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W1

The Investigation of Symptoms in Alzheimer's Disease: Toward Optimal Strategies of Treatment for the Disease

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Background: Cognitive dysfunction in Alzheimer's disease (AD) is usually accompanied by various behavioral and psychological symptoms of dementia (BPSD) and dysfunctions in daily activities. In general, it is considered that there is a worsening of functional decline with progressive impairment of cognitive decline in AD, but BPSD are worse in the middle stages with moderate cognitive impairment than those in the early stages with mild cognitive impairment, and even those in the late stages with severe cognitive impairment. For optimal strategies of treatment for AD, it is quite important to make sure the relations between cognitive function and BPSD/activities of daily living (ADL). Therefore, we investigated again these relations. In addition, based on the findings, we present the optimal strategies of treatment for AD.

Methods: The study patients were 114 visited between January 2019 and June 2019 at the Department of Psychiatry, Iwaki Clinic. Patients fulfilled the following inclusion criteria: diagnosis of AD according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-5) criteria. Patients with other types of dementia were excluded from the study. Neurocognitive function was assessed using the Mini-Mental State Examination (MMSE), and BPSD and ADL were assessed by the ABC Dementia Scale (ABC-DS). The ABC-DS, a novel assessment tool for AD is an effective tool in assessing symptoms and the severity of AD over time. The 13 items of the ABC-DS are grouped into three domains: domain A, related to ADL function; domain B, related to BPSD; and domain C, related to cognitive function, and the ABC-DS uses a 9-point scale for each question item, with lower scores indicating poorer function. The JMI software (Version 10.0.2) for Macintosh was used to perform the analyses. The clinical assessment scores were compared between the groups by analysis of variance (ANOVA), followed by post hoc comparisons (Tukey-Kramer HSD). The level of significance was set at $p < 0.05$. This study obtains the approval from Japanese association of Neuro-Psychiatric Clinics Study Ethical Review Board, and the data investigated were retrieved from databases and de-identified before data analyses. After data analyses, two cases with successful treatment by antidementia drugs are presented.

Results: The comparisons between three groups of mild (MMSE, >20), moderate (20-11), and severe (11>) cognitive impairment showed statistically significant differences in domain A and C, but not in domain B. However, for 35 antidementia drug naïve patients, the comparisons between two groups of mild and moderate cognitive impairment showed statistically significant differences in domain B, but not for 79 memantine-treated patients. Seventy-nine patients treated with antidementia drugs were as follows: donepezil, 22 (3mg/day, 1; 5mg, 5; 8mg, 1; 10mg, 15) ; galantamine, 32 (8mg, 1; 16mg, 6; 20mg, 1; 24mg, 23) ; rivastigmine, 24 (9mg, 1; 13.5mg, 1; 18mg, 22); memantine, 35 (10mg, 1; 20mg, 34). Among 35 patients treated with memantine, 33 were taking one of three cholinesterase inhibitors (ChEIs). Seven (9%) of 79 patients with antidementia drugs were on antipsychotics, 4 (5%) were on benzodiazepines, 9 (11%) were on hypnotics, 9 (11%) were on antidepressants, 2 (3%) were on mood stabilizers, and 5 (6%) were on Yokukansan, a traditional Japanese Kampo medicine. Case: A 75-year old woman. Mild AD. At initial diagnosis, ADAS-cog-J total score was 12.3. Rivastigmine patch started, and was increased up to 18 mg/day. Subsequently, memantine added, and was increased up to 20 mg/day. Rivastigmine was switched to galantamine, and galantamine was increased up to 24 mg/day. After 40-month treatment, ADAS-cog-J total score was 14.0.

Conclusions: There is a worsening of ADL decline with progressive impairment of cognitive decline in AD. BPSD are worse in the middle stages than those in the early stages, but controllable by the treatment. To inhibit decreasing of cognitive function in AD, and thus to prevent occurrence of BPSD and aggravation of ADL, it is critical to maintain initial good cognitive function as much as possible, besides early detection of AD and initiation of the treatment. For this purpose, we should always apply monitoring cognitive function under treatment, and manage limited four antidementia drugs effectively: e.g. combining memantine with a ChEI or switching a ChEI to another ChEI.

Keywords: Alzheimer's Disease, BPSD, Neurocognitive Functioning, Neurocognitive Assessment, ADL

Disclosure: Nothing to disclose.

W2

Computerized Functional Skills Training in Mild Cognitive Impairment: Preliminary Results From a Randomized Clinical Trial

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Background: Pharmacological interventions aimed at mild cognitive impairment (MCI) have not been successful to date. Recent meta-analyses have suggested that computerized cognitive training (CCT) may have beneficial effects on cognition in people with MCI. An additional feature of MCI is impairments in

everyday functional skills, revealed in particular with performance on tests of functional capacity. Functional skills training is facilitated by in many populations by CCT, while CCT alone often fails to yield functional benefits. This presentation presents the results of a randomized clinical trial of MCI patients and healthy controls where computerized functional skills training (CFST) was paired in half of the cases with CCT.

Methods: Healthy older (age>59) individuals (n=32) and similarly aged individuals who met diagnostic criteria for mild cognitive impairment (MCI; n=26) were randomized to receive 12 weeks of twice-weekly one hour CFST sessions or 12 weeks of two one-hour sessions split between CCT and CFST. The six functional skills trained were technology based and focused on banking, shopping, and medication management.

Results: Paired t-tests found that completion time for all 6 CFST tasks significantly improved from the baseline assessment to the final training assessment in both groups of participants, all ts >4.31, all ps < .001. Average improvement in time to completion was 45%. Further, none of the 6 tasks improved differentially in the MCI and HC samples, as indexed by percentage of improvement from baseline to end of training: all ts <1.66, all ps >.12. Finally, combined CCT plus CFST did not differ from CFST alone on any of the %-change score measures: all t <1.64, all p >.11.

Conclusions: Both groups evidenced substantial improvements in functional skills performance. CCT supplementation led to similar CFST gains with half as many CFST training sessions. Importantly, HC participants who received skills training alone required an average of only 6 sessions per task to perfect their performance. Both groups of participants demonstrated improvements in performance across all tasks; MCI participants required more training sessions but learned equivalently. These findings suggest that training interventions can bypass memory impairments in MCI and lead to gains in the ability to perform everyday functional skills.

Keywords: Functional Capacity, Mild Cognitive Impairment due to AD, Technology-Assisted Treatment

Disclosure: i-Function, Inc, Stock / Equity; Verasci, Inc, Consultant

W3

The Effects of Statins on Cognition in Older Women at Genetic Risk for Alzheimer's Disease

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Background: It is well established that the apolipoprotein epsilon 4 allele (APOE4) and being female are risk factors for late onset AD. Declines in verbal learning and memory are early and prominent cognitive symptoms of conversion to AD. However, it is unclear whether statin use could provide a protection against AD as there are conflicting findings regarding the effects of statin use on cognition. Therefore, this study sought to examine the effects of statin use on cognition, specifically with respect to verbal learning and memory, by APOE4 status in a sample of cognitively unimpaired women at risk for AD.

Methods: Neuropsychological, statin use, and APOE4 data were utilized as a secondary analysis from an ongoing longitudinal study at the Banner Alzheimer's Institute in Arizona. Subjects (N = 149) were cognitively unimpaired women aged 47-75 with a family history of probable AD in at least one first-degree relative. Neuropsychological outcome variables included total learning, immediate memory, and delayed memory scores from the Rey Auditory Verbal Learning Test (RAVLT). Statin use was defined by whether the study subject was taking a cholesterol lowering drug, including atorvastatin, fluvastatin, lovastatin, pravastatin, pitavastatin, simvastatin, rosuvastatin, lescol, mevacor, altocor, livalo, zocor, and crestor, at study enrollment.

Statistical Analyses: Linear regression analyses were conducted with age, education, body mass index (BMI), APOE4 status (i.e. presence of at least one APOE4 allele versus no APOE4 allele), and statin use included as model covariates in addition to the interaction term of APOE4 status by statin use.

Results: Statistically significant interactions were found between statin use and APOE4 status in relation to RAVLT total learning and RAVLT immediate memory. Among APOE4 carriers, statin use was associated with a 2-point drop in RAVLT total learning score and a 1-point drop in RAVLT immediate memory. Whereas, in the APOE4 non-carriers, statin use was associated with a 6-point increase in RAVLT total learning and a 1-point increase in RAVLT immediate memory.

Conclusions: Differential effects of statin use in women were found in relation to APOE4 status. Statin use in women APOE4 non-carriers was associated with better verbal learning and immediate memory performances whereas statin use in women APOE4 carriers was associated with worse performances on these same tasks. Our findings suggest that sex and APOE4 status are important factors in the consideration of statin use, and if replicated in a larger sample, would inform treatment guidelines for the statin drugs.

Keywords: Statins, APOE4 Allele, Alzheimer's Disease

Disclosure: Nothing to disclose.

W4

Functional Significance of Cortical Cholinergic Circuits in Cognitive Decline

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Background: Cholinergic neurons of the basal forebrain (BFCNs) send extensive projections to the cortical mantle and many subcortical regions of the brain including the hippocampus and the amygdala. These highly branched axonal arbors may be metabolically challenging to maintain, perhaps underlying the vulnerability of cholinergic axons to fragmentation and loss in the aging brain. Indeed, studies of post-mortem brains from patients with severe AD find significant loss of cholinergic fibers compared to their age-matched, cognitively intact counterparts. One region that is known to be vulnerable early on in aging is the entorhinal cortex (EC), a cortical region that receives extensive input from BFCNs. Using the EC as a model of early cortical dysfunction, we investigated the relationship between altered cholinergic integrity and cortical function in aging.

Methods: All studies were conducted in accordance with Stony Brook University's Institutional Animal Care and Use Committee (IACUC) and Institutional Review Board (IRB). In order to investigate the cholinergic system in vivo in humans, we used Positron Emission Tomography (PET) imaging with ligand 18F-VAT, a presynaptic cholinergic protein that serves as a marker of cholinergic synapse health. In parallel studies in rodents, we used transgenic mouse models specifically designed to selectively target and visualize the cholinergic system. By crossing these mouse models to an aging model (mouse model that exhibits accelerated aging pathology including amyloid plaques and hyperphosphorylated tau tangles) we were able to investigate

changes to cholinergic projection profiles and function in this AD-like model. Using specific cognitive tasks that assay cortical function, we can stratify the data on cholinergic system integrity by performance on cognitive tasks.

Results: First, to investigate the relationship between cholinergic system integrity and cortical function in aging in humans, we acquired PET scans in healthy participants and participants with cognitive impairment to visualize and quantify changes in the pattern and density of cholinergic innervation in health vs disease. Our preliminary findings show a decrease in 18F-VAT volume of distribution (VT) in the EC between healthy participants and patients. When we look across a continuum of cognitive score, we also find a significant correlation between cognitive performance and EC cholinergic terminal field density.

To understand the mechanism underlying altered VAT uptake, we used a rodent aging model that exhibits accelerated aging pathology. Confirming that the aging model mice have impaired performance on an EC-based memory task, we asked whether this was driven by alterations to cholinergic inputs to the EC. Our initial studies demonstrate dramatic fragmentation of cholinergic axonal fibers in EC. Further, we wondered how altered cholinergic input to the EC could change the circuit dynamics, and found elevated baseline firing rate in aging model mice, possibly due to an imbalance to the excitation-to-inhibition ratio. Ongoing studies are investigating the effects of altering endogenous acetylcholine levels using optogenetics and chemogenetics on firing rate and behavioral output.

Conclusions: The strength of these studies lies in our ability to apply high-resolution techniques in both rodents and humans to better understand the cholinergic system. Using related markers of cholinergic terminal fields, albeit with dissimilar techniques, has strengthened our view that the highly quantitative information gathered in rodents improves our interpretation of in vivo human imaging and allows us to make specific predictions about what altered VAT uptake can tell us about cognition and cognitive impairment.

Keywords: PET Imaging, Acetylcholine, Aging and Dementia, Translational Neuroscience

Disclosure: Nothing to disclose.

W5

Self-Reported Sleep Disturbances are Associated With Poorer Cognitive Performance in Older Adults With Hypertension: A Multi-Parameter Risk Factor Investigation

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Background: Sleep disturbances are associated with low-grade inflammation and metabolic dysfunction, both of which predict poorer cognitive outcomes in aging. Diminished physical mobility and elevated depressive symptomatology are also associated with poorer sleep and increased risk of cognitive decline in older adults. Given the evidence of multi-parameter risk factors in shaping cognitive outcomes, multi-dimensional investigations of how these factors impact cognition in older adults are warranted. In the present, we sought to determine the extent to which self-reported sleep disturbances, metabolic syndrome (MetS) risk factors, cellular inflammation, depressive symptomatology, and diminished physical mobility were associated with poorer cognitive performance in a cross-sectional cohort of older adults with elevated, though well-controlled, blood pressure.

Methods: Hypertensive, community-dwelling older adults (N = 145, 72.7 ± 7.9 years old; 66% women; systolic BP = 133 ± 18 mmHg) without recent stroke, neurological impairment, inflammatory disorders, or major depression, were recruited for a larger behavioral intervention study. Demographic variables and medication use were recorded via standardized interview. Average systolic and diastolic blood pressure were calculated from three consecutive seated measurements at 5 min intervals following 15 min seated rest. Timed Up and Go (TUG) was used to assess physical mobility. Unfasted lipids, glucose levels, and complete blood counts were assessed by commercial laboratory. Metabolic syndrome (MetS) risk factors, which include elevated waist circumference, serum glucose, blood pressure, and triglycerides, and lower high-density lipoprotein cholesterol, were quantified according to standard clinical thresholds. Depressive symptoms were assessed using the Beck Depression Inventory (BDI-II) and self-reported sleep disturbances using the Short Form Patient-Reported Outcomes Measurement Information System (PROMIS-SD) Sleep Disturbance Scale. Cellular inflammation regulation was assessed by ex vivo monocyte tumor necrosis factor (TNF) response to 4-hr LPS stimulation, quantified as the percent difference in TNF+ monocytes between LPS-only and LPS +isoproterenol treatment. Cognition was evaluated using the Montreal Cognitive Assessment (MoCA), and a threshold of 24/25 was used to differentiate impairment. Six domain-specific MoCA subscale scores were also calculated. Group comparisons were conducted using Chi-squared and Kruskal-Wallis tests. Univariate correlations between risk factors and cognitive measures were computed using Spearman's rho, and p-values adjusted using Benjamini-Hochberg. Binomial logistic regression was implemented with a non-parametric bootstrapping procedure to determine odds ratios for each risk factor in predicting cognition group membership. Relationships between risk factors and cognitive domain scores were assessed using bootstrapped linear regression.

Results: Of the 145 participants studied, fifty-four (37%) demonstrated evidence of cognitive impairment, and reported significantly greater sleep disturbances than normocognitive individuals (PROMIS-SD: 23.1 ± 0.62 vs. 21.0 ± 0.33; $t = 3.01$, $p < 0.01$). Lower MoCA total scores were significantly correlated with age ($\rho = -0.23$), TUG time ($\rho = -0.29$), and PROMIS-SD ($\rho = -0.27$) scores (all adjusted $p < 0.05$). PROMIS-SD scores were also negatively correlated with visuospatial ($\rho = -0.30$), executive function ($\rho = -0.21$), and language ($\rho = -0.19$) MoCA subdomain scores (all adjusted $p < 0.05$). In logistic regression analysis adjusted for age, sex, and native language, higher PROMIS-SD scores predicted a significantly increased risk of a low (≤24) MoCA total score [OR = 2.00 (1.26-4.87); $p = 0.004$]. MetS risk factor incidence [OR = 1.59 (0.98-3.12); $p = 0.06$] and anti-hypertensive medication usage [OR = 1.60 (0.97-3.36); $p = 0.07$] conferred somewhat greater risk of a low MoCA total score. In multiple linear regression analyses, higher PROMIS-SD scores were associated with significantly poorer scores on MoCA subdomains for executive function ($\beta std = -0.26$ [−0.51 − 0.06], $p = 0.005$), visuospatial performance ($\beta std = -0.20$ [−0.34 − 0.01], $p = 0.04$), and memory ($\beta std = -0.29$ [−0.29 − 0.01], $p = 0.048$).

Conclusions: Our results indicate that the deleterious impact of self-reported sleep disturbances on cognitive performance was prominent over other important risk factors in an older hypertensive population, and illustrate the importance of clinician evaluation of sleep in patients with or at risk of diminished cognitive function. Although replication by comprehensive neuropsychological evaluation is needed, certain cognitive domains, such as executive function, appear to be more affected by sleep disturbances. Future studies implementing more extensive neuropsychological testing and objective measurements of sleep quality are warranted to further explore these associations.

Keywords: Aging, Cognition, Sleep Disturbance, Hypertension, Cardiometabolic Risk

Disclosure: Nothing to disclose.

W6

JOTROL, a More Bioavailable Formulation of Resveratrol, Shows Potential in Targeting Alzheimer's Disease-Related Pathology

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Background: Alzheimer's disease (AD) is the sixth leading cause of death in the United States, affecting 1 in 10 people over the age of 65. Pathological hallmarks of AD include accumulation of amyloid plaques, intracellular neurofibrillary tangles resulting from the aggregation of hyperphosphorylated tau protein, and neuroinflammation. To date, all clinical trials involving single target treatments for AD have failed. Recently, a randomized clinical trial with the GRAS (Generally Recognized As Safe by the FDA) compound Resveratrol (3,5,4′-trihydroxy-trans-stilbene) has shown positive indications in AD patients (Turner et al., 2005; Moussa et al., 2017). Resveratrol, a polyphenol antioxidant found in plants such as red grapes, blueberries, and peanuts, has been reported to affect several AD-related and neuroprotective genes via activation of SIRT1. However, the high dose needed due to low bioavailability was reported to cause GI side effects. To address the low availability issue, JOTROL, a new oral formulation of resveratrol that shows significantly better pharmacokinetic properties than non-formulated resveratrol, was manufactured by Jupiter Orphan Therapeutics. We hypothesize that equimolar concentrations of JOTROL, compared to non-formulated resveratrol, will result in greater brain exposure to resveratrol, and more efficacious responses on AD biomarkers. Important, a reduction in dose regimen will likely result in reduced side effects.

Methods: Three-month-old male Sprague Dawley rats ($n = 3$ per treatment group) were administered a single oral dose of 50 mg/kg JOTROL 30 minutes prior to being euthanized and brains harvested. 15-month-old male triple transgenic (APPSW/PS1M146V/TauP301L; 3xTg-AD) AD mice were treated by oral gavage daily with 50 mg/kg JOTROL ($n = 5$) or vehicle ($n = 5$) for 36 days. Behavior effects were assessed using open field, novel object recognition, and Y-maze, after which mice were euthanized and organs extracted. All experiments were approved by the University of Miami Miller School of Medicine Institutional Animal Care and Use Committee and conducted according to specifications of the NIH as outlined in the Guide for the Care and Use of Laboratory Animals. RT-qPCR, ELISA and Western blot were performed to analyze inflammatory cytokine expression, enzyme activity and mitochondrial biogenesis. Unpaired Student's t test was used for comparisons of two means. One-way ANOVA or repeated measures two-way ANOVA with appropriate post hoc analyses were used for multiple comparisons. A P value < 0.05 was deemed to be of statistical significance.

Results: Increased bioavailability of resveratrol was confirmed in both rats and mice after oral treatment with JOTROL. JOTROL resulted in 7-fold greater concentration in rat plasma than non-formulated resveratrol ($p < 0.05$), and a significant increase in resveratrol concentration in mouse brain. In rats, a single oral administration of 50 mg/kg JOTROL significantly increased SIRT1 and α-secretase ADAM10 gene expression in the brain of rats at 30 minutes ($p < 0.0001$), and significantly decreased Tau gene expression ($p < 0.05$). In 15-month-old 3xTg-AD mice, JOTROL treatment significantly increased both mitochondrial biogenesis ($p < 0.05$) and ADAM10 expression in the brain ($p < 0.01$). We also found increased mitochondrial biogenesis ($p < 0.05$) and reduced inflammation markers TNF-α and IL-6 in 3xTg-AD mice livers ($p < 0.001$). These data show that JOTROL can address several AD-relevant targets in aged transgenic mice, supporting a multi-pronged approach.

Conclusions: Our data suggest that JOTROL may be beneficial for Alzheimer's disease through increased expression and activation of neuroprotective genes and suppression of pro-inflammatory genes. We plan on investigating the ability of JOTROL to prevent AD-like pathogenesis in AD mouse models.

Keywords: Alzheimer's Disease, Preclinical Alzheimer's Disease, Resveratrol, Natural Compound, Epigenetics

Disclosure: Nothing to disclose.

W7

Radio-Pharmacologic Perturbation of Alzheimer's Disease Hallmarks

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Background: There are currently no available methods to treat, attenuate, or reverse the progression of Alzheimer's disease (AD), the 6th leading cause of death in the United Sates. Traditional therapeutic approaches have mostly targeted the two main AD hallmarks, amyloid and tau pathologies, and have yielded disappointing results. We and others agree that a multifactorial approach is likely to better address the comorbidities present in AD. Previous work from our team has demonstrated that low dose (daily 2 Gy X 5 treatments) x-ray therapy of the brain of double transgenic AD mouse models over-expressing both the amyloid precursor protein (APP) Swedish and Presenilin 1 dE9 mutations (APP/PS1 mice) decreased plaque load, decreased pro-inflammatory cytokines and improved performance in the Morris water maze. In addition, we have shown that 3 months intraperitoneal (IP) administration of aged 3xTg-AD mice with the small molecule RGFP966, a selective HDAC3 inhibitor, reduced tau hyperphosphorylation in a brain region-specific and residue-specific manner, and improved performance in a battery of spatial and non-spatial learning and memory paradigms. We show here that low-dose combination therapy (LDCT) of x-radiation and HDAC3 inhibition has both additive and synergistic effects on modulating AD hallmarks.

Methods: Preliminary investigations to study changes in gene expression, APP processing, and pathogenic tau post-translational modifications (PTMs) were performed using the HEKAPPSw cell line. This cell line overexpresses amyloid precursor protein (APP) with the Swedish familial mutation at the beta-secretase cleavage site causing over-production of Aβ peptides which allows us to robustly measure changes in APP processing. Primary microglia from post-natal day 6 C57BL/6 mice and the spontaneously immortalized SIM-A9 microglial cell line have been used to analyze the innate immune response and alterations in phagocytic capacity. Cells were treated with either 1 Gy of x-radiation, 3 μM RGFP966, or a combination of the two for 6, 12, 24, and 48 hours. Control treatments included sham exposure in the irradiation cabinet and/or 0.03% DMSO. Additionally, a preliminary experiment was performed with 19 month-old C57/B6 male. The aged mice ($N = 6$ radiation, $N = 6$ sham) were dosed with 2% isoflurane (O2 flow-rate 2) for ~7 minutes while they received either whole brain x-radiation at a dose of 1 Gy twice per week or sham

irradiation for 4 weeks. All experiments were approved by the University of Miami Miller School of Medicine Institutional Animal Care and Use Committee and conducted according to specifications of the NIH as outlined in the Guide for the Care and Use of Laboratory Animals. RT-qPCR, Western blot, and immunofluorescence were performed to analyze inflammatory cytokine expression, protein expression, and phagocytosis. One-way ANOVA or repeated measures two-way ANOVA with appropriate post hoc analyses were used for multiple comparisons. A P value < 0.05 was deemed to be of statistical significance.

