in the latency to enter the light compartment after THC compared to vehicle control. With novelty induced hypophagia, THC increased the latency to drink condensed milk in the home cage as well as in the novel cage in adult rat that was attenuated by CBD cotreatment. In adolescent rat, THC increased the latency to drink condensed milk in the home cage but not in the novel cage, an effect that was not altered by CBD. An emotionality score was calculated for each rat based on the z-score from each test. This revealed very significantly elevated emotionality in adult rat after THC that was attenuated to control levels by cotreatment with CBD. In adolescent rat, there was a minor increase in the emotionality score after THC that was not affected by concomitant CBD.

Conclusions: Chronic THC in adult male rat and male monkey induced increased expression of dopamine D1-D2 heteromeric complexes in nucleus accumbens and dorsal striatum but decreased its expression in prefrontal cortex. CBD prevented the changes in striatal D1-D2 expression in both rat and monkey. In adolescent rat and monkey no changes in D1-D2 heteromer resulted after THC, with basal expression levels of heteromer lower than in adult brain. THC induced significant anxiety and anhedonia in adult rat, the greater magnitude in adulthood contrasting with a more minor effect in adolescent rat. Co-administration of THC with CBD reversed the behavioral impairments caused by THC in adult, but not adolescent rat. We postulate that the D1-D2 heteromer may mediate these THC-induced aversive symptoms and play a protective role in adulthood, by dampening subjective rewarding effects of drugs, therefore decreasing propensity to develop addiction. Conversely, lack of these aversive effects may contribute to adolescents exhibiting heightened reward sensitivity to THC with greater risk for abuse liability and the consequences of chronic cannabis use and abuse. Legalization of marijuana for medical or recreational use raises great concern regarding effects on adolescent brain development and maturation in modulating executive function and predisposing to mental health disorders, especially with the increasing concentration of THC with lower CBD in marketed preparations.

Keywords: Dopamine Receptor Heteromer, THC, Cannabidiol

Disclosure: Nothing to disclose.

T271

Whole Genome Approaches Reveal Novel Epigenetic and Gene Networks in the Amygdala After Acute Ethanol Exposure

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Background: Acute alcohol exposure is associated with anxiolytic effects and serve as an important factor in initiation and maintenance of alcohol use disorder (AUD). Neurobiological changes in the amygdala appear to be crucial in driving anxiety phenotypes and promoting drinking. Transcriptomic changes are under complex epigenetic regulation mediated by histone DNA chemical modifications. We have shown that acute ethanol exposure increases histone acetylation via inhibition of histone deacetylases (HDACs) in the amygdala and produces anxiolysis in rats. To identify novel molecular pathways that are associated with these changes, we utilized two genome-wide sequencing approaches- Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-seq) and RNA-sequencing.

Methods: Adult male Sprague Dawley rats were administered intraperitoneally with ethanol (1g/kg) or n-saline. One hour after exposure, we measured behaviors using elevated plus-maze (EPM) test. The amyloidoid tissues were collected from these rats and used for RNA-seq and ATAC-seq, along with gene expression and chromatin immunoprecipitation (ChIP) assay validations.

Results: Analysis of ATAC-seq data revealed an overall open or permissive chromatin state after acute ethanol and novel transcription factor binding footprints at the whole genome level. The RNA-seq data showed differential gene expression patterns in the amygdala. Merging the two data sets revealed gene candidates that had mRNA changes correlating to open chromatin regions near their transcription start sites. The promoters of Hif3a (Hypoxia inducible factor 3, alpha subunit) and Slc10a6 [solute carrier family 10 (sodium/bile acid cotransporter family), member 6] had ‘open’ chromatin regions (ATAC-seq peaks), which correlated with significantly increased levels of the activating epigenetic histone marks (H3K9/14 and H3K27 acetylation) and a decrease in DNA methylation at these regions. The transcripts encoded by these genes were significantly increased after acute ethanol and correlated with active epigenetic marks within the genome.

Conclusions: These data provide evidence that a low dose of ethanol changes the overall status of the epigenome and produces transcriptomic changes that may prime the amygdala for the development of AUD. Furthermore, acute ethanol produced anxiolytic effects and it is possible some of observed epigenetic and transcriptomic changes in the amygdala may be involved in ethanol-induced anxiolytic-like effects in rats. (Supported by NIH-NIAAA P50AA022538, U01AA019971, R01AA010005 and by the VA Merit and Senior Research Career Scientist award to SCP).

Keywords: Amygdala, Alcohol, Whole Genome, RNA-Sequencing, Epigenetics

Disclosure: Nothing to disclose.

T272

Changes in the Gut Microbiota and the Fecal Metabolome Differentiate Short- and Long-Term Alcohol Binge Drinking Baboons

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Background: Alcohol (EtOH) consumption affects all systems of the body, especially the gut. Approximately 14 trillion bacteria constitute the community residing in the gut. Diet and dietary energy intake may impact the composition of the gut microbiota. EtOH, in addition of being a drug, is a source of calories and may affect the gut microbiota. Very little is known on the role of the gut microbiota in alcohol use disorder (AUD) and whether the microbiota-gut-brain axis may be involved in the mechanisms that regulate EtOH binge drinking. Moreover, EtOH-induced changes in the gut microbiota composition and metabolic function may contribute to the link between EtOH-induced oxidative stress, intestinal hyperpermeability to luminal bacterial products, and the subsequent development of EtOH liver disease (ALD). Microbial action in the gut is responsible for the generation of metabolites derived from amino acids, bile acids, benzoate, and dietary sources. Yet, relevance of these microbial metabolites in EtOH-associated disorders is still unclear. This study aimed to investigate if EtOH binge drinking is associated
with significant traits of the gut microbiota and/or changes in fecal metabolites related to gut microbial dysbiosis.

Methods: Animals (N = 14) in this study were adult male baboons of the species Papio Anubis. They belonged to 3 cohorts: S = Short-term EtOH exposure group, daily self-administering EtOH for 2-3 yrs (N = 5); L = Long-term EtOH exposure group, daily self-administering EtOH for ≥10 yrs (N = 4); T = control group, daily self-administering orange-flavored Tang®, an isocaloric nonalcoholic beverage, for =8 yrs (N = 5). S and L consumed relevant amounts of EtOH 7 days/week and the resulting blood EtOH level (BAL) constantly exceeded 0.08% after a 2-hour self-administration period. Fecal samples were collected in 2 conditions: D = during 3 days of active EtOH/Tang Drinking and A = after 3-5 days of EtOH/Tang Abstinence. In each condition, over a month, 3 fecal samples, corresponding to 3 different time points, were collected per animal, for a total of 6 fecal samples. Gut microbiota analysis consisted in DNA extraction and 16S rRNA gene sequencing, analysis of microbial species diversity – α (Shannon Diversity Index) and β-diversity (Bray-Curtis dissimilarity) –, and comparison of family/genera relative abundances through Linear Discriminant Analysis Effect Size (LEfSe). Fecal metabolome analysis relied on UPLC-MS/MS, which identified 631 biochemicals. ANOVA and Mixed Model Contrasts analyzed biochemicals varying among the 3 cohorts.