Results: Combined at low doses previously shown not to be efficacious, these two multifactorial treatments show synergistic increase in primary microglia phagocytosis of oligomeric Aβ42 ($P < 0.05$; $N = 6$) synergistic increase in neuroprotective sAPPα production ($P < 0.05$; $N = 3$) and BDNF gene expression ($P < 0.0001$, $N = 6$) and a synergistic decrease in phosphorylated tau at Ser202 ($P < 0.05$; $N = 6$). Additionally, combination therapy steers microglial innate immune memory toward a tolerant phenotype as measured by a reduced inflammatory response to Aβ stimulation 48h after LDCT treatment compared to control ($P < 0.0001$, $N = 6$). Lastly, 19-month old x-irradiated mice showcased no significant behavioral or motor deficits in open field, Y-maze, and sub-threshold object location and object recognition memory paradigms.

Conclusions: While many additional questions remain to be addressed (transcriptomic changes, analysis of blood-brain barrier permeability, cellular metabolism, complementary genetic silencing and overexpression of HDAC3) these striking preliminary results support our hypothesis that radio-pharmacologic perturbation of the genome and epigenome can improve and potentially reverse AD pathology in a synergistic manner. We will continue to explore other epigenetic compounds that are currently FDA approved (i.e. Vorinostat) to maximize the translational potential of this strategy and apply this approach to 9-month old male and female 3x-Tg AD mice.

Keywords: Epigenetics, Alzheimers Disease, Neuroinflammation, Radiation, Low-Dose

Disclosure: Nothing to disclose.

W8

Pilot Magnetic Resonance Spectroscopy Study of Brain Metabolite Changes in Girls With PTSD After Treatment With Group Interpersonal Therapy

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Background: In the US, more than 250,000 girls and women are subjected to sexual assault or rape each year. Existing data suggest that 40-50% of these individuals will develop post-traumatic stress disorder (PTSD), a debilitating condition often associated with comorbid psychiatric disorders. Drug treatments for PTSD are inadequate in 40% of patients and only 20-30% of treated patients achieve full remission. Accordingly, we need a better understanding of the neurochemistry of PTSD to develop more effective treatments. Proton magnetic resonance spectroscopy (MRS) is a noninvasive imaging method that can be used to assess brain chemistry in vivo in humans. MRS has been used to quantify cerebral metabolites that may play important roles in the neurochemistry of PTSD, including glutamate. However, almost all prior MRS studies of PTSD published to date (32 of 33 by our count) used cross-sectional designs, which are unable to assess brain chemistry changes associated with treatment. Further, most studies used MRS methods that are suboptimal for quantifying glutamate. To overcome these prior limitations, this pilot study acquired multi-echo-time PRESS MRS scans (mTE) from girls with PTSD at baseline and after treatment with group interpersonal therapy (IPT) to study glutamate and other brain metabolite changes occurring over time.

Methods: This study was conducted in five adolescent girls aged 16±1.3 (mean±SD) years old presenting with PTSD to the Brazilian Public Health Service at the Perola Byington Hospital in Sao Paulo, Brazil. Clinician-Administered PTSD scale for DSM-5 (CAPS-5) scores were obtained before and after treatment. Subjects underwent serial clinical assessments and brain mTE scans on a 3 Tesla Philips Achieva scanner with a 32-channel headcoil before and after 15 weeks of treatment with IPT. MRS scans were acquired from an 8 cm3 voxel (2 x 2 x 2 cm) positioned midline over the dorsal anterior cingulate gyrus with TR=2000, TEs (24)=30,40,50,60,...,250,260ms, 2048 samples, Bandwidth=2kHz, averages=8, total scan time=8.8 min. Spectra at stepped TEs were summed prior to analysis with LCModel. A simulated metabolite basis set using the same stepped TEs was summed and used for metabolite quantification. The un-suppressed water signal from each voxel was used for eddy current correction and metabolite quantification.

Results: Baseline CAPS-5 scores averaged (±SD) 52.6 ± 16.2 and CAPS-5 scores declined after IPT by ≥20 points in all 5 subjects, indicating meaningful clinical improvement in all subjects. Water-normalized glutamate levels also declined in all 5 subjects while water-normalized myo-inositol levels increased in all 5 subjects (Ps < 0.05, 2-tailed t-tests).

Conclusions: This pilot study suggests that clinically-meaningful CAPS-5 score declines following IPT treatment for PTSD may be associated with lower glutamate/water ratios and higher myo-inositol/water ratios. The glutamate effect could reflect normalization of a hyperglutamatergic state and the myo-inositol effect could reflect astroglial proliferation associated with enhanced neural plasticity. These pilot findings illustrate the potential value both of prospective MRS studies of PTSD and of the use of MRS pulse sequences capable of quantifying glutamate. These findings warrant future studies with larger sample sizes to more fully characterize brain glutamate and other metabolite changes associated with PTSD and its treatment, which may help advance development of novel treatments for PTSD.

Keywords: 1H-MRS, Pediatric PTSD, Interpersonal Psychotherapy, Anterior Cingulate Gyrus, Brain MRI

Disclosure: Nothing to disclose.

W9

Trick or Threat: Neural Circuits Balancing Reward-Seeking With the Risk of Predation

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Background: Survival depends on a balance between approaching food and avoiding the risk of being attacked by a predator. However, which neuronal circuits integrate reward and fear information remains unknown. Neurons in the paraventricular thalamus (PVT) respond to both reward and fear-associated cues, making this region a potential candidate for this integration.

Methods: To test this hypothesis, Long-Evans adult rats previously implanted with guide cannulas, optical fibers or electrodes targeting the PVT were initially trained to press a bar

for sucrose in the presence of audiovisual cues (reward cues). Cat saliva was used to induce innate fear responses (predator odor). During a conflict test, predator odor and reward cues were simultaneously presented and rats needed to overcome their fear of predator cues to bar press for food.

Results: We found that rats exposed to predator odor alone exhibited robust defensive behaviors (increased freezing and avoidance responses), compared to controls exposed to neutral odors. Predator odor exposure also increased the expression of the neural activity marker cFos in the medial amygdala, ventromedial hypothalamus (VMH) and anterior portion of PVT (aPVT). Single-unit recordings from aPVT neurons revealed three distinct populations of neurons that changed their firing rate in response to predator odor, reward cues, or both, when compared to baseline (Z-score > 2.54 for excitatory and < -1.96 for inhibitory responses). aPVT responses to reward cues correlated with food-seeking behavior and were attenuated during predator odor exposure. Inactivation of aPVT with the GABAA agonist muscimol increased time approaching the food area and reduced time avoiding the predator odor area during the conflict test. Notably, inactivation of aPVT had no effect when the predator odor or the food-seeking tasks were carried out independently. In addition, chemogenetic inactivation of aPVT projections to the nucleus accumbens (NAc) reduced defensive responses and increased food seeking. Interestingly, predator odor exposure activated aPVT neurons expressing the stress neuropeptide corticotropin-releasing factor (CRF). Our neuroanatomical tracer study showed that aPVTCRF neurons send projections to VMH, bed nucleus of stria terminalis, central nucleus of the amygdala, and ventral hippocampus, with the NAc receiving the densest projection. Slice recordings from NAc neurons demonstrated that photoactivation of aPVTCRF fibers in the NAc elicited large excitatory postsynaptic responses, which were blocked by NMDA and AMPA/Kainate glutamate receptor antagonists. Thus, we speculated that aPVTCRF glutamatergic projections to NAc could play a role in food-seeking regulation. Consistent with our prediction, photoactivation of aPVTCRF fibers in the NAc reduced food-seeking and increased avoidance responses.

Conclusions: Taken together, all results suggest that activity in the aPVTCRF-NAc pathway regulates the balance between avoiding threats and searching for rewards.

Keywords: Paraventricular Nucleus of the Thalamus, Nucleus Accumbens, Approach/Avoidance, Fear, Reward

Disclosure: Nothing to disclose.

W10

Lower Translocator Protein (TSPO) Measured With PET in PTSD is Associated With Greater PTSD Severity and Peripheral Inflammation

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Background: Preclinical and clinical studies of peripheral, cerebrospinal fluid, and brain transcription levels of immune markers indicate that innate immunity and inflammatory responses are dysregulated in individuals with post-traumatic stress disorder (PTSD). In particular, higher levels of peripheral immune markers such as C-reactive protein (CRP) and the inflammatory cytokines IL-6, IL-1beta, IFN-gamma and TNF-alpha have been found in individuals with PTSD relative to controls and associated with greater severity of PTSD symptoms. In contrast, some studies have found lower peripheral immune markers in individuals with PTSD relative to controls, specifically TNF-alpha and IFN-gamma or no difference between groups in many of these markers. The goal of this study was to use positron emission tomography (PET) brain imaging to investigate levels of prefrontal-limbic translocator protein (TSPO), a marker of microglia, in vivo in individuals with PTSD compared to controls, and determine how this neuro-immune system marker relates to peripheral immune markers and severity of PTSD symptoms.

Methods: A total of 23 individuals with PTSD (13M, 10F) and 26 trauma-exposed and non-exposed otherwise healthy individuals (18M, 8F) were imaged. All subjects participated in a magnetic resonance imaging (MRI) study and one [11C]PBR28 PET scan with arterial blood sampling to measure the metabolite-corrected parent input function. [11C]PBR28 binds to TSPO. PTSD diagnosis was determined using the Clinician-Administered PTSD Scale for DSM-5. TSPO levels were quantified with [11C]PBR28 distribution volumes (VT) throughout the brain. There is a single-nucleotide polymorphism genotype, rs6971, which affects affinity of [11C]PBR28 for TSPO. Potential subjects were prescreened for a single-nucleotide polymorphism genotype, rs6971 which affects affinity of [11C]PBR28 for TSPO; individuals who were T/T homozygotes (low-affinity binders) were excluded and genotype was adjusted for in statistical analysis.

Results: Prefrontal-limbic TSPO availability was significantly lower in the PTSD group compared to the control group. Specifically, analysis in primary regions of interest (amygdala, anterior cingulate cortex, hippocampus, insula, ventromedial prefrontal cortex) revealed that [11C]PBR28 VT was significantly lower in the PTSD vs. control group (MANCOVA: $F_{5,41} = 2.46$, $p = 0.05$) with insula ($F_{1,45} = 2.16$, $p = 0.04$) and vmPFC ($F_{1,45} = 2.36$, $p = 0.02$) significant after correction for multiple testing. There were no significant differences in TSPO availability between the trauma-exposed and non-exposed healthy control groups. In the PTSD group, lower prefrontal-limbic TSPO availability was associated with lower IFN-gamma (beta = 0.86, $n = 14$, $p = 0.04$), and higher CRP (beta = −0.92, $p = 0.03$) levels, as well as with greater severity of PTSD symptoms (beta = −0.61, $p = 0.02$), particularly emotional numbing symptoms (beta = −0.47, $p = 0.03$).

Conclusions: Results of this study provide the first known in vivo evidence of neuroimmune dysregulation in PTSD. They further indicate that, in individuals with PTSD, greater suppression of neuroimmune function is associated with peripheral inflammatory markers and greater severity of PTSD symptoms. Collectively, these findings suggest that the neuroimmune system may be a promising target for novel treatment development in PTSD.

Funding: This research was supported in part by National Institute of Health Grants R01MH110674 and the Clinical Neurosciences Division of the U.S. Department of Veterans Affairs National Center for PTSD.

Keywords: PET Imaging, PTSD, Neuroimmune, Human Microglia, Brain

Disclosure: Nothing to disclose.

W11

Androsterone and DHEAS are Inversely Associated With Pain Symptoms in Male Iraq/Afghanistan-Era Veterans

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Background: Pain symptoms are common among Iraq/Afghanistan-era veterans, and many experience persistent pain symptoms

despite multiple pharmacological and behavioral interventions. Numerous pharmacological pain interventions are suboptimal in their efficacy and/or side effect profiles, and may have significant addictive potential. Safe and efficacious alternative treatment interventions are thus acutely needed. Emerging data suggest that neurosteroids may have biomarker and therapeutic utility for pain. Although the majority of findings have focused on allopregnanolone, a positive allosteric modulator of GABA-A receptors, other neurosteroids may also be involved in the pathophysiology of pain. Identification of endogenous markers associated with pain conditions may lead to the development of new safe and efficacious pain treatments. The current study thus determined the association between serum neurosteroid level (androsterone, DHEA, and DHEAS) and pain symptoms in Iraq/Afghanistan-era veterans.

Methods: The current study quantified serum DHEA and DHEAS levels (by radioimmunoassay), and androsterone levels (by gas chromatography/mass spectrometry) in a cohort of 484 male Iraq/Afghanistan-era veterans. Pain symptoms were assessed by items from the Symptom Checklist-90-R (SCL-90-R) querying headache, chest pain, muscle soreness, and low back pain over the past 7 days. Pain scores were dichotomized - participants reporting pain levels of 0 or 1 on an SCL-90-R pain item were designated to the "low pain" condition (i.e. "none" or "a little bit"), while participants endorsing pain levels of 2, 3, or 4 were designated to the "high pain" condition (i.e. "moderate" to "extreme" pain). Poisson regression procedures were conducted for each of the four SCL-90-R pain items and serum neurosteroid levels, controlling for age and smoking (variables previously reported to impact neurosteroid levels).

Results: Associations between pain ratings and neurosteroid levels were examined with linear regression analyses, controlling for age and smoking. Bivariate non-parametric Mann-Whitney analyses examining neurosteroid levels across high and low levels of pain were also conducted. Serum androsterone levels were inversely correlated with chest pain (P=0.03), headache pain (P=0.01), and muscle soreness (P=0.001). Serum DHEAS levels were inversely associated with chest pain (P=0.0004), headache pain (P = 0.0001), and low back pain (P=0.008). There were no significant associations between median DHEA levels and any self-reported pain symptoms.

Conclusions: Androsterone, a positive GABA-A receptor modulator, was inversely associated with self-reported chest pain, headache pain, and muscle soreness in male Iraq/Afghanistan-era Veterans reporting moderate/extreme pain. This finding supports literature demonstrating the analgesic effects of GABAergic neurosteroids such as allopregnanolone. To our knowledge, this is the first report that androsterone, also a GABAergic neurosteroid with characteristics similar to allopregnanolone, is inversely associated with self-reported pain symptoms. Our findings additionally demonstrated that serum DHEAS levels were inversely associated with chest pain, headache pain, and low back pain, providing support for the potential analgesic effect of DHEAS. Together, these results demonstrate that neurosteroids may play a role in the pathophysiology of pain and may be promising biomarker candidates for pain conditions with the potential for therapeutic development. Future research in larger Veteran cohorts are needed to corroborate our findings that lower androsterone and DHEAS levels are correlated with higher levels of self-reported pain.

Keywords: Androsterone, Dehydroepiandrosterone, Neuroactive Steroid, Neurosteroid, Pain

Disclosure: Avanir Pharmaceuticals, Centers of Psychiatric Excellence, Continuous Precision Medicine, Janssen Pharmaceuticals, Neurocrine Biosciences, Otsuka Pharmaceuticals, Teva Pharmaceuticals, Consultant

W12

Fear Memory Extinction is Facilitated After Systemic Antagonism of Nociceptin/Orphanin FQ (NOP) Receptors: Relevance for Preclinical Models of Traumatic Stress

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Background: FDA-approved pharmacotherapies for the treatment of post-traumatic stress disorder (PTSD) are limited and clinical studies of new entities often lack efficacy. We have implemented preclinical rodent models of traumatic stress using predator exposure and adverse associative learning, which show similar behavioral impairments to those reported in the clinical literature. The nociceptin/orphanin FQ (NOP) receptor system is implicated in central-mediated processes underlying PTSD, with reported increased levels of nociceptin in cerebrospinal fluid in a rat model of PTSD (Zhang et al., 2012), suggesting targeting this receptor system may have therapeutic potential. The relationship between the NOP receptor and fear memory extinction in preclinical models has yet to be fully characterized, despite reports on other aspects of fear learning and memory (acquisition, consolidation, and reconsolidation) (Ouagazzal et al., 2015; Andero et al. 2015; Rekik et al., 2017).Here, we characterized the effects of NOP antagonism in a rodent model of traumatic stress as well as in fear memory extinction.

Methods: We characterized NOP antagonists (J-113397, 2-20 mg/kg, i.p.; SB-612111, 0.1-4 mg/kg, i.p.) for acute and repeated administration effects in a behavioral test battery (measuring startle response, exploratory behavior, pain, and motor function) in adult male Sprague-Dawley rats. We also tested efficacy of these same NOP antagonists on behavioral performance recovery following a single-day protected exposure to predators (snake, ferrets, and cats). Finally, we tested the effect of NOP antagonism on fear memory extinction using SB-612111 (1-3 mg/kg, i.p., 1-h pre-treatment) in four, once-daily extinction tests beginning five days after fear conditioning (a single 35-min session of 20, 2-s 1 mA shock + light/tone stimuli pairings presented pseudorandomly).

Results: The NOP antagonists J-113397 and SB-612111 did not significantly degrade baseline behavioral performance at any tested dose, nor significantly alter behavioral recovery following predator exposure. In these experiments, neither NOP antagonist had effects on locomotor behavior. In fear memory extinction tests, SB-612111 (1 and 3 mg/kg) significantly reduced freezing behavior as compared to a vehicle-treated group as early as the first extinction test ($p < 0.01$), and remained significantly different from the vehicle-treated group across test days ($p < 0.05$).

Conclusions: NOP antagonism facilitates fear memory extinction in rats, suggesting elevated levels of the peptide ligand or its downstream signaling mediators may promote exaggerated fear responses. These results add to a growing body of evidence demonstrating that NOP-targeted compounds modulate aspects of learning and memory. Despite the beneficial effects of NOP antagonism on fear learning, we observed minimal improvement in behavioral recovery following predator exposure. The present results suggest NOP antagonists may hold therapeutic potential for associative fear memory in patients with PTSD. Importantly, as preclinical fear memory extinction sessions can be likened to prolonged exposure therapy in the clinic, these results suggest NOP antagonism may be a potential therapeutic to use in conjunction with prolonged exposure therapy sessions.

Disclaimer: Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted under an approved animal use protocol in an AAALACi accredited facility in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition. This work was supported by the US Army Medical Research and Materiel Command Military Operational Medicine Research Program and was conducted while the author (RMT) held a National Research Council Research Associateship award at the Walter Reed Army Institute of Research.

Keywords: Nociceptin/Orphanin FQ, Rodent Models, Fear Extinction, Traumatic Stress

Disclosure: Nothing to disclose.

W13

A Computational Network Perspective on Pediatric Anxiety

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Background: Current psychiatric taxonomy segregates pediatric anxiety symptoms into distinct diagnostic subtypes that are supported by behavioral observations and factor analyses. However, typical mixed symptom presentation poses significant challenges for diagnosis, treatment, and research.

Reconciling evidence for distinct domains and limited specificity, pediatric anxiety may alternatively be conceptualized as manifesting within a multivariate network of inter-connected domains in ways poorly captured by current classification systems. To evaluate this possibility, we utilize network analytic approaches to characterize pediatric anxiety as a network of symptom domains. Quantifying this network structure could inform our clinical conceptualization of pediatric anxiety, and, accordingly, clinical practice and research.

Methods: Participants were 4,964 youths (ages 5-17 years) from seven international sites, presenting with a wide range of symptom severity (healthy, non-selected, high-risk, or clinically-anxious youth). Participants were assessed using the child-reported Screen for Child Anxiety Related Emotional Disorders, a standard measure with strong psychometric properties for dimensionally assessing symptom severity along domains that follow pediatric anxiety DSM diagnostic categories: generalized anxiety disorder (GAD), separation anxiety disorder (SEP), social anxiety disorder (SOC), and panic disorder (PAN); additionally, school avoidance symptoms (SCH). We then applied computational network analytic tools to quantify the anxiety symptom network structure by estimating the magnitude of unique associations (edges) among symptom domains (nodes) using regularized partial correlations. Differences between networks were tested using permutation tests of network invariance.

First, using the full sample, we examined the extent to which pediatric anxiety manifests as a network of inter-connected symptom domains. Second, we tested whether variation in network structure related to diagnostic status (patients vs. healthy controls). Third, we examined whether variation in network structure related to age (3-year longitudinal assessments) and sex, key moderators of pediatric anxiety expression.

Results: Across analyses, anxiety networks were estimated with high accuracy. First, the full-sample anxiety network featured a highly inter-connected structure; all symptom domains were positively correlated but to varying degrees, with the strongest edges being GAD-PAN ($r = .39$) and GAD-SOC ($r = .31$), and the weakest edge being SOC-PAN ($r = .04$). Second, patients and healthy youth differed in symptom severity ($ps < .001$ in all domains) but demonstrated comparable network structure ($p = .14$). Third, network structure differed by sex ($p = .038$), with females showing stronger GAD-SCH association than males ($p = .005$). Furthermore, longitudinal data indicated structural changes during childhood ($p = .019$), with GAD-PAN association decreasing over time ($p < .001$). Across analyses, GAD and PAN symptoms consistently showed high centrality, indicating their importance within the symptom networks.

Conclusions: We show that pediatric anxiety does not typically manifest along one symptom domain; instead, it manifests along multiple, inter-connected domains, accounting for typical mixed symptom presentation. Using a large, heterogeneous sample, we quantify the nature of these inter-domain associations, as well as specific moderation effects by sex and age. These novel findings have important implications for the clinical conceptualization of pediatric anxiety. consequently, these insights could inform the diagnosis, treatment, and study of pediatric anxiety, as will be discussed in more detail in the poster.

Keywords: Adolescent Anxiety, Network-Analysis, Classification, Sex Difference, Anxiety development

Disclosure: Nothing to disclose.

W14

Efficacy of 3,4-Methylenedioxymethamphetamine (MDMA)-Assisted Psychotherapy for Posttraumatic Stress Disorder: A Systematic Review and Meta-Analysis

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Background: Posttraumatic stress disorder (PTSD) is a common psychiatric condition that can develop following a traumatic experience. PTSD is associated with significant disability, a large economic burden, and despite the range of therapies to treat PTSD, response to antidepressants is limited. A growing body of clinical research suggests the efficacy of 3,4-methylenedioxymethamphetamine (MDMA)-assisted psychotherapy in individuals with treatment-refractory PTSD.

Methods: To assess the effectiveness and safety of MDMA-assisted psychotherapy for reducing symptoms of PTSD, a systematic review and meta-analysis was undertaken. Six online databases were searched from inception to December 2018. Reference lists of relevant articles were manually searched as well as electronic sources of ongoing trials and conference proceedings. Researchers active in the subject were also contacted. Eligible studies included randomized and quasi-randomized clinical trials using MDMA-assisted psychotherapy for PTSD in comparison with other medications, placebo or no medication (supportive care). We used standard methodological procedures expected by the Cochrane Collaboration. Two authors assessed studies for inclusion and extracted data. Using random-effects meta-analysis with Cochrane's Review Manager 5.3, we obtained standardized mean differences [SMD] and rate ratios [RR] for reduction in PTSD symptomatology.

Results: A total of 5 trials met inclusion criteria, totaling 106 participants (average age: 35-40 years, 70% female). Studies were rated as moderate in quality. MDMA-assisted psychotherapy demonstrated a high rate of clinical response (RR = 3.47, 95% CI: 1.70, 7.06), remission (RR = 2.63, 95% CI: 1.37, 5.02), with a large effect size at reducing the symptoms of PTSD (SMD = 1.30, 95% CI: 0.66, 1.94). Available evidence indicates that MDMA was well-tolerated, with few serious adverse events reported across studies.

Conclusions: MDMA-assisted psychotherapy appears to be a potentially safe, effective, and durable treatment for individuals with chronic, treatment-refractory PTSD. However, future studies involving larger samples and longer durations of treatment and follow-up are warranted—and underway.

Keywords: Psychotherapy, Anxiety & PTSD, MDMA, Meta-Analysis

Disclosure: Nothing to disclose.

W15

Prefrontal Noradrenergic and Cholinergic Dynamics Define Global Arousal After Stress

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Background: Across the cerebral cortex, neurons increase their firing when the pupil dilates, reflecting neuromodulatory control of arousal. By recording only the pupil, the global arousal state of neurons throughout the cortex can be inferred. Pupil diameter is believed to relate to noradrenergic state; despite multiple studies relating neuronal spiking activity to pupillary diameter, measurements of neurochemical fluctuations in norepinephrine have been limited by available techniques. Precisely defining the neurochemical basis of dynamic changes in arousal may offer insight into the mechanisms of arousal dynamics and how they are altered by emotional states. We combine measurements of new optical neurotransmitter biosensors with in vivo calcium imaging and pupillary recordings to better define how arousal is altered by stress.

Methods: Mice (sample size n=7-10 in two groups, stress and control) are used in this study with a common experimental design: day 1 - pupillary recordings, day 2 - stress (Stress-Enhanced Fear Learning) or control, day 3 - pupillary recordings post-stress. Four cohorts of animals were used: 1) no brain imaging, 2) in vivo calcium imaging of mPFC neurons (n=458 stressed, n=372 unstressed neurons), 3) in vivo norepinephrine fluorescent sensor imaging (GRAB-NE2h), and 4) in vivo acetylcholine sensor imaging (GRAB-Ach4.3). Simultaneous pupil and imaging datasets were analyzed with spectral analysis, event-triggered analysis of neural response to pupil dilation/constriction events, and a Hidden Markov Model of this time series data. Mice used were male 8-12 week old C57Bl6 mice.

Results: Acetylcholine shows higher correlation to pupillary diameter than norepinephrine ($r = 0.64$ vs 0.36 $p < 0.01$). Acetylcholine shows greater coherence with the pupil at infra-slow (<0.2 Hz) whereas norepinephrine shows greater coherence with the pupil at slow (0.3 - 0.5 Hz) frequencies. Stress alters the Ach-NE balance by increasing infra-slow acetylcholine-pupil coupling and decreasing infra-slow norepinephrine-pupil coupling. Acetylcholine and norepinephrine dynamics differ in timing to pupillary events, with norepinephrine lagging acetylcholine by 2 seconds ($p < 0.05$).

Acute traumatic stress causes more rapid transitions between pupillary arousal states ($p < 0.05$). These rapid transitions in the pupil reflect shifts in cortical arousal. Population (average) mPFC activity is highly correlated to the pupil ($r2 = 0.56$), but the neuron-pupil correlation is reduced by stress ($p < 0.001$). mPFC neuron-pupil coupling is particularly reduced at low frequencies (<0.5 Hz) after stress ($p < 0.05$). mPFC neuronal activity state transitions occur 0.5 seconds before pupillary state transitions, but this relationship is disrupted after stress.

Conclusions: Acetylcholine and norepinephrine dynamics suggest that these neuromodulators function to structure arousal at different timescales. The balance of acetylcholine and norepinephrine is not constant but is plastic, and varies with emotional state. Prefrontal cholinergic dynamics are more reflective of arousal state after stress. Arousal dynamics change after stress, which is reflected in desynchronization of neural activity in the prefrontal cortex. Coordinated state-switching between pupil and mPFC neuronal activity is also disrupted after stress. These results extend our previous understanding of stress in modulating arousal states and have implications for the conceptualization of disorders involving stress-induced hyperarousal, such as PTSD.

Keywords: Arousal, Acetylcholine, Norepinephrine, Systems Neuroscience, Optical Biosensors

Disclosure: Nothing to disclose.

W16

Common and Dissociable Effects of Oxytocin and Lorazepam on the Neurocircuitry of Fear

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Background: Benzodiazepines such as lorazepam (LZP), which target the GABAA receptor system, represent the gold standard of anxiolytic pharmacotherapy; however, their clinical benefit is limited by side effects and addictive potential. Thus, from a clinical perspective, there is an urgent need for developing novel and safe anxiolytics. Notwithstanding an ever-growing insight into the neurocircuitry mechanisms regulating fear, with current concepts emphasizing a key role of the functional organization within the amygdala complex, a guided detection of candidate compounds along their ability to strengthen these inhibitory mechanisms is missing. An emerging target is the oxytocin (OXT) receptor system, given converging evidence of anxiolytic-like properties of OXT in animals and humans. Thus, despite acting via different neurotransmitter pathways, OXT and benzodiazepines may share similar effects on the neurocircuitry of fear. Therefore, the rationale of this ultra-high field functional magnetic resonance imaging study was to map and compare OXT and LZP effects on local and network responses to fear-related stimuli.

Methods: One hundred and twenty-eight healthy male participants volunteered in this randomized double-blind, placebo-controlled, parallel-group study. Prior to scanning using a well-established emotional face matching paradigm, participants were administered a single dose of OXT (24 IU), LZP (1 mg) or placebo.

Results: On the behavioral level, LZP caused mild sedation evident in a 19% increase in reaction times. On the neural level, both OXT and LZP inhibited responses to fearful versus neutral faces within the centromedial amygdala (cmA), but showed different effects on intra-amygdalar connectivity. OXT strengthened the coupling between the cmA and basolateral amygdala whereas LZP increased the interplay between the cmA and superficial amygdala. Furthermore, OXT, but not LZP, enhanced

the coupling between the cmA and precuneus and dorsomedial prefrontal cortex.

Conclusions: Our data provide first insights into the intra-amygdalar mechanisms of OXT and LZP in humans, thereby facilitating the development of individually adjusted treatment protocols based on the distinct anxiolytic mechanisms of both compounds. Collectively, OXT and LZP dampened responses to fear-related stimuli in the cmA as a common denominator of anxiolytic action, with only OXT inducing large-scale connectivity changes of potential therapeutic relevance.

Keywords: 7T fMRI, Amygdala, Anxiolytics, Oxytocin, Benzodiazepine

Disclosure: Nothing to disclose.

W17

Effects of SRX246, a Vasopressin Receptor (V1a) Antagonist, on an Experimental Model of Phasic and Sustained Threat in Humans

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Background: Arginine vasopressin (AVP) is a neuropeptide that modulates both physiological (i.e., hypothalamic-pituitary-adrenal axis) and emotional (i.e., anxiety) responses to threat (Hernández-Pérez et al., 2018). AVP receptors, such as V1a, are heavily expressed in the bed nucleus of the stria terminalis and amygdala (Dannenhoffer et al., 2018), both of which are regions implicated in anxiety (Davis et al., 2010). Until recently, drugs that target vasopressin receptors in the central nervous system were unavailable, leaving limited psychopharmacological treatments for disorders where dysregulation of AVP function may be an underlying neurobiological mechanism, such as anxiety and post-traumatic stress disorders (Rotondo et al., 2016). The development of a novel arginine vasopressin receptor inhibitor, SRX246, by Azevan Pharmaceuticals, Inc. now permits the experimental validation of AVP's role in the regulation of anxiety in humans. This study examined the effects of SRX246 on a translational behavioral paradigm of phasic and sustained aversive states in healthy humans.

Methods: The study used a double-blind, cross-over design. Each healthy volunteer ($n = 32$, 15M, 17F) received two treatments, placebo (PLC) and 300 mg (180 mg every morning, 120 mg every evening) of SRX246 (SRX), in a counter-balanced order, for 10-14 doses over 5-7 days. The wash-out period was 5-7 days between treatments. The "NPU" threat test was used to probe phasic and sustained threat responses. Threat was induced using mild electrical shocks. The NPU test consists of 3 conditions: Neutral, Predictable, and Unpredictable. During Neutral, participants are safe from shock. During Predictable, a geometric shape (Cue) indicates risk for shock, while the absence of the shape, i.e., No-Cue, indicates safety. During Unpredictable, participants are at risk for shock at any time. Online subjective anxiety ratings and startle amplitudes were converted to t-scores. Measures were analyzed using 3-way ANOVAs, with Drug (SRX, PLC), Condition (Neutral, Predictable, Unpredictable), and Stimulus (Cue, No-Cue) as within-subject factors. Post-hoc analyses included a 2-way ANOVA with Drug (SRX, PLC) and subjective anxiety rating quartiles across treatments.

Results: Regarding Drug effects, SRX had, at a trend level, a main effect on online subjective anxiety ratings ($F(1,31)=3.00$, $p = .09$). Anxiety tended to be lower in SRX compared to PLC sessions. To better understand this relationship, additional analyses were conducted after separating participants into 4 groups based on their level of anxiety (Very High, High, Moderate, Low) across all sessions. These exploratory analyses revealed a Drug x Group interaction ($F(3,28)=3.79$, $p < .05$). Participants in the Very High group reported lower anxiety with SRX compared to PLC ($t(7) = -2.90$, $p < .05$), while those in the other 3 groups reported similar anxiety levels in the SRX and PLC sessions. Inspection of the data also suggests that during SRX, anxiety was lower in the Very High anxiety than the other 3 groups. Drug had no statistical effect on startle. Other findings, not related to Drug effects, validated the task manipulations (Condition, Stimulus). Condition x Stimulus interaction was statistically significant ($F(2,62)=31.70$, $p < .001$), indicating higher anxiety ratings during Cue than No-Cue during the Predictable ($t(31)=5.87$, $p < .001$), but not Neutral or Unpredictable, condition. Condition and Stimulus had each a significant main effect (Condition: Unpredictable > Predictable > Neutral; $F(2, 62)=101.24$, $p < .001$; Stimulus: Cue > No-Cue; $t(31) = -1.81$, $p < .001$). Startle analyses also evidenced a Condition x Stimulus interaction ($F(2,62)=14.78$, $p < .001$), with the largest increase in startle from No-Cue to Cue during Predictable. Unpredictable had a larger increase from No-Cue to Cue than Neutral at a trend level ($t(31)=1.97$, $p = .06$). Here again, significant main effects were statistically significant for Condition (Predictable = Unpredictable > Neutral; $F(2, 62)=30.54$, $p < .001$) and Stimulus (Cue > No-Cue; $t(31) = -8.23$, $p < .001$).

Conclusions: Findings reveal that SRX246, a V1a receptor antagonist, reduces online subjective anxiety in individuals with the highest level of anxiety across all sessions. SRX246 did not modulate physiological (startle) responses to phasic or sustained threat differently than placebo. These data strongly support extending this work to anxiety patients.

Keywords: Vasopressin 1a Receptor Antagonist, Startle, Anxiety & PTSD, Phase II Clinical Trial, Threat of Shock

Disclosure: Nothing to disclose.

W18

Fighting Females: Neural and Behavioral Effects of Chronic Social Defeat Stress in Female Mice

Abstract not included.

W19

Sex Differences in Lipid Metabolism in PTSD

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Background: Post-traumatic stress disorder (PTSD) is associated with increased rates of hyperlipidemia and associated cardiovascular disease (CVD). Compared to men, women are at increased risk of PTSD, yet have decreased risk of CVD at ages pre-menopause. However, since the sex difference in CVD risk diminishes with increasing age, it has been hypothesized that the decline in sex hormones associated with ovarian aging may account for loss of protection from CVD risk factors in older women. While the primary functions of ovarian hormones are to control fertility and reproduction, estrogen, progesterone and their metabolites also provide protection from cardiometabolic risks, including ischemic damage, high-density lipoprotein and

triglyceride profiles, waist circumference, glucose levels, and hypertensive status. Glucose and lipid dysregulation have been found during the menopause-related decline in estrogen. Testosterone, the primary reproductive male reproductive hormone promotes reproductive tissue development and secondary sexual characteristics in males, is also decreased as a result of stress and aging, and has been associated with diabetes, hypertension and dyslipidemia, low-density lipoprotein, inflammation, and incidence of atherosclerosis, coronary artery disease, and CVD events. Whether sex steroids confer protection from elevated lipids in individuals with PTSD is unknown. This study examined the effect of sex and PTSD on plasma lipid metabolites and the role of sex steroids in this relationship. We predicted higher lipid levels in PTSD vs. controls and in males compared to females. We also predicted that lipid levels would be inversely related to sex steroids.

Methods: Metabolomic analyses were performed on plasma samples obtained from fasting male and pre-menopausal follicular phase female subjects with chronic PTSD (N = 44) and trauma-exposed, age-matched controls (N = 44). Participants were between the ages of 20 and 50, primarily civilians (89%), healthy, free of medications, alcohol and drugs, and limited to one cup of caffeine daily. Plasma samples were assayed for lipid and steroid metabolites using Time-of-Flight Mass Spectrometer (Agilent Technologies 6220 TOF) coupled with an Ultra HPLC. Lipid and sex hormone metabolites were tested for sex and PTSD effects using ANOVA with FDR correction. Chemical Set Enrichment (ChemRICH) analysis, a technique that utilizes structure similarity and chemical ontologies to map was used to visualize metabolite clusters that differed by PTSD status and sex. Lipid metabolites that were differentially expressed in PTSD vs controls were treated as outcomes in logistic regression models with sex steroids entered as mediators in males and females separately.

Results: Sex differences were found in 67 lipid metabolites. In comparing PTSD vs controls, 217 lipid metabolites were altered (mostly upregulated) in PTSD. The majority of lipid alterations in PTSD occurred in men, but not in women (138 vs 42 lipid metabolites). ChemRICH analyses indicated that PTSD was associated with alterations in 8 out of 11 lipid clusters in men, but only 4 in women. In the steroid panel, testosterone glucuronide, a downstream metabolite of testosterone, was decreased in both men and women with PTSD compared to controls (p's < .05). The testosterone metabolites, dihydrotestosterone and etiocholanolone, were elevated in PTSD in both sexes (p's < .05). Testosterone glucuronide accounted for alterations in lipid metabolites in women only. There were no associations of PTSD with estrogens.

Conclusions: A metabolomics approach was used to identify lipid and sex steroid metabolite alterations in PTSD in men and women. Complementing previous findings of hyperlipidemia in men and in PTSD, this study further identified sex differences in lipid alterations in PTSD. Metabolites in the testosterone pathway accounted for alterations in lipid alterations in women in particular. There were no associations of PTSD with estrogens, which were expected to be protective. However, conclusions about the role of female sex steroids were limited by the single timepoint blood draw that occurred during the early follicular phase, when estrogen and progesterone are low. Longitudinal assessment over the menstrual cycle would be important to evaluate the role of sex steroids on lipid metabolism in naturally cycling women with PTSD and in older individuals to examine effects of aging. These findings suggest that the testosterone-related compounds may account for sex differences in lipid profiles, potentially leading to differential health risks in PTSD.

Keywords: PTSD, Dyslipidemia, Sex Steroids, Metabolomics

Disclosure: Nothing to disclose.

W20

Neural Circuits Underlying Individual Variability in Avoidance Behavior

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Background: One goal of modern systems neuroscience is to better understand the circuits that regulate distinct behaviors. Approach and avoidance behaviors are of particular interest, as they are useful constructs for the study of motivation and anxiety. It is well known that individual mice can exhibit large differences in avoidance behavior, and that stress experience may further drive divergence in these behaviors. Here we have leveraged this individual variability with an unbiased whole brain anatomical analysis to identify novel circuits that regulate avoidance like behavior.

Methods: Mice – We performed all experiments in male and female DBH::Cre x L10a-GFP mice that were at least 10 weeks of age at the beginning of the experiment. Mice were singly housed, and had food and water ad libitum during the experiment.

Novelty induced hypophasia (NIH) paradigm – As per Bluett et al., Nature Communications, 2017, we expose mice to Vanilla Ensure (VE) in the home cage for 4 days. On day 5, mice are placed in a novel open cage with distinct bedding, VE and a bright light for 30 min. Their latency to drink from the bottle containing the VE, and the total amount consumed is recorded, as a measure of baseline avoidance behavior. Days 6-9 mice area allowed to rest in the home cage. Days 10-11 mice are re-exposed to the VE in the homecage. Day 11 mice received highly aversive stimuli (7 inescapable foot shocks). Day 12 mice are placed in a new novel open cage without bedding, with VE and a bright light. Their latency to drink from the bottle containing the VE, and the total amount consumed is recorded. An avoidance index is made of the latency to drink from the VE bottle in the novel environments on Day 12-Day 5.

iDISCO – Brains were cleared and stained for cFos expression using the iDISCO protocol (https://idisco.info/idisco-protocol/).

Light sheet microscopy -- Images were collected using the Lavision Ultramicroscope II light-sheet system and processed using CLEARMAP analysis package.

Approach/avoidance generalization - Marble burying, the elevated plus maze and light-dark box were used to explore if changes in avoidance behavior determined through the NIH assay generalized across other assays. We performed all behavioral assays during the dark cycle.

Results: We found that male and female mice showed a high degree of variability in their avoidance behaviors in the NIH assay following exposure to a highly aversive stimuli (shock). This variability generalized to significant differences across multiple assays of approach/avoidance behavior, suggesting this is a stable response strategy. Furthermore, in our preliminary study we found that male animals who had high levels of avoidance behavior (n=2) showed enhanced activation of multiple limbic and subcortical regions comparison to low avoidance counterparts (n=3). In contrast, the mice with higher levels of avoidance behavior had reduced activation prefrontal cortical regions.

Conclusions: Our data suggest that both limbic and cortical regions are differentially engaged by stress in mice with variable avoidance behavior.

Keywords: Stress and Anxiety Behavior, Avoidance, Whole-Brain Rodent Imaging

Disclosure: Nothing to disclose.

W21

Meta-Analysis Identifies a Robust Association Between Anxiety Disorders and Lower Urinary Tract Symptoms (LUTS)

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Background: Lower urinary tract symptoms (LUTS), including symptoms such as urinary frequency, pressure, urgency, pain when the bladder fills, and syndromes such overactive bladder (OAB), interstitial cystitis (IC) and bladder pain syndrome (BPS), are commonly reported by patients with anxiety symptoms. In addition, studies in individuals with LUTS have shown that these individuals are more likely to also have anxiety-related disorders. We conducted a systematic review and meta-analysis to examine the relationship between LUTS and anxiety disorders.

Methods: We used the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) criteria to conduct a systematic data collection and meta-analysis. We searched PubMed, CENTRAL, PsycINFO, and Google Scholar, and included all articles referencing at least one relevant anxiety-related diagnosis (e.g., generalized anxiety disorder, separation anxiety disorder, social anxiety disorder, obsessive-compulsive disorder, post-traumatic stress disorder) and at least one diagnosis related to LUTS (e.g., OAB, IC/BPS). We performed random-effect meta-analyses for anxiety-related disorders with pooled outcomes for LUTS.

Results: We identified 814 relevant articles. After detailed review, 94 articles were chosen for the systematic review of anxiety-related disorders, of which 23 had sufficient data for meta-analysis. The odds ratio for anxiety-related disorders among cases with LUTS was 2.94 (95% CI: 2.40,3.60, $P < 0.001$). When we excluded PTSD from the anxiety group, the odds ratio for the association between anxiety-related disorders and LUTS was 3.10 (95% CI: 2.47,3.89, $P < 0.001$). The odds ratio for LUTS among individuals with anxiety-related disorders was 2.76 (95% CI: 1.43,5.34, $P < 0.001$), consistent with a bidirectional relationship between anxiety-related disorders and LUTS.

Conclusions: The results demonstrate reliable associations between anxiety disorders and LUTS. We did not see evidence for sex differences in this association, although the number of included articles was small. This association appears to be bidirectional and clinically significant. There were very limited data exploring the relationship between LUTS and anxiety-related disorders in youth, which should motivate further study in this area.

Keywords: Anxiety Disorders, Obsessive-Compulsive Disorder (OCD), Lower Urinary Tract Symptoms (LUTS)

Disclosure: Nothing to disclose.

W22

Functional MRI Guided Targeting With Interleaved TMS/fMRI Readouts of Evoked Amygdala Responses

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Background: The amygdala is implicated in a variety of affective pathologies and is the focus of research on fear learning in non-human animals. Therefore, a method to non-invasively influence amygdala activity could be a highly valued tool in generating novel neuromodulatory interventions. A pilot TMS/fMRI study (n=14) in our lab suggested that the amygdala could be influenced by fMRI guided TMS and that the direction of fMRI BOLD response in the amygdala evoked by TMS depends on the cortical region where TMS is applied. Focusing on cortical targets that evoked a negative BOLD response in the amygdala (the putatively clinically useful direction), we sought to replicate the pilot results in a larger sample and focused on the most promising cortical zone.

Methods: In a healthy cohort (n=32), we used resting fMRI seeding the basolateral amygdala to find superficial cortical targets with high resting connectivity in the vicinity of the ventrolateral prefrontal cortex. The vlPFC hot spot for each participant varied but was identifiable in each individual FC map. We then applied 71 total single TMS pulses (120% motor threshold) with interleaved fMRI recordings to examine whether an amygdala response could be evoked. Using an identical stimulation and fMRI protocol (TR=2400ms including a 400ms gap in which TMS was applied), we also stimulated the hand knob of the primary motor cortex as a control site.

Results: Using a generalized estimating equation to account for within-subject correlations and including age, education, and stimulation intensity covariates, we found a negative BOLD response in the amygdala that was significantly different from the control site, Wald Chi-Sq=4.92, $p = 0.027$.

Conclusions: This replication extends our pilot work and supports a conclusion that individual fMRI guided TMS can effectively modulate the amygdala non-invasively. We are collecting data on a behavioral study to examine neuromodulation effects through this pathway as well as interleaved TMS/fMRI data in a depressed/anxious patient cohort.

Keywords: Interleaved TMS/fMRI, Amygdala, PFC, Anxiety & PTSD

Disclosure: Nothing to disclose.

W23

HIV Interacts With Childhood Trauma Exposure to Impact PTSD Symptom Severity and Psychophysiological Hyperarousal in African American Women

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Background: Many people living with HIV (PLWH) experience high rates of childhood trauma exposure, which is a significant risk factor for the development of posttraumatic stress disorder (PTSD). African Americans living in poverty and urban environments tend to be exposed to high levels of trauma, suffer from chronic PTSD, and are at increased risk for HIV infection. It is important to understand how HIV infection interacts with childhood trauma exposure to influence the presentation and psychophysiology of PTSD. Furthermore, the lack of knowledge surrounding how HIV status influences the presentation of PTSD may limit the generalizability of behavioral and pharmacological treatment strategies for PLWH and PTSD. Therefore, the overall goal of the current study was to characterize the extent to which HIV interacts with childhood trauma exposure to influence PTSD symptoms and psychophysiological hyperarousal in traumatized, African American women.

Methods: All participants (n = 89, 29 without HIV, 60 PLWH) were African American women between the ages of 18 and 65 years who were recruited from the Women's Interagency HIV Study (WIHS) in Atlanta, GA and provided informed consent. All subjects participated in a clinical interview conducted by a trained clinician on all psychological assessment instruments. Lifetime trauma history was determined by the 14-item Traumatic Events Inventory (TEI), which assesses for experiencing and witnessing traumatic events. Childhood trauma history was assessed via the Childhood Trauma Questionnaire (CTQ). The Clinician-Administered PTSD Scale (CAPS) was used to determine the current PTSD symptoms severity based on DSM-5 criteria. Psychophysiological hyperarousal was assessed by measuring skin conductance level (SCL) at baseline and during the trauma assessment portion of the CAPS using a mobile eSense SCL App for iPad. Usable SCL data was available from 27 participants (6 without HIV, 21 PLWH). ANOVAs controlling for income and HIV viral load (quantified by PCR) were used to assess the effects of HIV status, childhood trauma exposure and their interaction on PTSD symptom severity and psychophysiological hyperarousal (baseline, response to trauma reminder, and habituation over time).