Results: The 3 cohorts did not differ significantly in age, weight, and liver function, with the latter being normal, as demonstrated by liver enzyme values (ALT, AST, ALP) and veterinarians’ clinical evaluation. Fecal samples exhibited significant taxonomic diversity differences between cohorts T, S, and L, but not between sampling time points, as calculated with Shannon Diversity Index (p < 0.01). Tukey’s test showed a significantly decreased microbial diversity in L vs. T and S (p < 0.01 for both comparisons). In contrast, no shift was observed in T vs. S (p = 0.06). The microbial communities differed significantly between animals (p < 0.01). The PCoA of Bray-Curtis distances demonstrated a separation of L from both T and S, accounting for 22% of the differences (p < 0.01). LEfSe detected several clades showing statistically significant and biologically consistent differences among the 3 cohorts. The genera Lactobacillus and Streptococcus showed higher relative abundances in L. For S, the order Clostridiales and the family Ruminococcaceae showed high relative abundances vs. T and L. In T, members of the family Anaeroplasmataceae were more abundant. No significant difference was found between Conditions D and A. Changes in gut-generated aromatic amino acids were seen in L vs. T and S, suggesting differences in gut microbial catabolism and/or activity among the EtOH- vs. Tang-exposed cohorts. However, S had minimal impact on gut microbial metabolite changes vs. T, possibly due to altered gut microbial colonization kinetics dictated by the length of EtOH exposure. Increases in secondary biliary acids (BAs) were noted in both S and L vs. T under active drinking (D) and abstinence (A). Since they are generated by the gut microbiota, their increase in feces could indicate differential microbial BA metabolism in EtOH-exposed baboons. Furthermore, changes in phenolic metabolites were observed in both actively drinking (D) and abstinence (A) L vs. T, indicating altered gut benzoate metabolism with EtOH exposure.

Conclusions: Our findings suggest that in EtOH binge drinking baboons, compared to the control cohort (T), long-term protracted exposure to EtOH (L) leads to significant changes in the gut microbiota, whereas relatively short-term EtOH exposure (S) does not significantly alter it. These changes are not affected by acute forced withdrawal from chronic EtOH exposure. Furthermore, the analysis of the metabolic pathways underlying the differences among the 3 cohorts suggested important changes in aromatic amino acid metabolites, secondary bile acids, benzoate/phenolic metabolites, and markers associated with oxidative stress, inflammation, and intestinal tissue damage. Our findings are novel, given that they were generated from a nonhuman primate model of alcohol binge drinking, whose gut microbiota and fecal metabolome profiles have not been studied simultaneously before.

Keywords: Alcohol Use Disorder, Binge-Drinking, Animal Models, Gut Microbiota, Metabolomics

Disclosure: Nothing to disclose.

T273

Pharmacological Impact of μ-Opioid Drugs on Economic Choice Between Natural Reward and Opiates in Squirrel Monkeys

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Background: In order to improve preclinical models of opiate addiction which frequently lack non-drug alternative options, we developed a touch screen-based choice task in which squirrel monkeys made choices between different quantities of intravenous infusion of the fast-acting opioid remifentanil, and a natural reward, different quantities of sweetened condensed milk.

Methods: We demonstrated that subjects (n = 10) make economic choices based on the quantities offered and shift their choice preference in response to satiation with milk reward prior to the experimental session or saline substitution for remifentanil.

Results: Subsequently, we examined the impact of pharmacological pretreatment with an opiate agonist and antagonists. Pretreatment with the μ-opioid receptor agonist (1 mg/kg i.m. 15 min prior) reduced responding but had limited effect on the preference for remifentanil or milk. Saline pre-treatment had no effect on responding or preference. Pretreatment with the μ-opioid receptor antagonist naloxone (0.1 mg/kg i.m., 15 min prior), slightly reduced the preference for remifentanil, whereas pretreatment with a higher dose (1.0mg/kg, 15min prior) reduced preference for remifentanil (p = 0.023). Pretreatment with naltrexone (0.1 mg/kg i.m., 15 min prior) in 2 subjects, decreased the preference for remifentanil, whereas pre-treatment with a higher dose (1.0mg/kg, 15min prior) reduced preference for remifentanil (p = 0.016).

Conclusions: These data provide additional support for a non-human primate model of opiate use. Future experiments will examine the stability of preference with prolonged opiate intake, and the impact of pharmacological manipulation on drug preference.

Keywords: Opiates, Non-Human Primate, Opioid Use Disorder

Disclosure: Nothing to disclose.

T274

CRF Amplifies Drug Anticipation via Modulation of Accum-bens Dopamine: A Role for CRF-R2 in Cocaine-Seeking Behavior

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Background: Dopamine signaling within the nucleus accumbens core (NACC) is critical to generating motivated behavior in both appetitive and aversive contexts. Within the accumbens, various neuropeptides influence motivated output through modulation of

Mitochondria Dysfunction in Astrocytes Exacerbates Naloxone-Precipitated Morphine Withdrawal

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Background: Astrocytes play a leading role in metabolic support of neuronal activities in stressful and pathological conditions. However, whether and how abnormalities in astrocyte bioenergetics can contribute to the development of substance use disorders (SUD) remains poorly determined. This is particularly important for opioid addiction, as recent studies have indicated astrocyte expression of mu-opioid receptor (MOR). Here, we tested the hypothesis that abnormal astrocyte mitochondria respiration would affect the behaviors produced by chronic exposure to exogenous opiates. To test this hypothesis, we selectively deleted in astrocyte the Cox10 gene that encodes the protoheme IX farnesyltransferase, the terminal component of the mitochondrial respiratory chain and studied the effects of this deletion on morphine-produced behaviors.