Results: Rates of adult and childhood trauma exposure were not significantly different between PLWH and those without HIV (p's>0.05). Similarly, sociodemographic variables were not different based on HIV status, including age, education and employment (p's>0.05). Income level was however significantly greater in PLWH in the current study ($p = 0.02$). PTSD symptom severity as determined on the CAPS was influenced by a HIV status by childhood trauma interaction ($p = 0.05$). In women without HIV, higher childhood trauma exposure was significantly associated with greater PTSD symptom severity compared to HIV- women that experienced low levels of childhood trauma ($p = 0.001$). Similarly, high levels of childhood trauma exposure in PLWH were associated with greater PTSD symptom severity as compared to PLWH who experienced low levels of childhood trauma ($p = 0.07$). HIV was associated with greater PTSD symptoms severity only in women with low levels of childhood trauma exposure ($p = 0.06$). There was no impact of HIV status on PTSD symptom severity in women exposed to high childhood trauma ($p = 0.46$). Similar analyses yielded significant interactions of a HIV status by childhood trauma exposure on baseline psychophysiological arousal ($p = 0.05$) and psychophysiological reactivity to trauma reminders ($p = 0.015$). Higher childhood trauma exposure in HIV negative women was significantly associated with greater baseline SCL compared to HIV negative women with low levels of childhood trauma exposure ($p = 0.05$). There was no effect of childhood trauma exposure on baseline SCL in PLWH ($p = 0.69$). HIV was associated with lower baseline SCL only in women with high levels of childhood trauma exposure ($p = 0.08$). In women exposed to low levels of childhood trauma, psychophysiological response to trauma reminders was significantly lower in PLWH compared to women without HIV ($p = 0.06$). In women exposed to high levels of childhood trauma, HIV was associated with augmented psychophysiological reactivity to trauma reminders ($p = 0.06$).

Conclusions: Taken together, these findings suggest that HIV impacts PTSD symptom severity and psychophysiological hyperarousal in African American women in a manner that is dependent on levels of childhood trauma exposure. Given that HIV status impacts PTSD symptoms and baseline and trauma reminder-induced SCL, the current data may have high clinical significance for treating PTSD in PLWH. Ongoing analyses are being performed to directly assess the effects of HIV on fear acquisition, discrimination, and extinction.

Keywords: HIV, PTSD, Childhood Trauma, Women, Psychophysiology

Disclosure: Nothing to disclose.

W24

A Computational, Approach-Avoidance Framework for Predicting Behavioral Therapy Response for Generalized Anxiety Disorder and Major Depressive Disorder

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Background: Generalized anxiety disorder (GAD) is one of the most commonly diagnosed mental health disorders, with a 6% lifetime prevalence rate. Although there is significant symptom overlap with major depressive disorder (MDD), a comorbid GAD diagnosis conveys a much poorer prognosis. While cognitive behavioral therapy for GAD is superior to placebo, only 40-60% experience significant improvement. Understanding neurocognitive processes contributing to treatment response could help identify patients likely to be refractory and develop individualized treatment approaches. Given that GAD treatments typically focus on decreasing cognitive and behavioral avoidance and/or to manage internal rumination about potential threat/reward consequences, the ability to successfully arbitrate conflict (make decisions to approach or avoid) could theoretically contribute to propensity for treatment response. Computational approaches utilize mathematical models to precisely and quantitatively represent complex systems and examine the factors contributing to human behavior. Here, we present preliminary results from a study examining whether computational parameters and/or neural activation during approach-avoidance conflict decision-making predicts response to two different behavior therapy approaches for GAD.

Methods: A preliminary cohort of 48 individuals with GAD (41 female; age M = 35.69; 18 with current and 40 with lifetime MDD) have completed an approach avoidance conflict (AAC) task during functional magnetic resonance imaging (fMRI). In this task, individuals have to make "approach" or "avoid" decisions when faced with both "punishment" and reward outcomes (exposure to negatively-valenced images/sounds and 2, 4, or 6 cents earning per trial). After baseline assessment, participants were randomized to complete 10 weeks of either behavioral activation (BA) or exposure-based therapy (EBT), conducted in a group setting. Primary symptom outcome was GAD-7 score. Percent signal change (PSC) for a priori bilateral dorsolateral prefrontal cortex (dlPFC), amygdala, and striatum regions of interest were extracted for trials in which participants were faced with approach-avoidance conflict decisions. An active inference computational model was used to identify parameters related to the influence of the potential negative image, the potential points, conflict (ratio between these two), and policy precision. Linear regression analyses were conducted in R statistical package with PSC or computational parameters and treatment condition as predictors, post-treatment GAD-7 as outcome, and pre-treatment GAD-7, gender, and age as covariates.

Results: Preliminary analyses indicate that GAD-7 scores declined with treatment, in both treatment groups, with approximately 47% of individuals exhibiting a 50% score reduction. Baseline GAD-7 score related to bilateral amygdala activation during approach-avoidance conflict (Right: $t(44)=3.08$, $p = .004$, $\eta2 = 0.17$; Left: $t(44) = 2.87$, $p = .007$, $\eta2 = 0.15$) and greater conflict as estimated within the active inference model ($t(44) = 2.03$, $p = 0.048$, $\eta2 = 0.08$). Baseline conflict activation within bilateral dlPFC related not only to self-reported difficulty making decisions on the task (Right: $rs = 0.30$, $p = 0.043$; Left: $rs = 0.34$, $p = 0.021$), but also differentially predicted treatment

outcomes for BA versus EBT (Right: $t(40) = 3.36$, $p = 0.002$, ($\eta 2 = 0.17$; Left: $t(40) = 1.167$, $p = 0.007$, $\eta 2 = 0.13$), with greater activation predicting worse outcomes for EBT but better outcomes for BA. Less policy precision as estimated within the active inference model related to better treatment response for depression symptoms across both treatment modalities ($t(40) = -3.08$, $p = 0.004$, $\eta 2 = 0.11$).

Conclusions: These results suggest that amygdala activation and greater conflict between approach and avoidance drives is linked to GAD symptoms. However, different factors may be important in predicting outcomes to behavioral therapy. Less precision in one's model (i.e., greater uncertainty or variability in response patterns) may indicate malleability in terms of updating one's internal model with therapy. Additionally, activation within the dlPFC, considered important for decision-making and action selection, predicted how individuals responded to the different types of treatment. Increased dlPFC activation during approach-avoidance conflict may relate to greater neurocognitive effort or increased internal model complexity contributing to approach-avoidance behavior. Behavioral activation, by focusing on rewarding outcomes, may in some ways take threat out of the equation, thus relying less on explicit, conflict-based decisions as compared to EBT. Future research using computational tasks to more precisely model aspects of uncertainty in action selection, model complexity, learning rate, and sensitivity to reward/punishment, may have utility for informing precision medicine approaches to cognitive-behavioral therapies.

Keywords: Computational Psychiatry, Generalized Anxiety Disorder, Functional MRI (fMRI), Decision Making, Cognitive Behavioral Therapy

Disclosure: Nothing to disclose.

W25

Neuropeptide Y in CSF From Deployed Veterans is Positively Associated With Trauma Exposure but Negatively Associated With Lifetime History of mTBI

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Background: Neuropeptide Y (NPY) is a peptide neurotransmitter implicated in resilience following traumatic stress. NPY concentrations in cerebrospinal fluid (CSF) have been found to be decreased in combat Veterans with PTSD compared to combat Veterans without PTSD or healthy civilians. Animal work has suggested that one mechanism of NPY's impact on modulating anxiety-like symptoms may be via reducing noradrenaline (NA) release. NPY has also been implicated in either mediating or modulating symptoms following traumatic brain injury (TBI) including mild TBI (mTBI), although there is evidence the timecourse of these changes may be complex. However, NPY has not to our knowledge been examined in Veterans who have been assessed for both mTBI history and PTSD symptoms, nor in a context where interactive effects between NPY and NA in modulating symptom expression can be assessed.

We wished to test the idea that both mTBI and traumatic stress may lead to persistent increases in NPY, with lower levels of increase being associated with both increased symptoms of PTSD or postconcussive syndrome (PCS), as well as higher levels of NA release. To do this, we measured CSF NPY levels in a population of post-9/11 deployed Veterans for whom CSF NA, PTSD and PCS symptoms, and lifetime exposure to common traumatic stressors and mTBI had been previously assessed.

Methods: As part of an ongoing longitudinal study of blast mTBI, 66 Veterans (46 with a history of mTBI and 20 without; of the total, 33 met criteria for PTSD) underwent lumbar puncture, and the concentration NPY was quantified using a Millipore sandwich ELISA human NPY detection kit, while the concentration of noradrenaline was quantified using HPLC. PTSD symptoms were evaluated using the Clinician Administered PTSD Scale (CAPS) and the PTSD Checklist (PCL). Lifetime exposure to common traumatic stressors was assessed using the Life Events Checklist (LEC). Exposure to blast explosions and lifetime history of mTBI was assessed using a structured interview. For these analyses, lifetime history of mTBI resulting in loss of consciousness was used as a quantitative measure of mTBI exposure. All analyses were carried out using R, and all statistical tests are two-tailed.

Results: Consistent with our predictions, in a linear model including both trauma exposure and mTBI history, NPY concentration was significantly positively associated total combat trauma exposure ($p = .0054$), but contrary to our predictions, it was negatively associated with mTBI exposure ($p = 0.046$). Further, contrary to our predictions, in a linear model including NPY, trauma exposure, and an interaction term between the two, NPY concentration was not associated with NA concentration ($p = 0.95$ for NPY, $p = 0.93$ for interaction term). Finally, there were a number of relationships that were in the direction predicted based on previous findings and of a magnitude with the potential to be scientifically significant, but which were not statistically significant: NPY levels were higher (but not statistically significantly) in those with a history of traumatic stress exposure but without a diagnosis of PTSD than in either those with PTSD or trauma-exposed controls. And, in those with a history of traumatic stress exposure, NPY was positively associated (but not statistically significantly) with both PTSD and PCS symptoms while NA was negatively associated (not statistically significantly) with these symptoms across multiple measure types.

Conclusions: These results suggest that long-term changes in NPY levels may occur following combat trauma, but that the direction of the effect may vary between traumatic stress and traumatic brain injury. These results raise the possibility that repetitive mTBI may contribute to increased risk of PTSD diagnosis or symptoms at least in part by leading to long term inhibition of NPY, thus decreasing its availability to function as a resilience factor following traumatic stress.

Keywords: Neuropeptide Y, PTSD, Veterans, Noradrenaline, CSF Biomarkers

Disclosure: Nothing to disclose.

W26

Stress-Induced Impairments in Fear Learning are Causally Related to Increased Kynurenic Acid Formation in the Prefrontal Cortex

Abstract not included.

W27

Exploring 20% CO2 as a Probe for Human Anxiety and Avoidance Behavior

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Background: Inhalation of carbon dioxide (CO2) is one of the most commonly used methods for safely inducing anxiety in a laboratory setting. Most prior work has focused on inducing panic

using a single vital-capacity inhalation of 35% CO2. Much less is known about the anxiogenic effects of inhaling a single breath of lower dose CO2 and how the anxiety response changes following repeated inhalations. This study aimed to develop and test a novel probe for studying human anxiety and avoidance behavior using repeated exposure to a dose of 20% CO2.

Methods: A transdiagnostic sample of 20 male and female participants with high levels of anxiety sensitivity (Anxiety Sensitivity Index-3 total score ≥ 29) were recruited across the spectrum of anxiety disorders and compared to a demographically-matched sample of 20 healthy subjects (ClinicalTrials.gov NCT03925987). Each participant completed a 2-day CO2 habituation protocol where each day they were exposed to three self-paced inhalations of either 20% CO2 or compressed air. Real-time anxiety ratings were made throughout the protocol using a rating dial and avoidance was operationalized as the latency between inhalations. Respiratory measures (minute volume, O2 saturation, end-tidal O2 and CO2) were collected using a system developed by Hans Rudolph. All measures were analyzed by linear mixed-effects models using fixed-effects of Condition (air vs 20% CO2), Session (day 1 vs day 2), and Group (anxious vs healthy) and a subject-specific random intercept. Model comparison was carried out with Bayesian Information Criterion and considered all combinations of the main effects and interactions terms, with a significance threshold set at $p < .05$.

Results: All participants completed all rounds of CO2 exposure across the 2-day protocol with no adverse events. Oxygen levels remained stable throughout the protocol and there were no group differences in the total volume of CO2 inhaled with each breath. Despite being exposed to the same volume and dose of CO2, the anxious group reported significantly more anxiety in response to 20% CO2 as compared to both the healthy group and the air trials. Within-session, the anxious group's anxiety levels showed a pattern of sensitization, whereas between-sessions there was evidence for significant habituation of anxiety. The respiratory measures revealed that the anxious subjects exhibited significantly less minute ventilation in response to CO2, inhaling ~6 liters/minute of air less than the healthy subjects during peak CO2 exposure. The anxious group also exhibited significantly more avoidance behavior in response to CO2 (mean latency between each breath was 30 seconds longer in the anxious vs healthy subjects) and both groups showed significant between-session habituation such that the anxious group's avoidance behavior on day 2 was commensurate with the healthy group's avoidance behavior on day 1.

Conclusions: Repeated exposure to single breaths of 20% CO2 appears to be an effective and well-tolerated probe for studying human anxiety and avoidance behavior in clinically anxious populations. The marked between-session habituation in both anxiety and avoidance behavior highlights the potential of using CO2 as a form of interoceptive exposure therapy.

Keywords: Anxiety, Interoception, Exposure Therapy

Disclosure: Nothing to disclose.

W28

Sex Differences in Conditioned Flight

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Background: We developed a conditioning paradigm that elicits switching between different modes of defensive behavior. We used this paradigm to understand how neurons in the central amygdala mediate freezing and flight behavior (Fadok et al. 2017). Our previous work used exclusively male mice; therefore, we set out to determine whether there are sex differences in behavioral switches induced by our paradigm. Furthermore, we are investigating how the mouse ethogram changes over the course of conditioning. Our ultimate goal is to better understand the higher vulnerability of women to fear and anxiety disorders.

Methods: To facilitate investigations into the neurobiology controlling switches to more intense defensive

responding, we developed a conditioning paradigm based on a psychological theory known as the predatory imminence continuum. Our paradigm uses a serial compound stimulus, which is paired with a strong footshock during conditioning. Following conditioning, mice exhibit strong freezing responses to the tone component and a switch to a robust flight behavior in response to the noise stimulus.

Results: Equal numbers of male and female C57Bl6/J mice (N = 10 each sex) aged 10-12 weeks were subjected to the conditioned flight paradigm. When trials in a given condition were averaged together, we found a significant difference in the % freezing during the preSCS period (10s before SCS onset) on the first day of conditioning. We also see the emergence of higher freezing levels in female mice on a trial-by-trial basis. In addition to analyzing freezing and flight behaviors, we also scored several other emotional behaviors to determine the extent to which they are altered by traumatic experience in a sex-specific manner. We find that female mice exhibit more aggressive tail rattling behavior, whereas males exhibit higher levels of rearing and grooming behavior.

We are further investigating sex-dependent shifts in behavior that occur during fear conditioning by generating ethograms of mouse behavior. These data are allowing us to analyze the fine temporal details of behavioral changes.

We are also interested in understanding the extent to which behavioral phenotypes before conditioning correlate with the intensity of defensive behavior after conditioning. To begin, we are generating correlation matrices. These data will facilitate an understanding of behavioral variables that are predictive of behavioral responses to trauma.

Conclusions: Based on the results of these studies, we will subsequently investigate potential circuit differences between males and females, beginning in the central amygdala.

Keywords: Amygdala, Fear Conditioning, Sexual Dimorphism

Disclosure: Nothing to disclose.

W29

Disruption of Reconsolidation of Conditioned Threat Responses by Electroconvulsive Shock

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Background: The memory reconsolidation hypothesis posits that well-established memories become labile and susceptible to interference following reactivation or retrieval. An early study suggests that electroconvulsive shock (ECS) may disrupt the reconsolidation of threat conditioning in rats measured by the conditioned cue-controlled changes in licking behavior. However, the effects of ECS on other conditioned threat responses remain unknown. Here, we examined in rats the effects of ECS on reconsolidation of conditioned freezing responses.

Methods: Male rats underwent an auditory threat conditioning procedure consisting of a single 30s 5 kHz tone conditioned stimulus (CS) that terminated with a 0.6 mA 1 s electric shock to the footpads. On the following day, a group of animals was re-exposed to the CS in order to reactivate the memory and trigger

reconsolidation processes. Immediately after, animals were anesthetized with isoflurane and received 50 Hz, pulse width: 0.7 ms, duration: 1 s, 50 mA ECS (Retrieval-ECS) or not (Retrieval-Sham ECS). Another group of animals after a re-exposure to the CS received isoflurane anesthesia and ECS (or sham ECS) with a 4-hour delay (Retrieval-4hr delayed ECS and Retrieval-4hr delayed Sham ECS). An additional group of animals, on the day following threat conditioning, received either ECS (No retrieval-ECS) or sham ESC (No retrieval-sham ECS) under isoflurane anesthesia without a prior re-exposure to the CS. On the following day, all animals received the memory retention test (Long-Term Memory Test) consisting of exposures to the CS. In another experiment, on the day following threat conditioning using the protocol as described above, rats received memory reactivation session (as above) followed by ECS or Sham ECS (groups Retrieval-ECS and Retrieval-Sham ECS, respectively). Two hours later, all animals received the memory retention test (Short-Term Memory Test). Freezing behavior during all 4 CS presentations was averaged for analysis.

Results: Analysis of immobility or freezing behavior during the Long-Term Memory Test revealed that the Retrieval-ECS group displayed significantly less freezing than Retrieval-Sham ECS group ($p < 0.02$). In all other experiments (No Reactivation, Retrieval-4hr delayed, and Retrieval-Short Term Memory Test), there were no statistically significant differences in freezing behavior between experimental groups ($p > 0.05$).

Conclusions: This pattern of findings suggests that the ECS disrupted the reconsolidation of conditioned threat memories. Ongoing experiments are focused on: 1) assessing the effects of ECS on reactivated conditioned threat responses in female rats as studies show sex differences in threat conditioning and 2) determining brain network effects of ECS on the reactivated threat conditioned responses using expression of an early expression gene c-fos and functional connectomics approach.

Keywords: Memory Reconsolidation, Reconsolidation Intervention, Threat Conditioning, Network-Analysis, Functional Connectivity

Disclosure: Nothing to disclose.

W30

Endocannabinoid Signaling in a Septohabenular Circuit Regulates Anxiety-Like Behavior

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Background: The endocannabinoid (eCB) system can mediate anxiolysis, and exogenous cannabinoid agonists (e.g. Δ9-tetrahydrocannabinol) are frequently used for their anxiolytic effects. However, the neural circuits whereby cannabinoids exert these effects remain incompletely identified. The medial habenula (MHb) is a well-conserved epithalamic structure that is a powerful modulator of anxiety- and mood-related behavior in rodents and zebrafish and has been shown by MRI to be decreased in volume in humans with depression.

Methods: Using viral-genetic circuit mapping, optogenetics, and slice electrophysiology, we investigated whether the MSDB sends a direct, monosynaptic GABAergic projection to different subtypes of neurons of the MHb, whether this MSDB-MHb pathway is modulated by eCBs, and whether the eCB signaling in this pathway regulates anxiety- and depressive-like behavior.

Results: We report in adult male and female mice that the eCB 2-arachidonoylglycerol (2-AG) is released from neurons of the MHb, and that this eCB release retrogradely suppresses an atypical, excitatory GABAergic synaptic input from the medial septum and nucleus of the diagonal band (MSDB). We observed CB1 receptor-dependent depolarization-induced suppression of excitatory GABA currents (DSE-GABA) as well as a CB1 agonist-induced suppression of GABA postsynaptic currents in MHb neurons. Using optogenetics, we show that this occurs at MSDB axon terminals in the MHb. Viral-genetic knockdown of CB1 from MSDB neurons led to anxiety-like behavior in mice and abolished DSE-GABA in the MHb.

Conclusions: These results suggest that (1) the MSDB sends a direct GABA projection to the MHb; (2) the eCB 2-AG depresses GABA release from the MSDB to the MHb; and (3) eCB-mediated regulation of MSDB to MHb neurotransmission can produce anxiolytic behavioral effects. Thus, we have likely identified a novel circuit mechanism whereby eCBs control anxiety-like behavior.

Keywords: Endocannabinoids, Habenula, Anxiety

Disclosure: Nothing to disclose.

W31

Higher Levels of Anandamide in Positive History of Trauma and Higher Levels of 2AG in PTSD, in Individuals With Acute Psychosis

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Background: Trauma-induced Endocannabinoid (eCB) system alterations have been consistently reported in animal models of PTSD and few available human studies. However, there is no consensus on the nature of these alterations, with some reporting hyperactivation and others reporting suppression of eCB system. Moreover, eCB system has recently attracted great attention in etiology and treatment of psychosis. Though, there is high rate of trauma in both psychosis and PTSD, eCB system alterations has not been studies in comorbidity of psychosis and PTSD. In this study, we investigated the peripheral levels of eCBs in relation to severity of psychosis, current stress level, PTSD diagnosis, cannabinoid use, and history of trauma in individuals with acute psychosis.

Methods: This is a cross-sectional study of 118 psychotic patients admitted to a dual diagnosis unit at Mount Sinai Beth Israel hospital, New York. We used Positive and Negative Syndrome Scale (PANSS) to measure the severity of psychosis, Life Event Checklist (LEC) for history of trauma, Perceived Stress Scale for current levels of stress, urine toxicology for current cannabinoid use, and Mass Spectrometry to measure the peripheral endocannabinoid levels.

Results: Mean age was 35.98 (SD 12.06) and 69.8% of subjects were men. About half of the subjects (49.6%) had positive urine toxicology for cannabinoids (natural or synthetic). There was a high rate of lifetime history of trauma in our sample, with 89.1% of subjects reported being exposed to at least one type of trauma in their life. All subjects were diagnosed with acute psychosis, with mean PANSS score of 89.27 (SD 16.03). There were no significant differences in the severity of psychosis between subjects with positive or negative urine toxicology for cannabinoids and no correlations between severity of psychosis or cannabinoid use with eCB levels. Positive history of trauma was associated with higher levels of Anandamide (AEA) levels, controlling for age, gender, severity of psychosis, cannabis use and current perceived levels of stress (t 2.383, 95% CI .038-.428). Mean AEA levels were .37 in individuals with positive history of trauma and .16 in those without history of trauma (p-value <.0001). PTSD diagnosis was associated with higher 2AG levels, controlling for age, gender, severity of psychosis, cannabis use and current perceived levels of stress (t 2.783, 95% CI 7.940-47.897). Mean 2AG levels were 73.83

in individuals with comorbid PTSD diagnosis and 52.88 in those who did not have diagnosis of PTSD (p-value 0.029).

Conclusions: Peripheral eCB levels were elevated in individuals with history of trauma and PTSD, in subjects with acute psychotic symptoms. eCB alterations in PTSD and history of trauma propose important potential clinical implications in PTSD treatment.

Keywords: PTSD, Psychosis, Endocannabinoid System, Post-traumatic Stress Disorder

Disclosure: Nothing to disclose.

W32

Evaluating the Effects of CRP on Risk for Developing PTSD-Like Behaviors in Trauma-Exposed Mice

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Background: Increasing evidence suggests inflammation plays a role in psychiatric disorders caused by trauma exposure. Studies suggest post-traumatic stress disorder (PTSD) is associated with altered serum C-reactive protein (CRP) and CRP gene mutations. We examined the potential causal role of CRP in mouse models for PTSD, hypothesizing that CRP expression may confer a higher risk for PTSD-like behavior.

Methods: CRP knock out male and female C57BL6J mice were tested for baseline fear extinction by pairing five separate twenty-second tones (75 dB, 4 kHz) with terminal shocks (0.7 mAmps, 1 second). After forty-eight hours, mice were exposed again to thirty-two tones (20 seconds) within chambers containing altered visual, tactile, and odor dimensions to minimize contextual fear. Meanwhile, male wild-type C57BL6J mice received a single intra-jugular injection of 1011genome copies of either AAV8.CRP or AAV8.Null. Four weeks after infection, mice were exposed to either predator stress (10 minutes roomed with a laboratory cat) or handled (stress control). After one week, mice were tested for avoidance behaviors by open field and light-dark box tests. Two weeks post predator stress, avoidance of trauma cues were assessed by measuring exploration of a cue scented with dirty cat litter. Mice where cheek-bled one day after the trauma reminder to measure differential peripheral CRP protein expression, and thereafter subjected to the fear extinction assessment described above. Following fear conditioning, mice were sacrificed for further brain and peripheral tissue analysis.

Results: CRP KO female mice at baseline have improved fear extinction and improved recalled fear extinction ($F_{CRP}=1.794$, $n = 15$, $p = 0.0053$), though no difference was observed in male mice ($F_{stress}=1.051$, $n = 15$, $p = 0.3923$). However, AAV8-CRP overexpression did not confer a higher risk for PTSD avoidance-like behaviors or alter cued fear extinction in male mice after predator stress ($F_{CRP}=2.922$, $n = 17\text{-}19$, $p = 0.0944$).

Conclusions: CRP constitutive expression may disrupt fear extinction in females. CRP does not affect enduring trauma response or fear conditioning and extinction in males. Future studies will examine the contribution of CRP to enduring effects of trauma in female mice with CRP overexpression, as well as in both genders with the absence of CRP expression. Studies are also ongoing to determine how CRP expression alters the peripheral and central immune response to predator stress.

Keywords: CRP, PTSD, Trauma Exposure

Disclosure: Nothing to disclose.

W33

Low Dose Ketamine Infusion for Comorbid Post Traumatic Stress Disorder and Chronic Pain

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Background: Patients with PTSD and Chronic Pain (PTSD+CP) have two major central nervous system diseases that are exceedingly difficult to treat. Feder et al. (2014) reported that patients with PTSD respond to a single low dose of ketamine (KET) compared to midazolam. Albert et al. (2018) studied 16 VA patients with comorbid PTSD and treatment resistant major depression after 6 0.5 mg/kg i.v. KET infusion for 3 days for 2 weeks. Median relapse of PTSD was 41 days and depression 26 days. Furthermore, many clinical studies indicate low dose KET infusion is analgesic. The purpose of the present study was to investigate the effectiveness of KET infusion in VA patients with PTSD+CP or CP.

Methods: Twenty male or female veterans 18-65 years met DSM-V criteria for PTSD+CP and twenty met criteria with only CP. They were either free of psychotropic and/or pain medication for at least 6 weeks or were on stable doses of those medications prior to randomization for this study. There were 4 subgroups of 10 patients each. Subgroup one had PTSD+CP and was given 0.5 mg/kg of KET for over 40 min. Subgroup two with PTSD+CP was given an infusion of ketorolac (K) 15 mg over 40 min. Subgroup three and four were CP patients randomized to receive KET or K. To prevent nausea or vomiting, 4 mg i.v. ondansetron was given. Only research pharmacy was aware of drug identity. Trained raters administered clinical rating scales at pre-infusion baseline, 15, 40, 120, 240 min, 24h, day 1, day 2, day 7 after infusion.

Clinical measures included the Impact Event Scale-Revised (IES-R), Brief Pain Inventory Short Form (BPIS), Clinician Administered Dissociative States Scale (CADSS), Visual Analog Scale (VAS) and Patient Related Inventory of Side Effects (PRISE 20).

Results: 1. Primary outcome Day 1: Both KET and K improved IES-R, VAS, and BPIS scores in PTSD and pain measures. There were no differences in drug effectiveness.

2. Secondary outcome Day 7: All three of the above gradually returned toward baseline with both patient subgroups and KET and K.

3. The PRISE20 (measure of side effects) rapidly decreased from baseline by 40 minutes of infusion with KET and K in both patient groups.

4. Surprisingly, the CADSS showed tolerance to both KET and K, especially in the PTSD+CP patients who have two brain diseases.

Conclusions: 1. This preliminary study must be replicated with larger subgroups of patients.

2. It is important to emphasize that PSTD+CP patients in particular show tolerance to the effects of KET and K. Hence, brain circuits may be occupied with two brain diseases whereas KET and K act on one alone.

Keywords: Ketamine, PTSD, Chronic Pain

Disclosure: Nothing to disclose.

W34

Symptomatic and Preventive Effects of the PDE9 Inhibitor Bi 409306 Against Social Interaction and Dopaminergic Deficits in Adult Offspring From a Neurodevelopmental Mouse Model

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Background: Rodent models of maternal immune activation (MIA) are widely used to study neuropsychiatric disorders with developmental etiologies. One of these models is based on prenatal exposure to the viral mimic poly(I:C), which disrupts neuronal development and induces a number of behavioral abnormalities in the adult offspring, including deficits in social behavior, cognition, sensorimotor processing and dopaminergic dysfunctions [1]. BI 409306 is a selective phosphodiesterase-9 (PDE9) inhibitor [2] currently under development for the prevention of relapse in patients with schizophrenia taking antipsychotic medications (NCT03351244) and for early intervention in patients with Attenuated Psychosis Syndrome (APS) (NCT03230097). PDE9 inhibition is hypothesized to improve the NMDA-receptor signaling cascade by increasing cGMP levels, which subsequently leads to strengthening of synaptic plasticity. Abnormalities in glutamatergic transmission and excitatory/inhibitory network related to NMDA-receptor hypofunction, along with dysfunction of the dopaminergic circuitry, are believed to play a crucial role in the pathophysiology of APS and schizophrenia. Here, we evaluated symptomatic and preventive effects of BI 409306 in the poly(I:C)-based MIA model. In a first study, we investigated the effects of BI 409306 as add-on to the antipsychotic risperidone given in adult offspring of MIA-exposed and control mothers. In a second study, we administered BI 409306 during adolescent age to evaluate the drugs' efficacy to prevent the adult emergence of behavioral deficits.

Methods: Pregnant C57BL6/N mice were treated with poly(I:C) (5 mg/kg, i.v.) or control (saline, i.v.) solution on gestation day 12. In the first study, adult offspring ($n = 20$-23/group) were administered orally once daily with BI 409306 (1 mg/kg), risperidone (0.025 or 0.05 mg/kg), a combination of BI 409306 and risperidone (1 and 0.025 mg/kg, respectively), or vehicle starting at post-natal day (PND) 77. Two weeks after treatment initiation, the animals were subjected to a battery of consecutive behavioral tests until PND 119 while still receiving the corresponding drugs or vehicle solutions. The order of these tests was (1) social interaction to assess social behavior, (2) pre-pulse inhibition (PPI) for sensorimotor processing, and (3) amphetamine-induced hyperlocomotion to assess dopaminergic functions. In the second study, adolescent offspring ($n = 21$-22/group) were administered orally once daily with BI 409306 (1 mg/kg) or vehicle starting at PND 30 for 4 weeks. Behavioral testing (as above) was performed between PND 72 and 100. A subgroup of animals received BI 409306 (1 mg/kg) continuously during behavioral testing, whereas the other subgroup was tested "off-drug".

Results: Maternal poly(I:C)-induced immune challenge led to a significant decrease in social interaction ($p < 0.005$) and potentiation of amphetamine-induced hyperlocomotion ($p < 0.05$) in the adult offspring. PPI was not affected at a between-group level. Treatment of adult offspring with BI 409306 alone, or in combination with the subthreshold dose of risperidone (0.025 mg/kg), reversed the social interaction deficits ($p < 0.005$) and amphetamine hyper-responsiveness ($p < 0.01$). Risperidone alone at higher dose also showed efficacy against MIA-induced deficits in the social interaction and amphetamine-induced hyperlocomotion tests. When administered during adolescence and throughout behavioral testing in adulthood, BI 409036 abolished the MIA-induced social interaction deficits ($p < 0.005$) and amphetamine hyper-responsiveness ($p < 0.005$). When administered for 4 weeks during adolescence only, BI 409036 prevented social interaction deficits ($p < 0.01$) and partly attenuated amphetamine hyper-responsiveness in adulthood. Plasma exposure of BI 409306 determined in satellite mice was in the range as expected for the doses used in the ongoing clinical trials.

Conclusions: Firstly, our data show that chronic treatment of adult offspring with the PDE9 inhibitor BI 409306 alone, or in combination with an antipsychotic, reverse deficits in social behavior and dopaminergic abnormalities in a MIA-based neurodevelopmental disruption model relevant to schizophrenia. Moreover, we revealed that treatment with BI 409306 during adolescence is sufficient to prevent the adult emergence of social deficiencies and, to a lesser extent, dopaminergic dysfunctions, thereby providing support for the "vulnerable window" hypothesis of schizophrenia in a rodent neurodevelopmental disruption model. Overall, our preclinical findings support the current trials testing BI 409306 for prevention of relapse in patients with schizophrenia as well as early intervention in patients with APS.

Keywords: Attenuated Psychosis Syndrome, Neurodevelopmental Disorders, Cannabidiol, CBDV, Translational Models, Clinical Trials, Schizophrenia Novel Treatment

Disclosure: Boehringer Ingelheim, Employee (Self & Spouse)

W35

Microstructural Abnormalities in Deep and Superficial White Matter in Youths With Mild Traumatic Brain Injury

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Background: Diffusion Tensor Imaging (DTI) studies of traumatic brain injury (TBI) have focused on alterations in microstructural features of deep white matter fibers (DWM), though post-mortem studies have demonstrated that injured axons are often observed at the gray-white matter interface where superficial white matter fibers (SWM) mediate local connectivity. The objectives of this study were to examine microstructural alterations in SWM and DWM in youths with a history of mild TBI and examine the relationship between white matter alterations and attention.

Methods: Using diffusion tensor imaging (DTI) DWM fractional anisotropy (FA) and SWM FA in youths with mild TBI (TBI, $n = 63$) were compared to typically developing and psychopathology matched control groups ($n = 63$ each). Following tract-based spatial statistics, SWM FA was assessed by applying a probabilistic tractography derived SWM mask, and DWM FA was captured with a white matter fiber tract mask. Voxel-wise z-score calculations were used to derive a count of voxels with abnormally high and low FA for each participant. Analyses examined DWM and SWM FA differences between TBI and control groups, the relationship between attention and DWM and SWM FA and the relative susceptibility of SWM compared to DWM FA to alterations associated with mild TBI.

Results: Case-based comparisons revealed more voxels with low FA and fewer voxels with high FA in SWM in youths with mild TBI compared to both control groups. Equivalent comparisons in DWM revealed a similar pattern of results, however, no group differences for low FA in DWM were found between mild TBI and the control group with matched psychopathology. Slower processing speed on the attention task was correlated with the number of voxels with low FA in SWM in youths with mild TBI.

Conclusions: Within a sample of youths with a history of mild TBI, this study identified abnormalities in SWM microstructure associated with processing speed. The majority of DTI studies of TBI have focused on long-range DWM fiber tracts, often overlooking the SWM fiber type.

Keywords: Diffusion Tensor Imaging (DTI), Mild Traumatic Brain Injury, Attention

Disclosure: Nothing to disclose.

W36

Sex-Specific Effects of Air Pollution and Maternal Stress on Social Behavior, the Gut Microbiota, and Neuroimmune Signaling

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Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social interaction and communication, repetitive behavior, and a sex bias in prevalence (higher in males). Interestingly, gut microbiome signatures correlate with gastrointestinal (GI) pain in children with ASD (Luna et al., 2017), and fecal microbiota transfer improves both GI and behavioral symptoms of ASD (Kang et al., 2017). Furthermore, microglia, the resident immune cells of the brain, have been implicated in the pathophysiology of ASD and are sensitive to changes in the microbiome. Epidemiological studies suggest that exposure to air pollution during pregnancy is associated with increased likelihood of having a child with ASD (Volk et al., 2011; 2013) and maternal stress during pregnancy increases the severity of ASD symptoms (Varcin et al., 2017). Therefore, we developed a mouse model that combines exposure to diesel exhaust particles (DEP) and maternal stress (MS) during pregnancy. Previous work using this paradigm suggests that MS exposure serves as a second hit –unmasking effects of DEP on behavior (Bolton et al., 2013).

Methods: C57Bl/6 mouse dams were exposed to a combination of DEP and MS (limited nesting and bedding) during pregnancy. During adolescence, both male and female offspring born to either DEP/MS or vehicle/control (VEH/CON) dams were tested on a variety of behavioral assays, including sociability and social novelty preference tests. At postnatal day (PND)45 and PND80 brains, guts, and microbiome samples were collected. Immunohistochemistry was performed on brain tissue to analyze microglial density and structure, and qPCR was used to quantify mRNA, both in the brain and in the intestinal epithelium at PND45. At both PND45 and PND80, 16S sequencing was used to analyze the composition of the gut microbiome.

Results: We observed a decrease in sociability ($p < 0.001$), as well as a decrease in social novelty preference ($p < 0.001$) in DEP/MS offspring as compared to VEH/CON. These effects were largely driven by changes in the male offspring. We also found that DEP/MS exposure led to age-specific changes in the gut microbiome, in male offspring only. Specifically, we observed increased diversity of the microbiome at PND45, but decreased diversity at PND80 (Pielou's evenness; $p < 0.05$). Principle coordinates analysis revealed discrete clustering of DEP/MS vs. VEH/CON males at PND80, but not PND45 ($p < 0.05$). Changes in the microbiota were also accompanied by a sex-specific change in the expression of tight-junction protein mRNA in the small intestine (decreased in males but increased in females) at PND45, as well as structural changes in the intestinal epithelium. Finally, using 3Dmorph reconstructions, we observed an increase in the ramification index of microglia within the nucleus accumbens in DEP/MS male offspring as compared to VEH/CON males ($p < 0.01$). The nucleus accumbens represents a key node within the neural networks that support social interaction, in which we have also found decreases in socially relevant receptors, such as the dopamine D1 receptor, in DEP/MS males as compared to controls.

Conclusions: Our findings demonstrate that DEP/MS exposure induces ASD-relevant behavioral phenotypes in male offspring. In addition, DEP/MS exposure has sex-specific effects on the gut microbiome, intestinal epithelium, and microglial morphology. Together, these findings lend support for a growing body of literature suggesting that the gut microbiota may influence the development of social circuits in the brain, perhaps via a microglia-dependent mechanism.

Keywords: Autism Spectrum Disorder and Related Syndromes, Gut Microbiome, Microglia, Neuroimmune Mechanisms, Sex Differences

Disclosure: Nothing to disclose.

W37

Prenatal Stress Induces Intrauterine Dysfunction and Aberrant Behaviors in a Microbe- and CCL2-Dependent Manner

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Background: Prenatal stress (PNS) is associated with neuropsychiatric disorders in the offspring, including anxiety, depression, and autism. There is mounting evidence that these behavioral phenotypes have origins in utero. Maternal microbes, inflammation, and serotonergic dysfunction have been implicated as potential mediators of the behavioral consequences of PNS; however, whether and how these systems interact is unclear. C-C motif chemokine ligand 2 (CCL2) is a chemokine that recruits monocytes to the brain to elicit an anxiogenic response during stress. In addition to its role in the adult brain, CCL2 is highly expressed in uterine and placental tissue; whether CCL2 plays a role in fetal neurobiological and behavioral programming, however, is unknown. We used a prenatal stressor in pregnant mice to examine novel relationships between prenatal stress exposure, maternal microbes, intrauterine inflammation and serotonin, and adult behavior.

Methods: Pregnant WT C57/BL6, CCL2 -/-, and germ free (GF) dams were randomly assigned to either the stressed experimental group or non-stressed control group ($n = 10$-14/group). Mice were stressed between embryonic day (E)10-E16 for two hours a day using a well-validated restraint stress paradigm; controls were left undisturbed. In cohort one, placental tissue and fetal brains were collected at E17.5 from all mice, and examined for changes in gene expression and protein levels. HPLC was used to evaluate changes in serotonin metabolism. In a second cohort of WT and CCL2 -/- mice, adult male and female offspring behavior was assessed a social behavior paradigm. Multi-factorial analysis of variance (ANOVA) was performed to determine possible effects of sex. As the main effect of sex was not significant for any test, data were collapsed and unpaired t-tests were used to determine statistical significance between stressed and non-stressed groups. For groups with statistically different variances, unpaired t tests with Welch's correction were performed.

Results: Maternal stress resulted in an increase in CCL2 protein in both the placenta and fetal brain of WT mice accompanied by an increase in the inflammatory cytokines IL-6 and TNF-α in the fetal brain. Since microbes, or microbial products or components, can initiate an immune response by signaling through toll-like receptors (TLRs), we investigated TLR4 (which specifically responds to lipopolysaccharide (LPS) on gram-negative bacteria), in the placenta. We found a significant increase in TLR4 in PNS placentas when compared to control ($p < 0.05$). The increase in TLR4 in the placenta, and IL-6 and TNF-α in the fetal brain were not seen in GF mice, suggesting that microbes are required for certain inflammatory sequelae.

We next evaluated in utero inflammation following PNS in CCL2 -/- mice. CCL2 -/- mice did not demonstrate increased IL-6 in placenta or fetal brain, but did demonstrate a significant increase

in TLR4 in the placenta, suggesting microbial activation of that receptor is upstream to CCL2.

We next examine behavior in adult offspring. WT offspring exposed to stress exhibited social deficits when compared to control mice ($p < 0.05$), which was ameliorated in CCL2-/- mice.

In terms of serotonin (5HT), stress increased concentrations of 5-HT and its breakdown product 5-HIAA in the placenta ($p < 0.05$). While 5-HT was still elevated in CCL2-/- placentas, the increase in 5-HIAA was abrogated in the absence of CCL2, indicating a link between inflammation and serotonergic dysfunction in the intrauterine environment.

Conclusions: Here, we provide evidence of the critical role of microbes and CCL2 in mediating fetal brain inflammation following maternal stress. Furthermore, we demonstrate that maternal stress enhances 5-HT breakdown in the placenta and leads to aberrant sociability and anxiety-like behavior in the adult offspring in a CCL2-dependent manner. Altogether, these data provide supporting evidence that the behavioral sequelae of maternal stress have prenatal origins, and that maternal microbes and CCL2 are tantalizing targets for developing novel treatments.

Keywords: Prenatal Stress, Social Behavior, Microbiota-Gut-Brain Axis

Disclosure: Nothing to disclose.

W38

Genetic Variation Between Individuals With Autism and High or Low Levels of Aggressive Behaviors Matched on Clinical and Demographic Variables

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Background: Despite its clinical significance, relatively little is known about the underlying cellular and circuit basis of challenging behaviors such as irritability and aggression in the context of autism spectrum disorder (ASD). It is well established that de novo variants (DNVs) and rare inherited variants (RIVs) contribute to ASD in general. In this study, we sought to test whether individuals with ASD and high levels of aggression harbor a greater burden of DNVs and RIVs than individuals with ASD and low levels of aggression, when matched on key demographic and clinical variables. We also aimed to determine whether genetic variation clustered within specific cell types, brain regions, or functional pathways.

Methods: Individual subject data was obtained from the Simons Simplex Collection (SSC) version 15.0 (Total SSC: $N = 2759$, 86% male). Individuals with ASD whose Aberrant Behavior Checklist – Irritability Subscale (ABC-I) score fell within the first quartile (low aggression: ABC-I score 0 to 4) and fourth quartile (high aggression: ABC-I score 18 or greater) were subsequently manually matched on age, Social Responsiveness Scale (SRS) total raw score to measure overall ASD severity, full scale IQ, and Vineland-2 composite score to measure adaptive function. Only data from male subjects were included due to a small overall number of females which would underpower statistical comparisons between sexes. The final low and high aggression cohort consisted of $N = 260$ subjects in each group ($p > 0.35$ on each confounding variable above). De novo variants were ascertained using denovo-db v.1.6 (denovo-db.gs.washington.edu), while RIVs were ascertained using variant call format (vcf) data obtained from the SSC and filtering for high-confidence inherited variants using VCFtools v.0.1.14. Variant annotation was performed using ANNOVAR v.2019Apr16. Functional pathway analysis was conducted using Metascape (Zhou et al., 2019). Brain region and cell specific enrichment analysis (CSEA) was conducted using CSEA tool version 1.1 (http://genetics.wustl.edu/jdlab/csea-tool-2). Differences in genetic variation between groups were analyzed using the Mann-Whitney test.

Results: In the final matched sample, mean age was ~9 years old, mean full scale IQ was ~82, mean SRS total raw score was 100, and mean Vineland-2 composite was 74. Non-intergenic, non-intronic DNVs were significantly enriched in high aggression compared to low aggression subjects (mean DNVs per subject: 2.19 vs. 1.84, $p = 0.047$). Analysis of DNVs by function class demonstrated this difference was driven by an excess of DNVs in both 5′ and 3′ untranslated regions (UTRs) (mean DNVs per subject: All UTRs: 0.45 vs. 0.27, $p = 0.013$; 5′ UTR: 0.14 vs. 0.07, $p = 0.029$; 3′ UTR: 0.30 vs. 0.20, $p = 0.010$). Similar significant differences in 5′ and 3′ UTRs were obtained when DNVs were constrained to only those absent from the Exome Aggregation Consortium (ExAC) database. Unlike in probands, differences in UTR DNVs were not observed between the siblings of the high and low aggression cohorts. There were no significant differences in any other analyzed function class of DNVs, nor did we identify significant differences in RIVs. The UTR DNVs in the high aggression group were specifically enriched in D1+ and D2+ medium spiny neurons (MSNs) ($p < 0.05$, Benjamini-Hochberg corrected), while UTR DNVs from the low aggression group were not significantly enriched in any cell type. Finally, functional analysis revealed that high aggression subject UTR DNVs significantly clustered in post-translational modification, protein degradation, and dendritic morphogenetic pathways/processes, which were not enriched in the low aggression subjects.

Conclusions: Our results suggest an excess of DNVs in UTRs of individuals with ASD and high levels of aggression compared to individuals matched on ASD severity, age, full scale IQ, and adaptive functioning but with low levels of aggression. This genetic variation maps to MSNs in the striatum, and implicates a role for aberrant protein modification and protein degradation as well as dendritic morphology in high aggression within ASD. Both striatal MSNs and the functional pathways described above have been implicated in animal models with abnormal aggression. Thus, future studies will examine these neuronal types and pathways specifically in an ASD context to identify potential therapeutic targets.

Keywords: Autism Spectrum Disorder and Related Syndromes, Aggression, De Novo Mutation, Striatum, Rare Genetic Variants

Disclosure: Nothing to disclose.

W39

Intra-Individual Changes in Methylome Profiles: An Epigenetic 'Scar' of Early-Life Adversity?