Methods: To generate mice with conditional deletion (cKO) of the Cox10 gene, Aldh111-Cre/ERT2 BAC transgenic mice were crossed with Cox10f/lf mice. We induced conditional recombination in astrocytes with intraperitoneal injection of tamoxifen (100 mg/kg, daily, 3 days) in one-month-old male and female mice. Four weeks later, the protocol to produce morphine-dependence in mice was started. Control and cKO mice received in their home cages escalating doses of morphine sulfate (20, 40, 60, 80 mg/kg) or saline twice a day for four days. On Day 5, mice received single injections of morphine (100 mg/kg) or saline in their home cages. On Day 3 of the morphine injections, naloxone-precipitated conditioned place aversion (CPA) was initiated in the same mice. Pre-conditioning was done on Day 3 of the morphine injections, five hours after the first daily injection of morphine. During pre-conditioning, mice were allowed to freely explore the CPA apparatus. CPA was performed on Day 4 and 5 of the morphine injections by additionally injecting saline on Day 4 and naloxone (0.25 mg/kg s.c.) on Day 5 five hours after the first daily injection of morphine. CPA was tested on Day 6, 24 hours after the naloxone injection. The CPA score was calculated as the time spent in the naloxone-paired compartment minus the time spent in the same compartment on the pre-conditioning day. CPA was re-tested 3 and 6 weeks later in the same mice. In addition, we evaluated analgesia in cKO Cox10 and control mice in the hot place test before and 30, 60 and 90 min after s.c. injection of 5 mg/kg morphine. The anti-nociceptive response was calculated as a percentage of maximal possible effect (%MPE = [test latency − baseline latency] / [cut-off − baseline latency]).

Results: Compared to control mice, cKO Cox10 mice exhibited significantly stronger naloxone-precipitated CPA that persisted for 3 weeks. Specifically, CPA score was significantly different in the between cKO Cox10 mice in comparison to all other groups including control mice treated with saline, control mice treated with morphine and control and cKO mice treated with saline (n = 5-7 per group, two-way ANOVA with Bonferroni post hoc, p < 0.01). In addition, deletion of Cox10 in astrocytes significantly decreased morphine-induced analgesia. No sex-dependent group differences were detected in CPA or hot place tests.

Conclusions: Deletion of the nucleus-encoded mitochondrial gene, Cox10, in astrocytes exacerbates naloxone-precipitated morphine-produced withdrawal and decreases morphine-induced analgesia in mice in sex-independent manner. Our findings suggest that abnormal astrocyte bioenergetics may contribute to the development of opioid addiction-related behaviors. On-going studies aim at identification of the role of astrocyte MORs in mediating the neurobehavioral effects of opioids.

Keywords: Astrocytes, Morphine, Opioid Addiction, Mitochondria

Disclosure: Nothing to disclose.
regions. In structural brain imaging studies in humans, chronic cannabis exposure has been associated with gray matter volume reduction in the medial temporal cortex, temporal pole, para-hippocampal gyrus, insula, and orbitofrontal cortex; but the findings have been inconsistent. To date, there are no in vivo studies on the effects of cannabinoids on brain microstructure in humans. With the emission of the positron emission tomography (PET) radioligand, [11C]UCB-J for the synaptic vesicle glycoprotein 2A (SV2A), it is now possible to study the effects of cannabis exposure on brain microstructure. Synaptic vesicle glycoprotein 2 (SV2) is localized on the surface of synaptic vesicles. SV2A is the most monodisperse of three SV2 isoforms and expressed in virtually all active synapses. PET imaging of SV2A thus offers a unique method to measure synaptic vesicle density in the human brain in vivo.

Methods: This is a preliminary analysis of male cannabis-use disorder subjects (CUD) (n = 5) (Mean age = 24.8 ± 3.5 years), and age-, gender- matched healthy controls (HC) (n = 5) (Mean age = 26.5 ± 3.4 years) who underwent [11C] UCB-J PET imaging on the High-resolution Resolution Tomograph (HRRT) and 3T structural MRI for coregistration. Arterial sampling was used to measure plasma input function. [11C]UCB-J binding potential (BPND) was estimated using a one-tissue (1T) compartment model with centrum semiovale as the reference region. Verbal memory was assessed using the Rey Auditory Verbal Learning Task (RAVLT). Electroencephalography (EEG) was acquired during a 40 Hz auditory steady-state paradigm.

Results: Among male CUD subjects, the mean age of onset of cannabis use was 15.0 ± 2.4 years. The degree of cannabis use was 25.3 ± 7.8 days in the past month and 121 ± 73 occasions in the past 3 months. The injected activity dose was 617.90 ± 84.76MBq and 488.62 ± 189.74MBq; and mass dose was 0.020 ± 0.012 and 0.013 ± 0.004 mg/kg, in CUD and HC, respectively. There was no significant difference in [11C]UCB-J volume of distribution (VT) in the centrum semiovale between CUD (4.39 ± 0.48 mL/cm3) and HC (4.22 ± 0.87 mL/cm3), thus validating its use as a reference region. Relative to matched healthy controls (HCs), male cannabis-use disorder subjects (CUDs) (n = 5) showed significantly reduced [11C]UCB-J BPND in the hippocampus (~11%, p = 0.05, effects size 1.43) (i.e., > the ligand’s test-retest variability). CUDs performed worse on the verbal memory task (RAVLT (~41%, effect-size 1.08) and had lower EEG evoked gamma power by 40Hz auditory stimulation (~52%, effect size 1.21). Furthermore, gamma evoked power correlated with BPND of hippocampus (r = 0.71, p = 0.048) and temporal cortex (r = 0.68, p = 0.09), at a trend level.

Conclusions: Our results provide preliminary evidence of regional decreases in synaptic vesicle density in male CUD. The association between synaptic vesicle density, and EEG measure of information processing suggests that multimodal PET-EEG imaging is a useful tool to understand the pathophysiology of cannabis-related impairment in cognition.

Keywords: Cannabis Use Disorder, Positron Emission Tomography (PET), Synaptic Protein Imaging

Disclosure: Neurocine Biosciences, Grant

T277

Divergent Adaptations in KOR-Mediated Dopamine and Glutamate Signaling Along the Rostral-Caudal Axis of the Nucleus Accumbens in Male and Female Rats Exposed to Early Life Stress

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Background: Adolescent social isolation (aSI) in rats engenders enduring maladaptive behaviors linked to alcohol addiction vulnerability. We recently showed an aSI-induced increase in kappa opioid receptor (KOR) function at dopamine synapses in the NAc of male rats. In addition, we showed that aSI rats drinking significantly more ethanol compared to their group housed (aGH) counterparts and this potentiated intake is reversed with KOR blockade. However, this study did not examine females or investigate whether these KOR changes were limited to specific NAc subregions and dopamine transmission. In the current study, we have examined the impact of KOR overexpression specifically in ventral tegmental area dopamine neurons on ethanol drinking, as well as the effects of KOR activation on glutamate and dopamine transmission, and ethanol drinking in male and female rats exposed to control and impoverished living conditions.

Methods: Our housing manipulation consists of group housing (aGH; control; 3 to 4 rats/cage) and social isolation (impoverished; 1 rat/cage) of separate groups of rats beginning PND 28 and lasting throughout adolescence (at least until PND 70). We then conduct behavioral and neurobiological experiments. Specifically, we measured KOR-mediated changes in dopamine release and glutamatergic transmission using ex vivo fast scan cyclic voltammetry and electrophysiology, respectively. For behavioral experiments, rats were subject to two-bottle choice intermittent access paradigm (24 hours access on Monday, Wednesday, and Friday), during which we measured intake at 30 mins after bottles were introduced on each day. U50,488 (2.4 mg/kg; i.p) was administered 30 minutes before rats were given access to the bottles to examine the effect on KOR activation on ethanol drinking in aGH and aSI rats.