Abstract not included.

W40

Ventral Visual Processing Stream Abnormalities in Children With Williams Syndrome: Convergent fMRI Findings Across Three Independent Object Processing Tasks

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Background: Williams syndrome (WS) is a rare neurodevelopmental disorder caused by a well-delineated, stereotyped hemideletion of ~25 genes on chromosomal band 7q11.23. Affected individuals are behaviorally typified by increased social drive (often termed "hypersociability") and impairment in visuospatial construction ability1. The study of individuals with WS, in which both the affected genes and behaviors are circumscribed and well-defined, provides a unique opportunity to investigate how genetic influences are transduced to behavior through the brain.

Previous studies of adults with WS have identified structural and functional alterations in the dorsal visual processing stream as the neural underpinnings of the visuospatial deficit, while showing that object processing, which is subserved by the ventral visual stream, is relatively preserved2. However, the fact that the dorsal and ventral streams are extensively interconnected may influence the neural networks recruited for ventral stream tasks in WS. Indeed, previous research has shown that adults with WS have reduced functional connectivity between the parahippocampal place area (PPA), a ventral stream region crucial for object processing, and the intraparietal sulcus (IPS), a dorsal stream region involved in processing spatial information3.

Here, in children with WS who had IQs in the low-average to average range, and in unrelated typically developing children (TD), we leveraged our deep phenotyping of the WS population to demonstrate the convergence of findings across three separate, independently-administered task-based fMRI paradigms, each designed to probe function of the ventral visual processing stream.

Methods: 3T fMRI data were acquired for three tasks in 36 TD children (mean age=13, range 8-18, 20 males) and 19 children with WS (mean age=13, range 8-18, 5 males). For each trial of the first (Task 1), an aversive scene was presented as a target image at the top of the screen with two possible choices on the bottom, one of which was identical to the target; participants pressed a button to select the matching picture. For each trial of the second (Task 2), two images were shown sequentially, first an image was shown on the left side of the screen, and it disappeared before a second image appeared on the right; participants judged whether the second image was the same as the first and made a button press response. For the third task (Task 3), images of tools were presented one at a time; participants pressed a button when an image was the same as the preceding one. For each task, scrambled images presented in the same way and requiring the same button press served as a sensorimotor control.

All fMRI data were analyzed identically, including ART scrubbing for motion correction, warping to a study-specific template, and smoothing at 8mm FWHM. For each task, contrasts (i.e., scene/object matching versus sensorimotor control) were compared between groups, controlling for age and sex, with a statistical threshold of $p < 0.005$, uncorrected. Voxels with significant findings for each task were binarized and then added across tasks to create maps of convergent findings.

Results: Relative to TD children, children with WS had increased left PPA activation that overlapped across all three ventral processing stream tasks, alongside decreased IPS activation bilaterally that overlapped across Tasks 1 and 2. For the tasks with both PPA hyperactivation and IPS hypoactivation in children with WS (Tasks 1 and 2), we examined the relation between BOLD extracted from PPA and IPS clusters. For Task 2, there was a positive correlation for the TD group ($r = 0.61$, $p = 0.0001$) but not for the WS group ($r = -0.03$, $p = 0.90$).

Post-hoc analyses, with subgroups matched for sex ratios, indicated that findings were not due to group differences in the proportion of females.

Conclusions: In contrast to previous work in adults with WS, in our sample of children, we found differences in brain activation in the ventral visual processing stream. Specifically, relative to the TD group, the WS group had increased recruitment of the PPA during object processing. This increase in PPA activation was concomitant with a decrease in IPS activation, which may hint that these regions have differential functional connectivity with each other or with other brain regions in children with WS. The difference between our findings and those in adults with WS may point to developmental changes in the networks that subserve ventral and dorsal visual processing streams and the connections between them.

Several of the genes hemideleted in WS contribute to the development of white matter architecture, including LIMK1, which is also thought to be a key gene underlying the dorsal stream abnormalities in WS. These genes may impact the organization of ventral and dorsal processing stream networks and their functional cross-talk, pointing to a potential genetic mechanism underlying our findings.

Future multimodal, longitudinal work will examine the functional and structural connectivity of ventral and dorsal visual processing streams in children with WS and assess the longitudinal trajectories of these networks.

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Keywords: fMRI/Imaging Genetics, Neurodevelopmental Disorder, Genetic Disorder, Brain Imaging, fMRI

Disclosure: Nothing to disclose.

W41

Too Much Screen Time, Poor Physical Health, and Few Extracurricular Activities are Associated With Poor Neurocognitive Functioning in the ABCD Cohort of 9-10-Year-Old Youth

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Background: Fluid cognitive functioning (FCF) refers to the capacity to reason and solve novel problems, independent of past knowledge. It involves logical thinking and inductive/deductive reasoning, and it is a significant driver of long-term psychological well-being, academic achievement, and adoption of healthy behaviors. Previous research has found that FCF is adversely impacted by socioeconomic disadvantages (McLoyd, 1995; Noble et al., 2007), exposure to childhood maltreatment (Gould et al., 2012), poor parent mental health and substance abuse (Agha et al., 2017), family conflict (Jung et al., 2018), and current psychopathology (deVroege et al., 2018). Although a number of adverse predictors of FCF have been identified, previous investigations have studied relatively small samples and focused on a limited number of hypothesis-driven predictors in their analyses. This has limited the development of meaningful yet scalable preventive interventions for youth at risk for neurocognitive deficits. Given that pathophysiology of neurocognitive function is complex and likely influenced by numerous factors of potentially shared variance, more sophisticated multivariate statistical models employing large datasets are necessary to identify not only

generalizable associations, but also predictors of adequate prospective clinical utility. Here, we employ machine learning (ML) to (1) examine a large number of predictors of FCF in a national study of at-risk youth, and (2) identify predictors that may be targeted by scalable preventive interventions.

Methods: The Adolescent Brain and Cognitive Development (ABCD; Volkow et al., 2018) study is a large multi-site, longitudinal study designed to understand a multitude of factors influencing development and long-term outcomes. As part of the baseline assessment, 4521 adolescents completed the NIH Toolbox Neurocognitive Battery (Gershon et al., 2013), yielding age-corrected composite scores of fluid and crystalized cognitive functioning. Fluid cognitive functioning (FCF) scores are derived from measures of executive function, attention, inhibition, processing speed, and working and episodic memory. Predictors of FCF examined in the present analysis included (1) demographic variables from the PhenX survey toolkit (Stover et al., 2010; Barch et al., 2018); (2) parental psychopathology from the Achenbach Adult Self Report Questionnaire (Achenbach, 2009; Barch et al., 2018) and substance use; (3) youth mental health from the Child Behavioral Inhibition & Behavioral Activation Scales from PhenX (Carver & White, 1994; Barch et al., 2018); (4) youth physical health and involvement using the Sports and Activities Involvement Questionnaire (Huppertz et al., 2016), the National Health and Nutrition Examination Survey (CDC, 2016), Youth Risk Behavior Survey (CDC, 2016), and Sleep Disturbances Scale for Children (Bruni et al., 1996; Spruyt & Gozal, 2011); and (5) environmental factors such as neighborhood safety, economic insecurity, family conflict [Family Conflict subscale of the Family Environment Scale (Barch et al., 2018)], and youth chronic stress exposure. We used a ML pipeline as implemented in the caret package (version 6.0.82) in R (version 3.5.1) that combines predictions across multiple base learners (support vector regression [SVR], random forest [RF], and elastic net [ENET]) via stacking or meta ensemble approach. Performance of stack ensembles was assessed using a nested cross-validation (nCV) procedure with the inner loop building base and stacked models and the outer loop evaluating model performance, with 5-fold cross-validation in both inner and outer loops. Stacking was also used to assess for variable importance (VI), such that a single set of VI values was produced by averaging VI sets across folds. The whole nCV was repeated for 5 times.

Results: A ML model with all predictor variables explained 12% of the variance. Among the top 15 variables with the highest importance, 5 were demographic (parental education, income above $100,000, income less than $50,000, parents married, and majority race), 5 were related to psychological well-being (attention, aggression, and rule-breaking scores on the CBCL, and lack of positive urgency score from the impulsive behavior scale), and 5 were related to environmental factors (economic insecurity, parental self-perceived strength, youth weekday and weekend screen time activity, youth body mass index, and youth engagement in extracurricular activities).

Conclusions: The results of this data-driven investigation in a large sample of youth partly replicate previous findings and confirm the importance of socioeconomic, environmental, and mental health factors in FCF. Additionally, our analysis identified screen media activity, BMI, and engagement in extracurricular activities as important variables associated with FCF. Specifically, youth engaging in fewer social activities, spending more times with screen media activity, and being physically less active also had poorer neurocognitive functioning. The longitudinal data from ABCD will be able to begin to assess causality by examining how changes in these factors affect subsequent cognitive performance.

Keywords: Neurocognitive Functioning, Adolescent, ABCD Study

Disclosure: Nothing to disclose.

W42

Autism Traits in Parents, Age at Conception, and Polygenic Risk Score in ASD Children

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Background: Research has described multifactorial etiology in Autism Spectrum Disorders (ASD), where there may be a combination of genetic and environmental factors. Among the potential epidemiological factors, advanced parental age at conception (APA) has been associated with increased risk of autism in offspring [Wang, C. et al., 2017; Modabbernia, A., et al., 2017; Janecka, M. et al., 2017]. Although de novo mutations in male germ cells accumulated by APA have been proposed as the main explanation for the increased risk for autism [Malaspina, et al., 2001; Kong, A. et al., 2012], they account for only a part of the risk associated with APA. Alternative (but not mutually exclusive) explanatory models have been proposed, including the possibility of delayed fatherhood or motherhood in subjects with autistic traits, particularly studied in high-functioning subjects [Gratten, J. et al., 2016; Merikangas 2017; Sandin et al., 2012], with no data elucidating the possible mechanisms involved.

We aim to study whether autistic traits in parents of ASD subjects correlate with parental age at time of conception, and analzye to what extent this correlation differs according to the ASD subtype diagnosis. Secondly, we will explore the relationship between autistic traits in the parents and polygenic vulnerability.

Methods: ASD subjects were recruited from AMITEA [Parellada et al., 2013] at Hospital General Universitario Gregorio Marañón (HGUGM) in Madrid. Subjects were assessed with Autism Diagnostic Observation Schedule (ADOS), Autism Diagnostic Interview-Revised (ADI-R), and Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision and Fifth Edition (DSM-IV-TR and/or DSM-5) criteria for the diagnosis [Volkmar et al., 2014]. Parental autism traits were evaluated with the Spanish version of the autism-spectrum quotient for adults (AQ) [Baron-Cohen, S., 2003]. For the polygenic risk scores (PRS), we used exome data from 242 ASD trios from Madrid, Spain that were sequenced as part of the Autism Sequencing Consortium (ASC). PRS were calculated using this imputed data as the target sample and Genome-wide Association Study (GWAS) summary statistics from the Psychiatric Genomics Consortium (PGC) repository were used as a discovery sample [Grove et al., 2017], after an earlier analysis by our group [Costas et al., 2016]. Maternal and paternal combined PRS were used to study their correlation with AQ measures in the whole sample, and in the different ASD subtypes.

Statistical analyses were performed using R or SPSS (Statistical Package for the Social Sciences) 18.0 for Windows, with a significance p-value threshold set at < 0.05. In order to investigate firstly the relationship between autism traits in parents and age at the time of conception and secondly the relationship between those traits and PRS, bivariate correlation analyses were performed using Pearson and Spearman coefficients as needed. We also compared AQ, APA, and PRS of parents of children with different ASD subtypes.

The study was approved by the ethics committee at HGUGM. All participants or their legal representatives provided written informed consent.

Results: Ninety-two subjects with ASD (more than 80% male and Caucasian) and their parents (both) were included in this study. According to DSM-IV, 54% of the subjects had an Autistic Disorder (AUT), 19.6% had Asperger Syndrome (AS), and 26% had a Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). Age ranged from 3 to 36 years, mean 11.57, SD = 7.81. Mothers were

aged 22 to 44 years old, mean 32.54, SD = 4.654 and fathers were aged 22 to 47, mean 34.22, SD = 5.304.

We found a statistically significant positive relation between parental age at conception and AQ total scores (r = 0.252, p = 0.015). This correlation was present in fathers (r = .210 p = .045) and mothers (r = .225 p = 0.031), separately. In order to check if autistic traits were differentially present in parents of children with different subtypes of ASD, we divided the sample into AUT, AS, and PDD. APA and AQ positively correlated with mothers of AS subjects (r = .486 p = 0.041, N = 18).

We calculated PRS in trios with exome information available (N = 71 out of 92). As autistic traits did correlate with age at conception only in mothers, and particularly significantly in Asperger participants, we explored the AQ – PRS relationships in fathers and mothers independently, dividing the ASD population into AS and Non-AS. We observed a significant correlation between AQ and PRS in mothers of AS subjects (r = 0.574, p = 0.012), but not in mothers of AUT or PDD.

Conclusions: In a sample of parents of ASD subjects, fathers and mothers with more autistic traits conceive children later. This was true for both mothers and fathers separately. This was also true in mothers of children with a diagnosis of Asperger Syndrome. With regards to the relationship between self-reported autistic traits and polygenic risk scores derived from exome data, we found a positive correlation between those, but only in the case of mothers of Asperger patients. Thus, the PRS-AQ correlation, as a proxy of the AQ – APA relationship, is mainly driven by the maternal but not the paternal contribution.

The results of this study suggest that parents who delay conception tend to present more autism traits and probably, therefore, higher polygenic liability, particularly in mothers of AS subjects.

Keywords: Autism, Human Genetics, Polygenic Risk Score, Asperger's Spectrum Disorder, Gender Differences

Disclosure: Angelini, Otsuka, Sage, Lundbeck, Janssen, Acadia, Advisory Board, Self

W43

Differentiating Adolescent Suicide Attempters and Ideators Using Inflammatory Markers

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Background: Suicidality has been associated with elevations of inflammatory markers, including interleukin-6 (IL-6) and interleukin 1-beta (IL-1β) [Black 2015]. Recent studies have examined the complete blood count (CBC), and noted association between suicidality and elevations of inflammatory or hematological indices, including the neutrophil:lymphocyte ratio (NLR) and mean platelet volume (MPV) [Orum 2018]. However, few studies have examined a wider array of inflammatory molecules in an adolescent population to assess severity of recent suicidality. In a post-hoc exploratory analysis, we sought to examined hematological markers in a cohort of adolescents admitted to an inpatient psychiatric unit for suicidal attempt (SA) or suicidal ideation (SI), hypothesizing that adolescents admitted for SA would have elevated global inflammatory indices compared to adolescents admitted for SI.

Methods: We identified 142 adolescents admitted to an inpatient psychiatric unit for either suicidal ideation or attempt. Using retrospective chart review, we identified baseline demographic, medical, and reason for admission [suicide attempt (n = 51) or suicidal ideation (n = 91)] on patients, along with twenty blood hematological and immunological measures derived from the CBC. Both sexes were included in our study. Multiple logistic regression was used to examine the association of hematological measures with reason for admission, after controlling for age and gender. Sensitivity analyses were done to statistically adjust for the presence of serotonergic medications and prior suicide attempts.

Results: Participants were an average of 15 years old, 69.7% female, with 64% of participants having had a prior suicide attempt, 33.8% taking a serotonergic medication at blood draw, and 35.9% being admitted for suicide attempt. Patients admitted for suicide attempt were more likely to have had a prior suicide attempt (OR=29.4, p < 0.001, n = 137) and be older in age (OR=1.4, p = 0.008, n = 140). A lower percentage of eosinophils was found in patients who were admitted for suicide attempt, compared to those who were admitted for suicidal ideation (OR = −0.76, p = 0.042, n = 116). No differences were found in the NLR (p = 0.92, n = 121), MPV (p = 0.81, n = 121), or other hematological measures (p's>0.05). No changes in results were found on sensitivity analysis.

Conclusions: Based on this exploratory analysis, adolescent suicide attempts may be associated with a lower eosinophil count, potentially suggestive of selective immune system suppression of these inflammatory cells. Few studies exist examining eosinophil percentage in relation to suicidality, with one prior larger study identifying no relationship between eosinophils and PHQ-9 reported suicidality in a community sample of adults [Bergsman 2019]. However, our exploratory analyses suggest that suicide attempters and individuals with high levels of suicidality may differ in neuroimmune interactions, which has been suggested by other studies examining cortisol or cytokine mRNA levels [Melhem 2017]. Perhaps, in suicide attempters with greater neuroinflammation, white blood cell production nonspecifically skews to non-eosinophil white blood cells which more readily produce cytokines such as IL-6 or IL-1β. Such immune cells might include neutrophils, lymphocytes, or monocytes. In general, neuroinflammation may also relate to suicidality via well-described associations of inflammation and depression, or complex effects on neurobiological pathways, such as the hypothalamic pituitary axis, neurotransmitter alterations, neurocircuitry alterations, or the kynurenine pathway. However, more robust and larger studies are required to further assess these relationships with pathways and cytokines and the role of eosinophilia in adolescent suicidality.

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Keywords: Depression Inflammation Cytokine, Suicidality, pediatric

Disclosure: Nothing to disclose.

W44

Transcriptomic Analysis of Glucocorticoid Signaling and Immediate Early Gene (IEG) Expression in Balb/c Mice Following Treatment With a Prosocial Dose of VU0410120, a Novel Glycine Transporter Type 1 (GlyT1) Inhibitor

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Background: Impaired social communication is a core symptom, and anxiety is a common comorbidity (~40-50%) of autism spectrum disorder (ASD). Altered stress reactivity and glucocorticoid signaling and anxiety-like behaviors are observed in relevant mouse models of ASD. D-Cycloserine, a partial glycineB agonist, and VU0410120 (VU120), a glycine type 1 transporter inhibitor, improved sociability of Balb/c mice, a model of ASD, consistent with NMDA receptor (NMDAR) regulation of sociability and altered endogenous tone of NMDAR-mediated neurotransmission in this strain. Because glucocorticoid signaling may reflect or underlie social stress and anxiety phenotypes, we conducted a targeted hypothesis-driven comparison of gene expression profiles of 88 glucocorticoid signaling-associated genes and immediate early genes (IEGs) in frontal cortex (FC) and hippocampus (Hipp) of Balb/c mice treated with VU120. Changes in IEG expression show target engagement, the role of gene expression in therapeutic mechanisms of action, and the temporal and spatial selectivity of engaging these therapeutic targets.

Methods: 30-min after injection of VU120 (0.01 ml/g, i.p.) or vehicle, sociability and immobility were assessed in 4-week old Balb/c and Swiss Webster (SW) comparator mice (N≥21/condition) in a 3-chambered apparatus. Ranked social scores were used to designate global prosocial responses of individual mice to VU120 and assign groups of "High", "Medium" and "Low" prosocial responders for microarray experiments and transcriptomic analyses. Vehicle- and VU120-treated groups of Balb/c mice with the highest and lowest prosocial behaviors were used for microarray analysis (n = 4/condition); SW mice with a "medium" prosocial response were chosen for comparisons. RNA from FC and Hipp (n = 4/group) was extracted using RNAeasy Mini Kit (QIAGEN). RNA was analyzed and selected for analysis by microfluidic gel electrophoresis (RIN >8, Agilent 2100 Bioanalyzer). Transcriptome profiling was performed using the Affymetrix GeneChip Mouse Gene 2.1 ST array (ThermoFisher Scientific, Waltham, MA). Preliminary evaluation of differences in gene expression was determined using Affymetrix Transcriptome Analysis Console (TAC) software; fold changes (-1.25 ≤ or ≥1.25, $p < 0.05$) and False Discovery Rate (FDR, $p < 0.05$). Differences in expression of 88 glucocorticoid signaling genes and other exploratory comparisons were performed (e.g., differential expression of IEGs and gene ontology [GO] analysis [fold change -1.20 ≤ or ≥1.20, $p < 0.05$ and FDR, $p < 0.05$]) using Affymetrix TAC, DAVID (the database for annotation, visualization and integrated discovery) and/or Kyoto Encyclopedia of Genes and Genomes (KEGG). Heatmaps were used to visualize clustering analysis of related groups of genes based on their expression (R-studio).

Results: DAVID GO analysis showed a significant 5.4 fold-enrichment for "negative regulation of mTOR signaling" (EASE score, p = 0.013). Expression of five genes involved in this biological process was upregulated in FC of the VU120-treated Balb/c "non-responders": Akt1s1, Ddit4, Epm2a, Gsk3a, and Tmem127. Expression of Ddit4, a known negative regulator of mTORC1 signaling, was significantly increased in FC in VU120-treated Balb/c "non-responders", compared to both VU120-treated Balb/c "responders" and the VU120-treated SW "medium" social responders. Moreover, KEGG analysis also confirmed differences in mTOR signaling gene expression within VU120-treated Balb/c "non-responders" compared to the Balb/c "responders". Relatively increased expression of IEGs was selectively observed in FC of VU120-treated Balb/c mice (N = 8) compared to VU120-treated SW mice (N = 4). Consistent with a complex relationship between NMDAR "dysregulation" and ASD symptomatology, relative expression of several IEGs was increased in vehicle-treated Balb/c mice (N = 8) compared to VU120-treated Balb/c mice (N = 8). Irrespective of group assignment, Jun expression was increased in FC of VU120-treated Balb/c mice compared to VU120-treated SW mice. Moreover, relatively increased Jun expression in FC distinguished VU120-treated Balb/c mice in the "Low" sociability group (N = 4) from VU120-treated Balb/c mice in the "High" sociability group (N = 4).

Conclusions: Transcriptomic analysis revealed increased mRNA expression of Ddit4 (gene coding 'DNA damage inducible transcript 4') in VU120-treated Balb/c "Low" responders compared to both VU120-treated Balb/c "High" responders and VU120-treated SW mice displaying "Medium" levels of prosocial response. These data encourage additional studies of NMDAR activation on expression changes in mTOR signaling pathway-associated genes. Although preliminary, these data also support therapeutic inhibition of mTORC1 in ASD and a therapeutic role for NMDAR activation in the regulation (especially dampening) of the mTOR signaling pathway, which is disturbed in several paradigmatic models of ASD. IEGs are activity-regulated genes, which include many transcription factors that have important roles in regulating the properties of the neuronal membrane and synapse dynamics, as well as refining neural circuits. Strain differences in the regionally-selective "relative" suppression of IEG expression in response to GlyT1 inhibition observed in this gene expression analysis may be "surprisingly" relevant to proposed mechanisms of prosocial effects of VU120.

Keywords: Balb/c Mouse, ASD, Glycine Transporter 1, Immediate Early Gene, Glucocorticoids

Disclosure: Nothing to disclose.

W45

Translational Electrophysiological Biomarkers in Tuberous Sclerosis Complex, a Genetically Defined Neurodevelopmental Disorder

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Background: Genetically defined neurodevelopmental disorders provide an entry point for validating translational biomarkers of both disease manifestation and treatment response. Tuberous sclerosis complex (TSC) is a rare genetic disorder associated with autism spectrum disorder (ASD) and epilepsy. Mutation of the TSC1/TSC2 genes, causative of TSC, upregulates the mTOR pathway and results in alterations in axon outgrowth and synapse formation in animal and cellular models. In patients, the structural defects in connectivity manifest as loss of white matter integrity evident in imaging of patients. Mechanism-based therapies for the neuropsychiatric features of TSC are currently in clinical testing and therefore this disorder provides a unique model system in which to identify pharmacodynamic biomarkers. Electroencephalography (EEG) can be collected using similar techniques in both rodent models and patients and provides a functional readout of neural connectivity. Here we utilize parallel methodologies to quantify brain activity from EEG in patients with TSC and mouse models of the disorder to identify potential treatment response biomarkers.

Methods: Preclinical EEG: Utilizing hypomorphic Tsc2 mouse line (n = 7-8/group; male and female) associated with neuropsychiatric deficits and epilepsy, chronic in vivo EEG was recorded under baseline conditions and after either acute or chronic treatment with an mTOR inhibitor. Spectral features and auditory evoked potentials (AEP) elicited from auditory gating and mismatch negativity paradigms were calculated. Clinical EEG: High density EEG was recorded from children (n = 10/group; male and female) with TSC ages 4-14 under baseline conditions during

resting state and the same auditory paradigms used for the mouse study. All protocols were approved by the appropriate institutional review boards at Boston Children's Hospital.

Results: In baseline EEG, mice with reduced expression of Tsc2 have decreased oscillatory power in low frequency bands (theta; $p < 0.01$) but an increase in power in the high frequency bands (beta; $p < 0.01$) relative to littermate controls, which are modulated by circadian influences. The changes in spectral power are associated with a progressive seizure phenotype that ultimately results in the premature death of the animals. Mutant animals also have alterations in the evoked response to auditory tones, relative to control animals, characterized by enhanced early components and diminished late components of the response waveform (greater N1 amplitude $p < 0.05$ and smaller N2 amplitude $p < 0.05$). Patients with TSC exhibit a similar pattern of response to auditory tones and have an elevation in oscillatory power in the beta frequency. In the mouse model, acute treatment with an mTOR inhibitor has no effect on EEG based biomarkers. Chronic treatment with an mTOR inhibitor reduces the seizure burden of the animals and normalizes the auditory evoked responses (vehicle vs. drug $p < 0.01$) but intriguingly exacerbates the spectral phenotype.

Conclusions: Spectral power and AEPs are similarly disrupted in mouse models of Tsc2 deletion and children with TSC. Chronic, but not acute treatment, with an mTOR inhibitor normalizes AEPs but not spectral power in the mouse model. Upon validation, this suggests that AEPs, but not oscillatory power could be used as a biomarker of target organ engagement or treatment response in ongoing clinical trials with mTOR inhibitors in TSC. Evidence of a parallel treatment response in patients with TSC and the mouse models would suggest EEG could be a powerful translational approach to increase preclinical confidence in novel pharmacotherapies for neurodevelopmental disorders.

Keywords: Autism Spectrum Disorder and Related Syndromes, Tuberous Sclerosis Complex, EEG Biomarkers, EEG/ERP Electrophysiology, Translational Biomarker Development

Disclosure: Nothing to disclose.

W46

Maternal Prenatal Stress Phenotypes Associate With Sex at Birth and Perinatal Outcomes

Abstract not included.

W47

A Longitudinal Follow-Up Study on the White Matter Integrity of Autism Spectrum Disorder in Taiwan

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Background: Studies have suggested atypical developmental trajectory of white matter in the individuals with autism spectrum disorder (ASD). Tract-based Spatial Statistics study further revealed that the of white matter integrity was age dependent. However, little is known about whether the changes of white matter integrity with age differ between ASD individuals and typically developing controls (TDC). Meanwhile, most current studies investigating age effect on white matter tracts adopt a cross-sectional design, a longitudinal follow-up study may better answer the question regarding neurodevelopment. This study aims to investigate white matter integrity by diffusion tensor imaging in ASD by a longitudinal follow-up study.

Methods: We recruited 75 individuals with ASD (male 92.0%) and 84 TDC (70.2%). The mean ages of first enrollment were 15.3 ± 4.2 in ASD group and 14.5 ± 4.8 in TDC group. The follow-up latencies were 4.7 ± 1.9 in ASD (range 1.3~8.8 years) and 4.5 ± 1.3 in TDC (range 2.3~8.5 years). The mean ages were not different between ASD and TDC at both time points. We compared the fractional anisotropy (FA) of white matter integrity of 76 tracts bilaterally between the ASD and TDC (by analysis of variances) and between the two time points (by mixed model). False discovery rates (FDR) were adopted for multiple test correction. Group-by-time interaction was examined in the statistical models.

Results: We found significant time effect on most white matter tracts in either ASD or TDC groups. As for diagnosis effect, some tracts had significantly different FA between ASD and TDC but the significance disappeared after FDR correction. As for group-by-time interaction, we found that left geniculate fibers, left fornix, and callosal fibers connecting bilateral dorsolateral prefrontal cortices and those connecting temporal poles showed significant interaction, with greater FA increase of callosal fibers connecting bilateral dorsolateral prefrontal cortices and fewer FA increase of the other tracts.

Conclusions: Our findings suggested that although there was no significant group difference of white matter integrity at either time point, ASD may have altered development of several white matter tracts compared to TDC. Our findings provide evidence to support the developmental alterations of white matter integrity in ASD. These findings await further validation.

Keywords: Autism Spectrum Disorders, Longitudinal MRI, Diffusion Spectrum Imaging

Disclosure: Nothing to disclose.

W48

Gender Difference of Gaze Fixation Patterns in 5-year-old Children: The Usefulness of Early Detection of Girls With Autism Spectrum Disorder

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Background: It has been suggested that early intervention can improve developmental outcomes of Autism Spectrum disorder (ASD). Delay in the identification of ASD impedes early access to interventions and causes negative developmental outcomes. However, identifying children with ASD is difficult, especially in girls. We need biomarker considering gender differences. Recently, a lot of studies have identified unique gaze fixation patterns in individuals with ASD using eye-tracking systems. Such gaze fixation patterns in individuals with ASD are considered to be associated with social attention. Recently Gaze fixation patterns have attracted attention as the indicator of sociality. The purpose of this study is to clarify the gender difference of gaze fixation patterns in 5-year-old children and analyze these results statistically along with other existing tools, after then to examine the utility for early diagnosis of ASD.

Methods: In the community health check-up spanning three years (2013-2015, N = 3804), the participants screened in the community health check-up were 2923 children. The local government officers invited 440 children (included 31 applicants) to additional assessments and an interview based on the results of the screening. We measured the percentage of gaze fixation time allocated to particular objects depicted in movies by preschool children in a community health check-up (n = 438) by double-blind method. Subjects of analysis are ASD (n = 64) who

diagnosed by DSM-5 criteria and Typical development (TD, n = 68) who had no abnormalities in all the experiment. We compared gaze fixation percentage between the two groups and determined the cut-off point by ROC analysis using all 200 girls. In addition, we compared the fitness and the Odds Ratio of using Gaze fixation patterns with other existing tools by logistic regression.

Results: Analyzed the gaze fixation percentage of 'People' by gender, there was a significant difference only in girls ($p < 0.05$, ES = 0.96). As a result of ROC analysis in girls, AUC was 0.762 ($p < 0.001$). It indicates moderate predictive ability and diagnostic ability. When we set the cut-off point to gaze fixation percentage 50% (sensitivity 90%, specificity 59%) and combine it with Autism Spectrum Screening Questionnaire (ASSQ) and Strengths and Difficulties Questionnaire (SDQ), the fitness of logistic analysis and the Odds Ratio rose to 3.3 times. In girls, the combination of Parent-interview ASD Rating Scale - Text Revision (PARS-TR) short-version and gaze fixation percentage 50% showed sensitivity 71.5%, specificity 88.7%.

Conclusions: This study suggested the gender difference in the evaluation of sociality in preschool age. We have to select images considering age and gender. Gazing fixation pattern is useful in objectively evaluating social development. There is the possibility to contribute to early identification of girls with ASD by adding gaze fixation pattern.

Keywords: ASD, Gaze Fixation, Infant

Disclosure: Nothing to disclose.

W49

Towards Improving Infant MRI Segmentation Using Convolutional Neural Network Deep-Learning Approaches

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Background: Magnetic Resonance Imaging (MRI) is a non-invasive approach for understanding brain-behavior relationships in humans and will play an important role in unraveling how gene by environment interactions shape the neurobiology underlying behavioral as well as physical phenotypes of interest. One critical avenue of research is understanding the role these factors play in shaping neural circuits during early infancy (0-1 year of age), as this critical period of development can influence more apparent neurobehavioral and physical phenotypes across life. A major obstacle to this research is that relative to adolescents and adults, the MRI methods for infant scan preprocessing for subsequent analysis, particularly in regard to brain tissue segmentation, are extremely underdeveloped.

As opposed to MRI scans from adults for which various automated pipelines are effective (e.g., FAST, SPM), in infants the segmentation step is by far the most difficult to implement owing to several factors that create difficulty in discerning voxel intensity values corresponding to different tissue types such as gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF), as well as cortical and subcortical structures. This leads to 'partial volume' effects, or ambiguity regarding the tissue identity of specific voxels particularly along boundaries of tissue types and structures, causing ineffective segmentation across the majority of methods attempted to date. Compared with adults, infant brains 1) have a much lower contrast-to-noise ratio due to the relative lack of myelination and shorter scan times; 2) lower resolution due to the smaller overall volume of the brain; 3) reversal of GM/WM voxel intensity values before 6 months of age due to the transiently lower intensity of unmyelinated WM fibers relative to GM; and 4) similar GM/WM intensity values between 6-8 months, which reach adult values by 1 year of age. Another difficulty in applying a standardized approach is that the early infant brain experiences dramatic shifts in brain development over the span of weeks. This creates difficulty in adapting the few extant infant tissue atlases, generally limited to a single age, to guide segmentation of scans acquired at other, even proximal ages.

Methods: Recently a new technique—convolutional neural networks (CNN), which is a highly modifiable artificial intelligence-based image segmentation technique pioneered in the field of computer vision and medical imaging, which has been applied to MRI and shown to yield unprecedented levels of accuracy in automated tissue segmentation of adult scans, with similar potential demonstrated for infant scans when validated against manually traced images. To test and validate the idea of utilizing CNN to achieve accurate tissue segmentation for infants during 0 to 1 year old, we are planning to build a repository of high-quality, multimodal infant scans across a few institutions include Columbia, UCI, Brown, and Rochester University. But before the repository built up, we used 10 manual segmented structural MRI scans (T2) from Baby Connectome Project (BCP) challenge, 5 F /5 M, term born without any pathology (40±1 weeks of gestational age). MR scans were acquired on a Siemens head-only 3T scanners with a circularly polarized head coil. During the scan, infants were asleep, unsedated, fitted with ear protection, and their heads were secured in a vacuum-fixation device.

Results: As proof-of-principle to examine the feasibility and promise of our proposed approach, we used a classic U-Net CNN approach utilizing the basic ResNet-34 encoder to segment the tissues of 2D axial T2 infant images. We built three separate binary classifiers corresponding to WM, GM, and CSF. 788 2D axial slices were used as training dataset, and 196 slices as validation dataset.

As a comparison, we segmented the same scans using standard pipelines adapted for segmenting the infant brain – dHCP, SPM, and FAST. The performance of pipelines was calculated using the dice coefficient, and compared using ANOVA with post-hoc testing. As compared with standard pipelines, CNN delivered by far the best segmentation accuracy of tissues (GM, WM, and CSF, $F(3,116)=197.9$, $p < 10\text{-}5$, eta squared=0.84; post-hoc t-test p's < 10-5).

Conclusions: In this project, we showed the feasibility and potentials of achieving better tissue segmentation with a convolutional neural network for the infant MRI scans. We aim to address several limitations to infant brain segmentation to generate a robust, age-diverse infant MRI training and validation set for CNN development and create an accurate and reliable CNN-based pipeline for infant brain segmentation in future.

Keywords: Multivariate Approaches, Deep Learning, Neurodevelopment, Infant, Structural MRI

Disclosure: Nothing to disclose.

W50

Computational Network Identifies Patterns of Psychiatric Disorders and Traumas Associated With Adolescent Suicide Attempts

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Background: Suicide is the second leading cause of death among adolescents in the US and the rate of adolescent suicide is increasing. The drivers underlying this trend remain poorly understood and current gaps in knowledge on suicide risk factors among youth are a critical barrier to preventing suicide in this

vulnerable population. Although psychiatric disorders are a major contributing factor, affecting more than one-third of all adolescent suicide decedents, suicide is often not attributable to a single cause, but a consequence of complex interactions of multiple risk factors. Conventional statistical approaches often fall short in capturing these interacting factors. Here, we applied network analysis to investigate the interplay of risk factors for suicide attempts among adolescents with psychiatric disorder.

Methods: We analyzed electronic health records of adolescents aged 11 to 24 who attempted suicide prior to hospitalization for a psychiatric disorder at one of 141 HCA Healthcare facilities (n = 16,120). Information on self-reported suicide attempt and history of psychological traumas were extracted from a standardized behavioral health assessment tool administered by nursing staff at admission. A computational network was developed to model the inter-relationships between psychiatric disorders and psychological traumas experienced by adolescents who attempted suicide. Eigenvector centrality was quantified to measure the influence of individual risk factors in the network. To identify distinct patterns of psychiatric disorders and traumas associated with suicide attempts, we derived network cliques representing clusters of risk factors that co-occurred more frequently than by chance.

Results: Pre-admission suicide attempts were reported in 5,515 (34.2%) admissions and suicide attempt was the cause for hospitalization in 514 (3.2%) admissions. The risk of suicide attempts was elevated among those with depression (OR 1.95; 95% CI 1.75-2.16; p < 0.0001), personality disorder (OR 1.42; 95% CI 1.23-1.64; p < 0.0001), alcohol use disorder (OR 1.39; 95% CI 1.18-1.63; p < 0.0001), adjustment disorder (OR 1.36; 95% CI 1.18-1.58; p < 0.0001), and anxiety disorder (OR 1.14; 95% CI 1.03-1.26; p = 0.0149). Those who attempted suicide were also more likely to be exposed to psychological traumas (OR 1.17; 1.12-1.22; p < 0.0001), 48% of whom experienced one or more traumatic events. Being a victim of bullying was the strongest psychological trauma associated with suicide attempts (OR 1.50; OR 1.27-1.68; p < 0.0001). Application of network analysis offers insights into the context in which traumatic events may have precipitated suicidal behavior. For example, we identified a network clique comprising the following psychological traumas: "bullying", "neglect", "witness harm", and "family loss"; the clustering of these traumatic events suggests that victims of bullying who attempted suicide were more likely to be exposed to family adversity that may have reduced their resilience to bullying victimization. Of the risk factors included in the network, "neglect" and "family loss" were the dominant eigenvectors and appeared in most network cliques (Fig), further reinforcing the role of family adversity in precipitating suicidal behavior among adolescents. We further observed the clustering of personality disorder with sexual trauma and victimization, concurring with published evidence demonstrating a high prevalence of sexual abuse among individuals with personality disorder. Sexual trauma and victimization were also more likely to co-occur among those with adjustment disorder and anxiety disorder. In contrast, depression and alcohol use disorder did not appear to be linked to psychological traumas, suggesting that they were risk factors of suicide attempts independent of exposures to traumatic events.

Conclusions: Application of network analysis revealed the complex interplay of psychiatric disorders and traumas associated with suicide attempts among adolescents. Notably, suicide attempts caused by psychological traumas often occurred in contexts characterized by significant social disruption and family adversity. Adolescent suicide prevention efforts are therefore more likely to succeed if the effects of family adversity can be ameliorated.

Keywords: Suicide Attempt, Suicide Risk Factors, Adolescents, Psychiatric Comorbidity

Disclosure: Nothing to disclose.

W51

Association of Adverse Prenatal Exposures With Psychopathology at Age 9-10 in the Adolescent Brain Cognitive Development (ABCD) Study

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Background: The prenatal environment is thought to influence postnatal brain development in ways salient to psychopathology risk. A patchwork of specific risk factors (e.g., pregnancy complications, maternal substance use) have been studied in regard to individual mood, anxiety, and thought disorders. However, a more comprehensive assessment of these relationships, including cumulative effects of prenatal exposures on standardized, dimensional measures of psychopathology, would provide a clearer picture, help quantify risk for clinical outcomes, and take important steps toward discerning intermediate biology. The Adolescent Brain and Cognitive Development (ABCD) study, an ongoing, large, U.S.-based epidemiologic cohort, provides the necessary scope and scale to approach these goals, while also controlling for both measured and unmeasured potential confounds. We determined effects of individual and cumulative exposure to adverse prenatal events on standardized, dimensional measures of psychopathology, replicating our results in an exposure-discordant, sibling-pair sample.

Methods: We studied baseline data from 9,292 non-adopted youths (47.3% female), age 9-10, from singleton pregnancies, including 7,898 unrelated youths (non-sib group) and 697 sibling pairs with short inter-birth intervals (sib group). Child psychopathology was rated using the Child Behavior Checklist (CBCL), and prenatal exposures (unplanned pregnancy; exposure to alcohol, marijuana, or tobacco; pregnancy complications; birth complications; pre-term delivery; caesarian section) were determined through maternal interviews. Within the non-sib sample, we first identified prenatal factors that independently predicted CBCL total psychopathology, covarying for age, sex, site, race/ethnicity, socioeconomic status, and numerous other potential pre- and post-natal environmental confounds; all risk factors and covariates were entered simultaneously into the model to account for overlapping effects. The sum of these significant exposures was then determined for each participant; these cumulative exposure scores were then entered in a logistic regression model to determine odds of clinically significant CBCL score (>=60) as a function of number of exposures. Loading of significant exposures from the non-sib group was then calculated for each individual in the sib group; effects of load on odds of CBCL score >=60 was determined using mixed model logistic regression to control for family-level effects, as well as for the same covariates as before. Finally, effects of exposure load differences on CBCL differences within sibling pairs were evaluated using repeated measures ANOVA, controlling for age and sex differences between sibs.

Results: In the non-sib group, 5 exposures (unplanned pregnancy, exposure to alcohol or tobacco early in gestation, pregnancy complications, and birth complications) independently predicted CBCL total score (p's=.02 to <.001). The presence of >1 such exposure, in any combination, predicted significantly increased odds of having a CBCL score in the pathologic range; the presence of 4 or more factors (n = 423) increased these odds by 2.74-fold (95% CI 1.92 to 3.90, p < .001). Across the sib group (n = 1,394), loading of >1 of the same 5 exposures again predicted CBCL score >=60, with 4 or more exposures increasing odds by 3.05-fold (95% CI 1.30 to 7.15, p = .011). Within sib pairs, greater discordance in exposure loading predicted greater differences in

CBCL score (p = .031). Post hoc analyses indicated similar effects of exposure load across individual CBCL domains.

Conclusions: These findings associate numerous adverse fetal exposures with additive risk of generalized psychopathology in school-aged children. While retrospective recall of prenatal factors is a limitation of this design, replication across a unique set of near-age siblings with highly overlapping genetic and postnatal risk factors -- but different degrees of prenatal exposure -- reduces concern over unmeasured confounds. This work underscores the importance of adequate prenatal planning and care to brain health in childhood, and calls for additional studies to elucidate biological mechanisms and discover actionable, protective measures early in life.

Keywords: Prenatal Exposure, Children and Adolescents, Epidemiology, Alcohol, Tobacco

Disclosure: Nothing to disclose.

W52

Functional Annotation of Rare Structural Variation in the Human Brain

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Background: Structural variants (SVs) contribute substantially to risk of many brain related disorders including autism and schizophrenia. However, outside of a small number of loci that are frequent enough and have larger enough effect sizes to be statistically associated with disease, annotating SVs for their potential contribution to disease remains a major challenge. The integration of high-resolution SV calling from genome sequencing and genome-wide transcriptional measures from RNA-sequencing in human brains presents an opportunity to characterize the transcriptional consequences of SV and annotate them for potential contribution to disease.

Methods: Here, we leverage a collection of 755 human post-mortem brains with genome-sequencing (150bp paired end) where 637 also have RNA-sequencing from at least one brain region (dorsal lateral prefrontal cortex [DLPFC] and/or anterior cingulate cortex [ACC]) to quantify the dosage and regulatory effects of SV. Structural variants were generated from an integrated pipeline leveraging 8 distinct calling approaches. Gene expression was generated using STAR aligner and featureCounts, normalized using voom/limma and adjusted for hidden confounders using surrogate variable analysis. For each gene, we calculated z-scores for expression that used only non-carriers for calculation of mean and standard deviation. For cis-regulatory elements, we used brain-based enhancers, CTCF sites, enhancer-gene targets and topological associated domains (TAD) from publicly available resources such ENODE and PsychENCODE.

Results: We show that genic (p = 5.44x10-9) and regulatory SV (enhancer p = 3.22x10-23, CTCF p = 3.86x10-18) are seen at significantly lower frequencies than intergenic SV after correcting for length. We demonstrate a significant quantitative and directional relationship between the proportion of genic and regulatory content altered by a copy number variant (CNV) and the expression of that gene. The size of the effect a CNV has on expression is inversely correlated with the intolerance of the gene. Further, we present a joint linear model that leverages genic and regulatory annotations to predict expression effects of rare CNV in independent samples (R2 = 0.21-0.41). We defined an approach to annotate CNV that aggregates the predicted expression across all affected genes weighted by intolerance score to represent the total regulatory disruption. Applying this score to an independent set of 14,891 genome-sequenced individuals where SV were called using the same pipeline showed a significant increase in regulatory disruption scores for deletions implicated as pathogenic for neurodevelopmental disorders from ClinGen. Rank ordering individuals based on highest regulatory disruption identified individuals carrying pathogenic deletions that would not have been prioritized by frequency or length alone.

Conclusions: This work demonstrates the ability to quantify the effects of SV when combining genome-sequencing and RNA-sequencing for both dosage and regulatory effects and provides a simple approach for functionally annotating SV in the human brain that has potential to be useful in larger SV studies and can only be improved with more regulatory annotation information and more sophisticated approaches.

Keywords: RNA Sequencing, Whole Genome Sequencing, Functional Genomics, Copy Number Variation, Brain

Disclosure: Nothing to disclose.

W53

White Matter Microstructure in Peri-Pubertal Adolescents: Associations With Hormones and Adverse Experiences

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Background: The peri-pubertal time frame (age 8-12) represents a sensitive period in terms of brain development. Until puberty (~12 years of age), gray matter volume increases, after which time a loss of gray matter occurs in association with synaptic pruning (Giedd et al., 1999; Sowell et al., 2003). In contrast, white matter (WM) volume continuously increases in frontal, temporal and parietal lobes during this time period (Brouwer et al., 2012). Further, sex differences in WM maturation have emerged with respect to volume, with boys showing a greater rate of increase in volume (reviewed in (Schmithorst & Yuan, 2010); however, studies investigating WM integrity have been less conclusive. During puberty, endocrine events, including changes in adrenal and gonadal hormones, appear to affect brain development (Giedd et al., 1999; Lenroot et al., 2007; Sowell et al., 2002), and may mediate changes in WM volume and microstructure (Blakemore, Burnett, & Dahl, 2010). Adverse events occurring in childhood and early adolescence are also likely to have a significant impact on WM during this sensitive period, but little is known about interactions between trauma, hormones, and WM microstructure. As such, the goal of this study was to examine relationships between WM microstructure (as measured by fractional anisotropy, FA), early life trauma exposure, and levels of primary sex hormones in male and female adolescents aged 8-13 years.