Results: Bath application of cumulative concentrations of U50488, a KOR agonist, revealed augmented KOR function at dopamine synapses in the caudal NAc in both male and female aSI rats. Specifically, KOR-mediated inhibition of dopamine release was greater in male and female rats exposed to the impoverished housing condition (aSI). A comparison of the sexes showed that KOR-function was enhanced at the dopamine terminal in caudal NAc in male aSI rats. Notably no effects of early life stress (and sex) were observed in the rostral NAc. Electrophysiological studies revealed augmented glutamatergic excitation in recordings from female aSI NAc slices. Specifically, our data suggest that this change is primarily presynaptic and restricted to the caudal, but not rostral, NAc of aSI slices. Finally, to test whether the increased ethanol drinking behavior in aSI rats is driven by augmented KOR function in dopamine neurons, we used tyrosine hydroxylase-Cre (TH-Cre) and temporal cortex (r = 0.68, p = 0.09), at a trend level.

Conclusions: Our results provide preliminary evidence of regional decreases in synaptic vesicle density in male CUD. The association between synaptic vesicle density, and EEG measure of information processing suggests that multimodal PET-EEG imaging is a useful tool to understand the pathophysiology of cannabis-related impairment in cognition.

Keywords: Cannabis Use Disorder, Positron Emission Tomography (PET), Synaptic Protein Imaging

Disclosure: Neurocine Biosciences, Grant

T277

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Conclusions: Together, these data indicate that NAC KOR-function is enhanced following aSI, primarily in the caudal subregion, and contributes to the escalation in ethanol intake promoted by this model. Furthermore, these data show that adolescent social isolation mediated facilitation in KOR function is restricted to presynaptic changes and affect both dopamine and glutamate transmission. Finally, our behavioral data in conjunction with viral manipulation suggest that KORS may play a causal role in stress induced excessive ethanol consumption.

Keywords: Kappa Opioid Receptor, Adolescent Stress, Ethanol, Dopamine, Glutamate
T278
Effects of a Fruit-Flavorant on Nicotine Consumption and Preference Throughout Adolescence and Young Adulthood

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Background: High school rates of e-cigarette (e-cig) use increased by 78% between 2017 and 2018. The majority of adolescents (~80%) initiate nicotine use with flavored products and ~74% of adolescent e-cig users vapes a flavored product. Furthermore, clinical research suggests that adolescent e-cig use leads to a transition towards combustible cigarettes. However, it is not understood if this transition has to do with social factors (e.g. cost, decreased stigma after nicotine experimentation) or if it is related to altered nicotine reward. As regulatory bodies debate the issue of flavored nicotine products, animal models offer crucial advantages, such as the ability to perform longitudinal studies in a short period of time and the ability to separate social from biological factors.

Methods: We studied both male and female C57BL/6J mice using a 2-bottle choice paradigm, in which adolescent mice had access to 1 bottle of flavored nicotine and 1 bottle of unflavored nicotine. We measured preference for both nicotine bottles and total nicotine consumption (mg/kg). A control group had two bottles of unflavored nicotine. Once mice entered young adulthood (PND 50), they began a classical nicotine 2-bottle choice paradigm, in which they were given 1 bottle of unflavored nicotine and a control bottle (no nicotine). During this phase, nicotine preference and dose (mg/kg) were monitored daily. This was meant to model a transition to unflavored nicotine products.

Results: Adolescent mice preferred a fruit-flavored nicotine solution over an unflavored solution. In addition, mice with access to flavored nicotine consumed higher daily doses of nicotine than mice that only had access to unflavored nicotine. However, this increase in nicotine dose did not persist into the second phase of the experiment, during which mice only had access to unflavored nicotine.

Conclusions: These data recapitulate clinical data which has shown (1) a higher preference for and (2) increased consumption of flavored nicotine compared to unflavored or tobacco-flavored nicotine. Previous research has shown that sweet flavorants can reduce aversion to bitter tastes (e.g., nicotine) that can persist even after the sweetener is removed. However, our data do not suggest that flavored-nicotine consumption in adolescence alters unflavored nicotine preference in young adulthood. This suggests that a transition from e-cigs to combustible cigarettes may have more to do with social factors than with altered nicotine preference.

Keywords: Flavored Nicotine, Nicotine Addiction, Adolescence

Disclosure: Nothing to disclose.

T279
Activity-Dependent Epigenetic Alterations in the Nucleus Accumbens Induced by Cocaine Self-Administration

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Background: Substance use disorder is a behavioral disorder characterized by cycles of abstinence, drug seeking, and relapse. At the core of this phenotype is the persistence of symptoms long after the cessation of drug use. The neural basis of these behavioral changes has been linked to receptor-based changes in neural circuits across the brain, however, the molecular drivers that allow for these changes to persist beyond the life-span of any individual protein remain opaque. Work from our lab identified a self-administration-induced remodeling of the transcriptome as well as a novel transcriptional signature that is induced by cocaine following a history of self-administration that predicts addictive behaviors. To understand SUD, it will be critical to define the link between neuronal activation, and longer-term changes in transcription that control drug seeking. Epigenetic adaptations – where DNA-protein interactions are modified to alter the probability of targeted transcription - have been implicated in the long-lasting changes in neural circuit function and the resilient nature of drug-seeking behavior. For example, histone acetylation, a generally permissive epigenetic mark, is induced following re-exposure to cocaine and cocaine-associated cues, suggesting that the epigenetic enzymes regulating histone acetylation are key regulators for drug-induced gene networks. KAT2A is a histone acetyltransferase known to regulate activity-dependent transcription and hippocampal memory. While several KAT2A histone targets are modulated by cocaine, the role of KAT2A in cocaine-associated behaviors remains unknown. As such, we hypothesize cocaine-induced gene networks are underpinned by recruitment of various histone modifications, a subset of which are regulated via KAT2A.

Methods: To test the role of KAT2A in cocaine-induced epigenetic changes, C57BL6/J mice receive cocaine (20mg/kg, I.P.). Following 1, 7.5, 15, 30, and 60 min post-cocaine exposure, brains were collected, and chromatin was generated from nucleus accumbens punches. Using co-immunoprecipitation and western blotting, we assessed KAT2A occupancy at and subsequent post-translational modifications of Histone H3. To further link changes in KAT2A function to cocaine-associated behaviors, male and female mice were implanted with jugular intravenous catheters and trained to self-administer cocaine (1mg/kg/infusion, i.V) or saline. Following intravenous self-administration, nucleus accumbens tissue was collected and analyzed for post-translational histone modifications induced by: 1) acute cocaine, 2) repeated cocaine, or 3) persisted 24 hours following self-administration. Moreover, using co-immunoprecipitation, we assessed cocaine-mediated effects of KAT2A occupancy on Histone H3.

Results: We identified temporally specific changes in KAT2A occupancy of Histone H3 in response to acute cocaine in the nucleus accumbens. Moreover, this change in KAT2A occupancy is also linked with changes in Histone H3 post-translational modifications, including pH3S10 and acH3K14. In response to cocaine self-administration, we also characterize changes in KAT2A activity within the nucleus accumbens. Lastly, we characterize an epigenomic network within the nucleus accumbens linked to cocaine consumption following self-administration.

Conclusions: The nucleus accumbens has been extensively studied for its role in regulating reward and drug-associated behaviors. Moreover, various epigenetic changes within the nucleus accumbens have been linked to altered accumbens function and response to drugs of abuse. The results of this study provide evidence for long-lasting cocaine-induced changes to the epigenome. In addition, we provide data linking these changes in epigenetic state to cocaine-seeking behavior. Future studies will identify a causal link between changes to epigenetic gene regulation and NAc circuit function during cocaine-seeking behaviors.

Keywords: Reward, Epigenetics, Substance Use Disorder, Reinforcement, Histone Acetylation

Disclosure: Nothing to disclose.
The Alpha-7 Nicotinic Acetylcholine Receptor in the Mesolimbic System: Persistent Upregulation Produced by Adolescent Alcohol Exposure and Regulation of Dopamine

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Background: The alpha-7 nicotinic receptor (α7) is the only receptor in the brain that has been shown to be simultaneous ionotropic and metabotropic actions. The α7 receptor system modulates immune, neuroimmune, multiple neurotransmitters, and hormonal and epigenetic pathways throughout the body. Alterations in the expression of the α7 receptor is associated with multiple neuropsychiatric disorders including Alzheimer’s, schizophrenia, major depression, anxiety disorders, bipolar depression, and many more. Replicable effects of adolescent alcohol consumption on the adult brain include a hyper-dopaminergic response to stimuli, reduction in choline acetyltransferase (ChAT), alterations in neuroimmune function, and non-specific modulation of epigenetic factors.

Methods: A series of experiments were performed to determine; 1) the ability of a α7 receptor agonist to stimulate the mesolimbic dopamine system, 2) alterations in the expression of the α7 receptor within the mesolimbic system produced by adolescent alcohol exposure, and 3) if adolescent alcohol exposure alters the ability of α7 receptor to stimulate the mesolimbic dopamine system. The first experiment examined the alteration in dopamine in the nucleus accumbens shell (AcbSh) produced by microinjections of the α7 receptor agonist SR-17779 directly into the posterior ventral tegmental area (pVTA). The second experiment examined the alteration of the genetic expression of α7 receptor in the AcbSh and pVTA during adulthood following experimenter administered binge-like level of intoxication during adolescence via the NADIA AIE model. The third experiment examined the effects of voluntary alcohol consumption during adolescence in alcohol-prefering (P) rats on protein levels of the α7 receptor in the pVTA in the pVTA during adulthood. The final experiment examined the effect of adolescent alcohol exposure on the ability of SR-R17779 micro-injected into the pVTA to stimulate DA release in the AcbSh.

Results: Microinjections of SR-R17779 into the pVTA stimulated dopamine release in the AcbSh (concentration range 500 pM – 500 nM). The increase in dopamine release in the AcbSh ranged from 98-273% over baseline. Experimenter administered binge-like levels of alcohol during adolescence (NADIA AIE protocol) increased the genetic expression (mRNA) level of Chrna7 in the pVTA and AcbSh in both male and female Wistar rats during adulthood. Voluntary alcohol consumption during adolescence in P rats increased protein levels and IHC detection of the α7 receptor during adulthood. Preliminary data indicate that binge-like alcohol exposure during adolescence (NADIA AIE) alters the ability of SR-R17779 microinjected into the pVTA to stimulate dopamine release in the AcbSh (leftward and upward shift in dose-response curve).

Conclusions: Overall the data indicate that under multiple exposure conditions and rat lines, adolescent alcohol exposure increases the expression of the α7 receptor in the mesolimbic pathway during adulthood. The α7 receptor system could be a vital pathway that mediates the effects of adolescent alcohol exposure on adult drug responsivity.

Nothing to disclose.
Conclusions: Overall, the presented work will illustrate the potential of the EAB sensor platform to greatly increase the number of neurochemically important molecules that can be monitored in the blood and/or in brain in real time and will provide unique opportunities to measure their variation on the behaviorally relevant, seconds to subsecond timescale. This, in turn, would significantly advance our view into the dynamics of psychoactive drugs, of neurotransmitters, and of the intersection of the two with health, abuse, and disease.

Keywords: Pharmacology, Behavioral Pharmacology, Neuropsychopharmacology

Disclosure: Nothing to disclose.

T282
Endogenous Cannabinoids and Suicidal Behavior in Combat Veterans
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Background: Combat veterans are at increased suicide risk. The aim of this study was to test the hypothesis that combat veterans who have made a suicide attempt post-deployment can be distinguished from combat veterans who have never made a suicide attempt based on differences in psychological and biological variables. For the latter, we focused on endogenous cannabinoids, neuroendocrine markers that are associated with stress.

Methods: Demographic and clinical parameters of suicide attempters and non-attempters were assessed. Only suicide attempters who made an attempt within 5 years prior to the day of evaluation were included in the attempter group. Blood samples were assayed for anandamide (AEA), 2-arachidonoylglycerol (2-AG), and cortisol.

Results: Suicide attempters had higher Scale for Suicidal Ideation (SSI) scores in comparison to non-attempters (p < 0.001). Controlling for gender, 2-AG levels were higher among suicide attempters in comparison to non-attempters (p = 0.046). Cortisol levels positively correlated with 2-AG levels (p = 0.021) and negatively correlated with SSI scores among non-attempters (p = 0.046) but not among attempters. AEA levels negatively correlated with SSI scores among attempters (p = 0.031) but not among non-attempters.

Conclusions: Our results indicate that there are psychological and biological differences between combat veterans with or without a history of suicidal attempt. Our findings also suggest that clinically observed differences between the groups may have a neurobiological basis.

Keywords: Endocannabinoids, Suicidal Ideation, Suicide Attempt

Disclosure: Nothing to disclose.

T283
Task-Related Modulation of Intrinsic Functional Connectivity Networks: Correlations With Psychopathology in a Young Adult Sample
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Background: There has recently been interest in assessing intrinsic functional connectivity (iFC), not just during rest, but also during tasks. Findings indicate that network iFC during rest and task reveal a similar network structure and may allow for more robust assessment of iFC, given the potential for longer data collection and therefore higher reliability. At the same time, networks do modulate according to task, and the degree of modulation might be related to psychopathology. Here, we utilize data from a sample of young adults with a spectrum of “distress” and psychopathology, to assess this task-related modulation. We aimed to assess which network edges changed between each task vs. rest across the sample, and whether the degree of change differed according to clinical characteristics.

Methods: 254 young adults 18–25 years old, 123 (48%) of whom were seeking help for psychological distress, irrespective of diagnosis, were recruited. A battery of self-report and clinician-rated measures were utilized to assess anxiety, depression, reward sensitivity, and impulsivity; these included the Hamilton Depression and Anxiety rating scales, UPPS-P, BIS, and BISBAS. Participants underwent an hour-long scan which included a 6-minute resting state paradigm (REST), as well as (1) implicit processing of emotional faces (dynamic faces; DYNFACE), an n-back task with emotional/neutral face distractors (EFNBACK) (2) and a card-guessing task (REWARD). We analyzed data using the Data Processing & Analysis for Brain Imaging (DPABI) toolbox; data were realigned and slice-time corrected; nuisance regression was conducted; adjusting for motion parameters, global signal, and task-related events; regressors were also entered for each time point with framewise displacement > 0.5. Images underwent temporal filtering (0.01–0.08 Hz), normalization (using DARTEL), and smoothing (6 mm kernel). We excluded participants who had <75% of any run remaining after censorship. Tasks were truncated at 240 volumes (6 minutes), the length of the shortest run (REST), to be consistent across tasks. Time series were extracted using networks of interest from the Gordon parcellation (Cingulo-opercular network; CON, Default Mode Network; DMN, Frontoparietal Network; FPN, Salience Network; SAL, and Ventral Attention Network; VAN) and subcortical regions (SUB; bilateral amygdala and ventral striatum), and z-scored adjacency matrices were constructed. Difference matrices were calculated, subtracting observed REST iFC from each task. We conducted a factor analysis that included 23 scales/subscales for the purposes of data reduction. Next, we assessed, for each task vs. rest, which edges showed a significant increase or decrease (p < 0.005) across group with task. Within this selection of task-sensitive edges, we used network-based statistic (NBS) (threshold t = 2.5, 5000 permutations) to assess for networks where task-related differences were modulated by psychopathology; all models adjusted for gender, age, IQ, scanner, and framewise displacement.

Results: At an uncorrected threshold (p < 0.005), a substantial percentage of edges showed a change in iFC with task (DYNFACE: 19% increased and 15% decreased; EFNBACK: 18% increased, 18% decreased; REWARD: 22% increased, 22% decreased). For DYNFACE vs. REST, iFC between salience networks (SAL/CON) and subcortical regions increased, while iFC between SAL and other cortical networks (DMN and FPN) decreased. Similarly, for EFNBACK vs. REST, iFC between SAL and subcortical regions increased while SAL-DMN iFC decreased; in addition, iFC within VAN and CON increased and CON-DMN iFC decreased. For REWARD vs. REST, (1) iFC within VAN, DMN, and CON increased; (2) iFC between CON and SAL increased, and both became less connected to DMN; and (3) FPN became more connected to CON, and within-FPN iFC decreased. Regarding the factor analysis, a 4-factor solution was chosen based on a scree plot. These factors were: anxiety/depression, impulsivity, sensation seeking, and drive. Of these factors, only anxiety/depression (ANX/DEP) showed...
significant task-related iFC changes. In response to DYNFACE vs. REST, higher ANX/DEP was associated with a greater increase in a network centered around the superior frontal gyrus (SFG) and precuneus, largely consisting of edges between DMN and other cortical networks (CON, VAN) (k=57, r=0.002); and a smaller increase in a network centered around the right insula, consisting primarily of CON-CON and CON-amygdala connections (k=25, r=0.05). In response to EFNBACK vs. REST, higher ANX/DEP was associated with a greater increase in a network centered around SFG and supplementary motor area (SMA), consisting mostly of CON-DMN edges (k=29, r=0.02); and a smaller increase in an insula- and amygdala-centered network, mostly CON-CON and CON-amygdala iFC (k=39, r=0.009). No significant differences were observed for REWARD vs. REST.

Conclusions: While canonical networks are preserved during task vs. rest, a substantial proportion of edges are modulated in response to task. We found that the degree of modulation differed according to ANX/DEP. In response to two tasks with emotional faces (DYNFACE, EFNBACK), more ANX/DEP was associated with greater task-related increase CON-DMN iFC and a smaller task-related increase in CON-CON and CON-amygdala iFC. This novel analysis indicates that task-related iFC modulation of networks may provide insight into individual differences in depression and anxiety.

Keywords: Human Neuroimaging, Intrinsic Functional Connectivity, Depression

Disclosure: Nothing to disclose.

T284

Tensor Factorization-Based Identification of Brain Subnetwork Level 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, features that describe each network are computed, and these graph theoretical measures are used for analysis. However, predefining features limits access to potentially informative latent network structure. Alternatively, network edges may be directly used for analysis, but requires correction for large numbers of multiple comparisons, which severely limit statistical power. In this study, we present a simple tensor decomposition-based method for the detection of brain sub-networks that correlate with clinical measures of disease burden that allows for subjects to be represented by latent network structure and avoids the limitations of multiple comparisons.

Methods: Data used are diffusion tensor imaging based structural connectome data 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). Data are arranged in an n x n x m tensor where n=90: number of nodes and m=89: number of subjects. The proposed method may be broken down into three steps: 1) community detection to find network structure across all subjects (as determined with Louvain modularity on the average connectome), 2) CANDECOMP/PARAFAC tensor decomposition of sub-networks identified in the previous step, and 3) Spearman correlation of the decomposed subject mode embedding with clinical measures (the Depression Anxiety and Stress Scale, DASS, was used in this study). Subjects, therefore, are concisely represented by their embedding from the decomposed subject mode of the data tensor for each subnetwork, providing a scalar for each subject to be correlated with their clinical metrics.

Results: In a preliminary analysis, out of eleven subnetworks identified, one significantly positively correlated with DASS. This subnetwork is composed of the bilateral precuneus and anterior, mid and posterior cingulum (r=0.341, p=0.00107, uncorrected). Interestingly, the precuneus and posterior cingulum are hubs of the default mode network, a major subnetwork thought to be involved in the pathology of depression and anxiety. Correlations were performed using the decomposed subject mode from each subnetwork tensor and DASS scores across all subjects (HC and PT).

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 such a subnetwork that includes brain regions that have been previously implicated in depression and anxiety. These results provide evidence to suggest that regions within the default mode network may be transdiagnostic markers of disease across the swath of internalizing psychopathologies.

Keywords: Connectome, Rdoc, Computational Modeling, Default Mode Network

Disclosure: Embodied Labs, Advisory Board, Blueprint, Advisory Board, Keywise, Stock / Equity

T285

Temporal (De-)Synchronization of Arousal Networks With Spontaneous Eye-Blinks During Task and Resting fMRI

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Background: Neuropsychological measures of eye-blink rates are believed to reflect the state of arousal controlled in part by catecholaminergic systems. Though a few fMRI studies have assessed BOLD activity related to spontaneous eye-blinks, the temporal relationship of eye-blinking with arousal networks is not well known. Here we explored temporal regional brain dynamics associated with spontaneous eye-blinks during choice reward task and resting state fMRI. We aimed to characterize pre-blink and post-blink brain networks and their modulation due to task state.

Methods: Sixteen healthy subjects (8 males, 8 females; ages: 21-63, mean = 41.2) underwent two fMRI sessions; each consisting of three blocks of gambling task scans and two blocks of eyes-open resting state scans. Scans were done on a 3T Magnetom Prisma scanner (Siemens Medical Solutions USA, Inc., Malvern, PA) using a 32-channel head coil and a standard echo planar imaging (EPI) sequence (sequential interleaved acquisition, repetition time 2.13s, echo time 30 ms, flip angle α = 79˚, 64 x 64 pixels in-plane resolution, 40 slices, slice thickness 3.5 mm, voxel dimensions 3 x 3 x 3.5 mm3, field of view 192x192 mm2). Stimuli were presented on a black background under dimmed room lighting using a liquid-crystal display screen (BOLDscreen 32, Cambridge Research Systems; UK). During scanning we measured eye-blinks with an ASL eye-
Information-processing based on internal goals. De-Frohlich*
Justin Riddle, Trevor McPherson, Amber McFerren, Flavio
Alpha and Theta Frequency Neuronal Oscillations Dynamically
synchronized with eye-blinks during a choice reward task
Wager, & Bianciardi, 2019) that were temporally synchronized and
visual and emotional arousal systems (Satpute, Kragel, Barrett,
caudate and putamen with correlations peaking ~3 seconds post-
ventral cerebellum). Post-blink synchrony was also observed in
peak activity in cerebellar regions (dorsal cerebellum earlier than
ventral cerebellum). Post-blink synchrony was also observed in
caudate and putamen with correlations peaking ~3 seconds post-
blink only for task. In contrast, amygdala, orbitofrontal and motor
regions showed blink and post-blink asynchrony for both rest and
task.

Conclusions: Our results revealed a wide-range of regions in
AAN but also in visual, striatal and limbic areas associated with the
visual and emotional arousal systems (Satpute, Kragel, Barrett,
Wager, & Bianciardi, 2019) that were temporally synchronized and
d-e-synchronized with eye-blinks during a choice reward task condition but not at rest.

Keywords: Blink, Arousal Networks, Resting and Task fMRI

Disclosure: Nothing to disclose.

T286

Alpha and Theta Frequency Neuronal Oscillations Dynamically
Guide Internal Attention

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Background: Cognitive control is the ability to guide
information-processing based on internal goals. Deficits in
cognitive control are comorbid with a variety of psychiatric
illnesses. These deficits arise from an inability to either suppress
irrelevant information or prioritize relevant information. Our
previous research found that auditory hallucinations in schizo-
phrenia are causally linked to reduced alpha frequency neuronal
oscillations over auditory cortex (Ahn et al., 2018); and symptoms
of chronic pain are causally linked to reduced alpha oscillations
over somatosensory cortex (Ahn et al., 2019). Alpha oscillations in
these clinical populations suppress irrelevant auditory or sensory
information. In addition, theta oscillations in the prefrontal cortex
support prioritization of relevant information. For example,
recent evidence shows that reduced prefrontal theta oscillations
in a cognitive control task predict treatment outcomes for brain
stimulation interventions in depression (Bailey et al., 2017). We
previously reported that alpha and theta oscillations are
antagonistically organized in the ferret model (Stitt et al. 2018).
Thus, the network substrate of suppression and prioritization of
information may be mediated by dynamic interaction of alpha
and theta oscillations. To test this hypothesis, we designed an
experiment that simultaneously drives suppression and prior-
itorization cognitive demands to characterize the behaviorally
relevant functional interactions between theta and alpha
oscillations.

Methods: Participants performed a working memory (WM) task
in which colored squares were presented in the left and right
visual hemifield. Participants were required to encode the colors
and locations into WM. After a brief delay, memory for one of the
visual hemifields was tested by a probe. During the delay period,
either a retro-cue was presented that was informative of which
hemifield (left or right) would be tested or a neutral-cue was
presented that provided no information. We hypothesized that
participants would show increased WM capacity (Pashler's metric)
for trials with retro-cues relative to neutral-cues. We hypothesized
that, following the cue, participants would generate lateralized
alpha oscillations in parietal cortex to suppress contralateral
irrelevant information and theta oscillations in prefrontal cortex
to prioritize relevant information. During performance of this
cognitive control task, we recorded high-definition electroence-
phalography (EEG) in 22 participants (16 female) and analyzed
task-evoked oscillatory activity using Morlet wavelet convolution.
This study was approved by the UNC-Chapel Hill IRB and is
registered as NCT03828734.

Results: Participants were screened for their ability to success-
fully utilize the retro-cue to improve performance. During the EEG
recording, we verified that participants used the retro-cue to
increase their WM capacity (t(21) = 3.81, p = 0.0010, d = 0.81)
relative to the uninformative neutral cue. In our time-frequency
EEG analysis, we found increased theta oscillations [5–8 Hz]
in prefrontal cortex for the retro-cue relative to the neutral cue from
100–500 milliseconds after the cue and increased alpha oscilla-
tions [8–12 Hz] in left posterior parietal cortex for left versus right
retro-cues from 500–900 milliseconds. These task-evoked neuronal
oscillations were behaviorally relevant: the degree to which
participants recruited alpha oscillations to suppress irrelevant
information in the parietal cortex correlated with the benefit of
the retro-cue to WM capacity (r = 0.55, p = 0.008). In an
exploratory analysis, we calculated the composite metric of alpha
minus theta oscillations in posterior parietal cortex and found that
this metric predicted inter-participant variability in their ability
to utilize the retro-cue (r = 0.67, p < 0.001).

Conclusions: Our findings support the theoretical model that
alpha and theta oscillations underlie cognitive control in the
suppression and prioritization of information, respectively.
Furthermore, we were able to explain inter-participant variability using a
composite metric of alpha and theta neuronal oscillations. A
better understanding of the neuronal oscillations that underlie
psychological control provides targets for therapeutic intervention
using non-invasive brain stimulation and predictors of treatment
response.

Keywords: Cognition, Alpha Oscillations, Working Memory, Cognitive Control

Disclosure: Pulvinar Neuro, Stock/Equity, Pulvinar Neuro, Consultant
T287

Optimizing Reliability for Clinical Measurement in Resting State Functional MRI

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Background: A fundamental, and often unexamined, choice in functional connectivity analyses is whether to interrogate the brain as fewer, larger regions or more, smaller regions. Larger regions are more likely to involve heterogeneous activity, but will contain more of the same voxels over time, and are subject to fewer alignment problems. Smaller regions are more likely to be homogeneous in terms of tissue and functions, but are more difficult to identify consistently and may be more subject to motion artifacts. Our laboratory has a longstanding interest in evaluating how these trade-offs affect individual difference measurements given their importance for statistical power and interpretability. Here we examined the impact of resting-state functional connectivity metrics on retest reliability using two approaches: 1) independent components analysis (ICA) that parcelled the brain across different granularities (25, 50, 100, 150, 200, 250, 300 or 350 components) and 2) applied out-of-the-box anatomical and functional parcellations (Gordon, et al., 2014; Glasser, et al., 2016).

Methods: We examined resting state functional connectivity (rsFC) from four fMRI scans (15’ each) from 1003 participants studied by the Human Connectome Project. Motion denoising included ICA-FIX artifact removal and 24 motion parameter regression. We conducted ICA and dual regression (Beckmann and Smith, 2004) and categorized the resulting components with the seven canonical resting-state networks (Yeo et al., 2011). We computed retest reliability for two rsFC measures: (within-component or region-of-interest) coherence and (between-component or region-of-interest) connectivity using intraclass correlation coefficients. For connectivity correlations, we compared 1) full (i.e., Pearson’s) correlation; 2) partial correlation with L2 regularization; and 3) applied out-of-the-box anatomical and functional parcellations (Gordon et al., 2014; Glasser et al., 2016).

Results: For components in the frontoparietal, cingulo-opercular, dorsal attention and default mode networks, the coherence regressed mean FD in each run out of rsFC measures and L1 regularization; and 3) partial correlation with L2 regularization. With most ICC’s ≤ 0.60 found variance was merely a functioning of connectivity variance or strength, we found variance ≥ 0.2 was necessary for good reliability. Furthermore, while high ICC’s did not predict high connectivity, ICC’s ≥ 0.4 appeared to be necessary to observe between-component correlations ≥ 0.4.

Conclusions: These findings provide guidance for variable selection and parameter optimization for rsFC analysis, and importantly highlight that these decisions are hypothesis-dependent. In particular, network location is an important factor that affects the general level of reliability, optimal ICA dimensionality, and whether ICA or other parcellations are preferred. For general purposes, cortical ICA with a dimensionality of 150 may provide an optimal balance between parcellation fineness, reliability, and burden for multiple comparison corrections.

T288

Elucidating the Cellular Basis of Dopamine and Oxytocin Interactions Underlying Pair Bonding

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Background: Pair bonds are long-lasting attachments that form between mating partners. While pair bonding is common among humans, the majority of mammals, including laboratory rats and mice, do not exhibit this trait. Instead, socially monogamous prairie voles, which form life-long pair bonds, provide an excellent model for studying attachment in adults. Previous work has shown that concurrent dopaminergic D2 receptor and oxytocin receptor activation in the nucleus accumbens is required for pair bond formation. However, it remains unclear whether concurrent signaling is occurring at the same or different cells within the nucleus accumbens.

Methods: We generated probes to detect prairie vole dopamine D1 receptor (Drd1), dopamine D2 receptor (Drd2), and oxytocin receptor (Oxtr) mRNA. Using RNAscope fluorescent in situ hybridization, we mapped the cellular distribution of these receptors at five separate levels of the core and shell of the nucleus accumbens in sexually naive male and female prairie voles. All tissue (12 um thickness) was stained under the same conditions, and each level of the nucleus accumbens was imaged using the same scanning parameters on the same day. Two confocal stacks of the core and three confocal stacks of the shell were obtained for each level of the accumbens on right and left sides for each animal at 40x. Confocal stacks were projected into single images using the maximum fluorescence. Total number of cells within an image were identified via DAPI staining, and DAPI staining was used to create a mask for each cell. Cells were identified as positive for a probe if >1 dot/cell was observed within the cell mask at each individual wavelength. The threshold level was set manually for each wavelength, and kept at the same level for analysis in images obtained from the same level of the nucleus accumbens. Co-expression of probes was defined by number of cells with >1 dot/cell of 2 or more probes.

Results: We successfully labeled mRNA for Drd1, Drd2, and oxytocin receptors in the nucleus accumbens core and shell of sexually naive male and female prairie voles (n = 3/grp). Of the total number of cells identified in the nucleus accumbens shell, 68% (males) and 65% (females) were positive for Drd1; 70% (males) and 59% (females) were positive for Drd2; and 56% (males) and 29% (females) were positive for Oxtr. Similar results were obtained for all 3 receptors in both sexes for the nucleus accumbens core, with preliminary analyses suggesting slightly lower expression in the core as compared to the shell for each receptor type. There was no statistically significant difference between females and males for any of the mRNA receptor levels examined in either the shell or the core. When investigating the distribution of dopamine receptor positive cells in the shell, preliminary data suggests 12% are negative for either Drd1 or Drd2; 28% are positive for Drd2 only, 21% are positive for Drd1 only, and 40% are positive for both Drd1 and Drd2. When examining which proportions of cells are double and triple labeled, preliminary data suggests that in the nucleus accumbens shell, oxytocin receptor labeled cells are co-labeled with Drd1 (19%) or Drd2 (30%) with a slight bias towards Drd2 neurons. Additionally, 47% of oxytocin receptor positive cells are labeled...
with both Drd1 and Drd2. Ongoing work is investigating whether receptor distributions change following pair bonding.

**Conclusions:** Oxtr is expressed in all types of nucleus accumbens neurons identified and is not limited to a single dopamine receptor class. This suggests that the requirement for concurrent dopaminergic and oxytocinergic signaling during mating and bond formation is not occurring in a single cell type. In addition, oxytocin receptor protein levels in the nucleus accumbens are highly variable across voles, and our data suggest that this is likely due to differences in the number of oxytocin-receptor-expressing cells rather than a global reduction in mRNA levels. Finally, the large number of Drd1+Drd2+ co-labeled neurons is surprising. In mice, these two receptors are rarely co-expressed in the same cells in the striatum. This may suggest a substantially different organization of dopamine receptors in prairie voles compared with mice.

**Keywords:** Vole, Pair Bond, Nucleus Accumbens, Dopamine, Oxytocin

**Disclosure:** Nothing to disclose.