Methods: Forty-eight male and female adolescents between age 8-13 (8.1 years-13.3; SD = 1.5 years) were recruited as part of a longitudinal study of brain development and trauma exposure (MH100122). Diffusion-weighted images were acquired using a Siemens 3-Tesla TIM-Trio MRI scanner. FA maps were generated using DTIfit in the FMRIB Diffusion Toolbox, and using TBSS (Smith et al., 2006) were aligned to a common space using a nonlinear registration tool. A mean FA image was created and thinned to produce a mean FA skeleton representing the centers of all tracts common to the group. Voxel values of each participant's FA map were projected onto the skeleton. FA values were extracted from specific ROIs that were defined anatomically using the

probabilistic Johns Hopkins University White Matter Atlas (Hua et al., 2008) provided by FSL. The corpus callosum (CC), cingulum bundle (CB; segmented into anterior and posterior regions), fornix, and uncinate fasciculus (UF) were selected as ROIs given that volume and integrity of these regions has been previously associated with pubertal status and trauma exposure. The Violence Exposure Scale for Children-Revised (Fox & Leavitt, 1995), a self-report assessment of the child's exposure to community violence, was administered. Levels of progesterone, testosterone, and estradiol were assessed using assays obtained from saliva samples; hormone data were available for 32 participants.

Results: Age (in months) was significantly correlated with FA in the CC ($r = .32$, $p = .03$), right UF ($r = .36$, $p = .01$), and left UF ($r = .36$, $p = .01$). In males, after controlling for age, progesterone was negatively correlated with FA in the right UF ($r = -.66$, $p = .01$) and left UF ($r = -.6$, $p = .03$). No relationships were observed between WM microstructure, estradiol and testosterone in boys. In girls, after controlling for age, a significant negative correlation was also observed between progesterone and FA in the left UF ($r = -.64$, $p = .006$) with a marginally significant correlation observed for the right UF ($r = -.5$, $p = .05$) and for the CC ($r = -.47$, $p = .06$). Similar to boys, no relationships were observed between WM microstructure, estradiol and testosterone in girls. After controlling for age, there were no significant relationships between Vex-R (total types) and FA for any paths.

Conclusions: In this sample of adolescents, we found that trauma exposure were not related to WM microstructure in fronto-limbic pathways after controlling for age. However, WM microstructure was positively associated with age, and negatively associated with levels of progesterone in both boys and girls, particularly within a WM tract that connects regions associated with emotion regulation, the amygdala and ventromedial prefrontal cortex. We will explore potential mechanisms that may contribute to the direction of these findings, exploring the role of progesterone in the development of this fronto-limbic white matter pathway.

Keywords: White Matter, Puberty, Hormones, Early Trauma

Disclosure: Nothing to disclose.

W54

Exaggerated Theta-Gamma Phase Amplitude Coupling (PAC) in Fragile X Syndrome

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Background: Fragile X Syndrome (FXS) is a leading cause of inherited Autism and intellectual disability and is caused by transcriptional silencing of the FMR1 gene. Available evidence suggests disruptions in excitatory and inhibitory mechanisms disturb circuit homeostasis. Cross-frequency phase amplitude (PAC) coupling between the phase of slow neural oscillations and amplitude of high frequency activity is a proposed mechanism of cortico-subcortical interaction associated with sensory, motor, and behavioral functions. To date, PAC findings have not been reported in human FXS literature. Thus, we were interested if 1) patients with FXS would have exaggerated PAC and 2) changes in PAC following acute challenge with the GABA-B agonist baclofen.

Methods: We conducted two experiments, a large case control study of FXS versus control subjects and acute dose challenge RCT (placebo control) in a subgroup of FXS subjects (NCT02998151). Patients underwent 5-minutes of dense array (128-channel) resting state EEG and clinical testing. Resting state EEG was repeated four hours following acute dose challenge. In addition to conventional spectral measures, we implemented Modulation Index (MI) from [Tort et al., 2010], which quantifies the degree of modulation from an empirical phase-amplitude distribution. MI value of 0 indicates a totally random relation between phase and amplitude; and larger MI values mark stronger phase-amplitude modulation [Tort et al., 2008]. We selected 10 equal size intervals in the range 0.5-20 Hz with and 0.5 Hz overlapping as phase frequencies, and 24 equal size intervals ranging from 3.5 Hz to 100 Hz with 1Hz overlapping as amplitude frequencies. Cluster-based permutation analysis was performed to assess for significant differences in spectral power and PAC between groups and pre-post drug challenge.

Results: 1) Patients affected by FXS ($n = 49$; 28 males; mean age=22.4 years) have disrupted PAC compared to matched controls ($n = 45$; 26 males; mean age=24.2 years). In FXS, increased coupling between theta phase (4 to 8 Hz) and gamma amplitude (30 to 80 Hz) and beta phase (13-30 Hz) and gamma amplitude. The spatial distribution of these findings was widespread including fronto-occipital coupling, temporo-parietal, and temporo-occipital coupling. In contrast, healthy controls, on the other hand, had increased alpha-phase (8-12 Hz) and gamma amplitude coupling. Spectral analysis revealed that FXS had a marked increase in theta and gamma relative power compared to controls. 2) Eight males with FXS (mean age=27.9 years) received a 30 mg single dose of Baclofen or placebo. Following baclofen dose, mean relative theta power was increased and posterior gamma power was decreased when compared to placebo response. Following baclofen, initial results indicate coupling between beta phase (13-30Hz) and gamma amplitude increased between fronto-occipital electrodes and decreased in the placebo group. We are in the process of correlating clinical measures and completing PAC analysis across all channels and frequency bands to better understand the clinical relevance of this physiological finding which will be completed for the poster presentation.

Conclusions: Data from a large cohort of FXS subjects demonstrate that PAC may represent a non-invasive biomarker of putative network abnormalities and can be sensitive to drug response. To the degree that such measures can reflect the degree of clinical symptomatology, such a biomarker could be a control signal for pharmacotherapy, brain stimulation, or behavioral therapy.

Keywords: Fragile X Syndrome, EEG, Baclofen

Disclosure: Nothing to disclose.

W55

Estimating the Heritability of the Brain's Structural Connectivity and its Association With Changing Symptoms of Attention Deficit Hyperactivity Disorder

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Background: Twin studies show that age-related change in symptoms of attention deficit hyperactivity disorder (ADHD) is heritable. However, the heritability of the development of the neural substrates that underlie ADHD is unknown. Here, we use longitudinal data to estimate the heritability of developmental change in the microstructural properties of white matter tracts and determine associations with change in ADHD symptoms.

Methods: 133 children from 51 nuclear families (34 with ADHD; 84 males), all with two assessments (age at baseline: 9.2 ± 3.1 years; follow up: 11 ±3.3) from which the annual rate of change in

ADHD symptoms was determined. Diffusion tensor imaging (3T; 60 non collinear directions) estimated voxel level axial diffusivity (AD) and radial diffusivity (RD). Additive genetic heritability (h2r) of the annual rate of change in microstructural properties was calculated using Sequential Oligogenic Linkage Analysis Routines. Permutation tests corrected for multiple comparisons by assessing cluster-size significance over the white matter skeleton.

Results: Rates of change in microstructural properties were heritable in two voxel clusters within the left uncinate fasciculus (AD: h2r =.48±.09, p = 0.004) and the forceps minor (RD: h2r =.52 ±.12, p = 0.01). Improvement with age in inattentive symptoms was associated with AD change in the left uncinate (t=3.41, p = .003), and with RD change in forceps minor (t=2.05, p = .06). The uncinate cluster was also associated with improvements in hyperactivity/impulsivity (t = −3.39, p = .003).

Conclusions: We demonstrate heritability in the development of microstructural properties of some white matter tracts, and find these properties are also associated with age-related change in ADHD symptoms.

Keywords: Heritability, White Matter Fractional Anistropy, ADHD

Disclosure: Nothing to disclose.

W56

Sibling Risk Across Neurodevelopmental and Psychiatric Disorders, and Developmentally Low Cognitive Ability

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Background: While phenomenological classifications such as DSM and ICD define psychiatric disorders as separate diagnostic entities, many symptoms, risk factors genetic associations and treatments are shared across diagnostic entities.

The objective of the current study was to utilize diagnostic data from screening of an entire population to further understand shared familial, possibly genetic risk, across psychiatric diagnostic groups.

Methods: Subjects were adolescents (ages 16-17) undergoing mandatory screening for eligibility to serve in the Israeli military, between the years 1998-2014. The risk of psychiatric disorders was compared between siblings of cases with psychiatric disorders and siblings of controls matched for age and gender. Cases were diagnosed with psychotic disorders (7902) personality disorders (24,816), anxiety disorders (10,606) mood disorders, (9,572), severe/profound intellectual disability (2,128) ASD (2128), substance/alcohol abuse (791) and ADHD (3,272). There were also 2,770 (0.3%) cases with Type-1 diabetes, 30,199 (3.4%) cases with hernia, and 931 (0.1%) cases with hematological malignancies and a group of low cognitive ability (defined as IQ < 2 standard deviations below population mean; (n = 31,186). Odds ratios (OR's) were adjusted for gender, socio-economic status and year of birth.

Results: Siblings of cases with all psychiatric disorders were at increased risk for all psychiatric disorders examined and, for low cognitive ability (most ORs ranging 2-3). Higher risks were found among siblings of cases with psychotic disorders for psychotic disorders (OR=9.27, 95% CI=8.66-9.92); among siblings of cases with intellectual disability for intellectual disability (OR=9.53, 95% CI=8.81-10.31) and for ASD (OR=7.53, 95% CI=6.33-8.97); and among siblings of probands with ASD for intellectual disability (OR=6.87, 95% CI= 5.97-7.92) and for ASD (OR=11.53, 95% CI=9.23-14.40). In comparison, siblings of probands with non-psychiatric illnesses (type-1 diabetes or inguinal hernia) were at increased risk for concordant disorders, but not for psychiatric diagnoses.

Conclusions: In this large population-based study, there appears to be a large shared risk across different psychiatric diagnostic groups, with specifically increased genetic risks in psychotic disorder, intellectual disability and ASD. Psychiatric disorders are co-segregated such that risk is only shared for psychiatric, and not for general medical conditions. Molecular studies should continue in their attempts to identify both the shared and the specific genetic variations associated with different psychiatric diagnostic groups.

Keywords: Neurodevelopmental Disorders, Autism, Siblings, Risk Assessment, Epidemiology

Disclosure: Nothing to disclose.

W57

Novel Tooth-Matrix Biomarker to Quantify Temporal Patterns of Pre- and Postnatal Inflammation

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Background: Many neuropsychiatric diseases are influenced by early life events. Although precise etiology remains elusive for all such diseases - genetics, environment and inflammation have all been implicated and likely interact in complex ways. Current methods for assessing endogenous and exogenous factors predating the onset of neuropsychiatric susceptibility versus resilience are limited to highly restrictive sampling of e.g. blood, urine, hair, or saliva in the mother and/or child. Thus, there is a critical need for novel tools that enable high resolution time-sequenced acquisition of pre- and post-natal environmental factors associated with future disease burden. Interestingly, the dentin in primary teeth begins forming in utero, laying daily layers akin to developing tree-rings. Thus, dentin is hypothesized to act as a biological hard drive, storing incremental snapshots of an individual's intrinsic and extrinsic environment during important fetal and postnatal periods. Using this approach, the Arora lab previously developed methods to measure time-sequenced elemental data at key developmental critical periods. Our goal here was to extend these methods to measurements of time-sequenced fetal and postnatal inflammation in shed baby teeth.

Methods: Dentin Matrix Protein 1 (DMP1) and collagen, two dentin scaffolding proteins, were used in the development and validation of a protein-sparing tooth decalcification and sectioning protocol. A screen of over two dozen biomarkers associated with inflammation and/or brain development was then performed using commercially available antibodies and immunohistochemical approaches. Biomarkers included inflammatory molecules (such as TNF-alpha, INF-gamma, IL-6, IL-10, and CRP), hormones (such testosterone and cortisol), and growth factors (such as MeCP2, BDNF and EGF).

Results: Of 27 biomarkers, only one showed consistent yet distinct staining in all tested samples: CRP. Antibody specificity was validated using primary antibody omission and antigen controls. The highly stereotyped "banding" effect served as an additional control given known developmental growth patterns. Staining in non-adjacent sections showed high within but not between subject signal cross-correlation, further validating stained bands to be developmentally generated.

Conclusions: We have established and validated a novel method for measuring developmental time-resolved inflammation taking advantage of CRP trapped during dentin development. Due

to the nature of dentin development, which begins forming in the second trimester of fetal development and continues throughout the life of the deciduous tooth, this method can offer an unprecedented look at how the temporal dynamics of pre- and post-natal inflammation contribute to the development neuropsychiatric susceptibility and resilience.

Keywords: Dentin, Inflammation, CRP, Development

Disclosure: Nothing to disclose.

W58

Identifying Individual Differences in the Functional Organization of the Neonatal Brain

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Background: Connectomics is a powerful method to understanding the functional organization of the brain by dividing the brain into a set of distinct brain regions (labeled nodes) and connections between these regions (labeled edges). Studies of the connectome (or the wire diagram of the brain) have begun to link individual differences in the brain to individual differences in behavior across development. Currently, most resting-state functional connectomic research based on functional magnetic resonance imaging relies of group-wise atlases. These group-wise atlases generally reflect a division of functionally relevant nodes that are "on average" optimal for a group of subjects. Nevertheless, this approach neglects individual differences in functional organization for each subject and ignores a potentially informative marker of brain organization. We have recently developed an exemplar-based parcellation approach to customize a group atlas to an individual that accounts for individual differences in functional variations and maintains the correspondence of nodes and edges across individuals. While this and other approaches have highlighted the individual differences in functional organization for adults, less is known about when in development do these differences emerge. Here, we explore the individual variability of functional organization of the neonatal brain and compare these individual differences in functional organization to those observed in the adult brain. We hypothesize that the functional organization of the whole brain of neonates compared to young adults will have less individual differences. We also hypothesize that there will be regionally distinct patterns of individual differences for neonates and young adults.

Methods: Resting-state imaging data from forty healthy neonates were downloaded from the Developing Human Connectome Project. Imaging data were acquired between 40-44 weeks postmenstrual age. Standard resting-state functional connectivity preprocessing was performed. We used the exemplar-based parcellation approach from Salehi et al. (2018) to individualize the Scheinost 95 node neonatal atlas. Briefly, this approach initially identifies an exemplar for every group-defined node in an individual's brain by maximizing a monotone nonnegative submodular function. Then, every voxel in an individual's brain is assigned to the closest functional exemplar. This results in an atlas for each individual where the boundaries of each node are shifted to best fit that individual's functional organization. Next, we quantify the change in size for each individualized node in reference to the size of the original group-wise nodes and calculate the variance of each node's size over the group of neonates. For comparison, we performed the same procedure on 500 young adults (ages 21-35) from the Human Connectome Project using the Shen 268 node atlas.

Results: For neonates, 6 of the 95 nodes (the frontal lobe, left occipital lobe, fusiform gyrus, hippocampal gyrus, motor cortex, and basal ganglia) exhibited significant ($p < 0.05$) individual differences in functional organization. In contrast, for young adults, all 268 nodes exhibited significant individual differences ($p < 0.05$). Large variability in functional organization was observed in the occipital lobe for both neonates and adults. In contrast, neonates exhibited little variability in the frontal lobes; whereas, young adults exhibited large variability, in alignment with known developmental trajectories of the frontal lobe.

Conclusions: We investigated an individualized functional atlas in resting-state fMRI data in neonates. Neonates showed a significant amount of individual differences in functional organization. Though, these individual differences were significantly less than the variability in young adult functional organization. Large variability in functional organization was observed in the occipital lobe for both neonates and adults. In contrast, neonates exhibited little variability in the frontal lobes; whereas, adults exhibited large variability, in alignment with known developmental trajectories of the frontal lobe. The current study highlights that individual differences in the functional organization of the brain are present at birth, and evolve regionally in the brain through young adulthood in developmental expected patterns Future work will look to determine the impact of prenatal and postnatal factors that influence the trajectory of these individual differences. This will allow us to better understand the influence of life experiences versus biological factors on individual differences in the emerging human connectome. Additionally, we will look to extend this approach into the fetal period with fetal fMRI to determine the earliest periods of observable individual differences in functional organization using fMRI.

Keywords: Resting State Functional Connectivity, Infant, Individual Differences

Disclosure: Nothing to disclose.

W59

White Matter Correlates of Cognitive Flexibility in Youth With Bipolar Disorder and Typically Developing Children and Adolescents

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Background: Prior studies using behavioral tasks and functional magnetic resonance imaging (fMRI) have shown children and adolescents with bipolar disorder (BD) have deficits in cognitive flexibility—defined as the ability to adapt to changing rewards and punishments. However, no study has examined the white matter microstructural correlates of cognitive flexibility in BD youth. We sought to address this gap by testing the hypothesis that greater cognitive flexibility deficits would be associated with greater degree of white matter microstructural deficits in BD vs. age-matched matched typically developing controls (TDC).

Methods: Twenty-eight children with BD and 26 TDC youth participated in this IRB-approved study (age range 8-17 years old and full-scale IQ>70). Graduate-level clinicians administered the Child Schedule for Affective Disorders and Schizophrenia Present and Lifetime version (K-SADS-PL) to verify their BD vs. TDC status (Kaufman et al., 1997). Cognitive flexibility was assessed with the Cambridge Neuropsychological Testing Automated Battery (CANTAB)'s Intra-Extra Dimensional Set Shift task (ID/ED) as in prior BD studies. White matter microstructure was assessed via diffusion

tensor imaging (DTI) on a Siemens 3 Tesla scanner (64 gradient directions, voxel size 1.8x1.8x1.8 mm, interleaved axial slice acquisition, number of slices=70, TR = 10,100 ms, TE=103 ms, b-value=1000 s/mm2, at least one B0 volume without diffusion weighting). DTI data were analyzed using FSL for preprocessing (including skull-stripping, Eddy current and slice-to-volume motion correction, and replacement of outlier slices) and Tract-Based Spatial Statistics (TBSS). FSL's randomise algorithm was used to correlate ID/ED Simple Reversal stage error rate and DTI metrics of fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD) (in the TBSS white matter skeleton), incorporating diagnosis in the model, and controlling for age (5000 permutations, corrected for multiple comparisons using threshold-free cluster enhancement $p < 0.05$).

Results: We found significantly different correlations between FA and cognitive flexibility performance in BD and TDC youths, in multiple sections of the TBSS skeleton, including white matter regions projecting to frontal lobes (such as the Anterior and Superior Corona Radiata, Genu of the Corpus Callosum and Superior Longitudinal Fasciculus) and other regions (Posterior Corona Radiata, Posterior Thalamic Radiation, Posterior Limb of the Internal Capsule). Further evaluation showed significant between-group differences in the slopes of the Simple Reversal Stage Error rate vs. FA, with BD participants having greater slope than TDC youths—indicating an aberrant relationship between cognitive flexibility and underlying white matter microstructure in BD youth. There were no significant interactions for the remaining white matter microstructural indices (AD, RD or MD).

Conclusions: Our results highlight the importance of understanding specific cognitive flexibility-neuroimaging relationships in youth with BD. Future longitudinal studies are warranted to define the developmental trajectories of white matter microstructure in relation to cognitive flexibility deficits and illness course in BD (relative to TDC).

Keywords: Bipolar Disorder, Child, Adolescent, Cognitive Flexibility, Diffusion Tensor Imaging

Disclosure: Nothing to disclose.

W60

Motivated Maternal Approach Behaviors, Salience Processing of Infant Cues and Associations With Childhood Maltreatment Exposure in Mothers

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Background: Childhood adversity is common and associated with psychiatric disorders across the lifespan. Specifically, childhood maltreatment exposure (CME) in mothers is associated with differences in behavioral and neuroendocrine outcomes in offspring. While substantial work has examined neural circuitry of maternal depression, fewer studies have addressed the impact of maternal CME on mothers' neural response to infant stimuli how it leads to motivated maternal approach behaviors. We adapted behavioral and neuroimaging face processing tasks to assess motivated maternal approach, its association with "real-life" maternal-infant interaction. In this pilot study, we hypothesized that mothers with higher CME would have decreased differential behavioral approach response to own vs. other baby in the computerized task, and that these responses would be associated with decreased maternal sensitivity to own baby in mother-infant observation. Further, we hypothesized that mothers with higher CME would have decreased differential salience network activation to own vs. other in the fMRI task.

Methods: A community sample of mothers (18-45 yo) with varying CME level (Childhood Trauma Questionnaire) performed implicit face processing behavioral ($N = 28$) and fMRI ($N = 8$, sample subset) tasks with conditions of identity (own/other baby matched for valence/age/sex/race/ethnicity) and emotion (happy/distress). Demographic differences, peripartum medical history, breastfeeding history, primiparous vs. multiparous, psychiatric diagnoses (Structured Clinical Diagnostic Interview for DSM-5), and PTSD symptoms (PCL-5) were assessed. Behavioral task tested mothers' tendency to approach own vs. other baby, while fMRI task assessed salience network activation (ACC/amygdala/insula) when viewing own vs. other. In the behavioral task, infant faces are presented initially as "further" or "closer", simulated by size, after which mothers are prompted to "Nudge" the baby closer (down arrow) to or further from them (up arrow), with each baby photo re-presented as larger/smaller. To decrease habituation, 2 photos (counterbalanced) of each baby and emotion (happy/distress) are used; demand characteristic is decreased by short initial stimulus presentation (400ms) that is nonetheless adequate for face detection and instructing mothers to respond as quickly as they are able. Adapted fMRI task had 3 conditions: identity (own/other), emotion (happy/distress), direction (approach/withdraw). For mothers to respond but not explicitly to emotion, we colorized babies and mothers responded with color (red/blue). Lastly, we recorded 10-minute observations of each dyad, coding maternal sensitivity using the Emotional Availability Scale, a macro-coding scheme. Given relatively small sample sizes, we constrained analyses to target effects of identity including interaction with CME for both analyses (and maternal sensitivity for behavioral task analysis), and covaried to account for emotion effects. For both tasks, linear mixed effects models were employed with fixed effects of group and task conditions, random effect of participant, and for significant interactions, contrasts were tested within condition.

Results: We divided our community sample of mothers into those with moderate-severe CME ($N = 7$) vs. those with none or low CME ($N = 21$) based on CTQ cut scores in the literature. There were no group demographic or clinical differences in scales/diagnoses except peripartum medical illness history and PTSD symptoms (high>low CME), which were included in models. Logistic regression for behavioral approach task revealed Identity x Group x Sensitivity interaction ($F=12.9$, $p < 0.001$, $OR=0.14$, CI: 0.05-0.41), with convergence in approach response to own infant among the two groups in mothers with greater sensitivity. While high CME-low sensitivity mothers approached own baby >95% of the time, high CME mothers-high sensitivity mothers approached own baby at 80%. Low CME-high sensitivity mothers approached 80% for own baby, while low CME-low sensitivity was associated with 50% approach. This dissociation between the interactions suggests that sensitivity may have different optimal levels in mothers based on CME, or that the sensitivity measure may have coding variability related to group, which might be addressed with more comprehensive micro-coding approaches in the future. Linear mixed effects fMRI model in bilateral salience network regions (corrected $\alpha=0.008$ for multiple ROIs) revealed Identity x Group interaction in L anterior insula ($F1,42=11.1$, $p < 0.005$). Low but not high CME mothers had greater activation to own vs. other baby ($t1,6=3.1$, $p < 0.005$); low CME mothers showed insula activation to own baby, while high CME mothers showed deactivation ($t1,6 = -2.5$, $p < 0.05$). This dissociation between the imaging and the behavioral data may suggest a compensatory response with over-responsivity in mothers with higher CME.

Conclusions: The different relationships between behavioral approach task performance and maternal sensitivity in mothers associated with CME suggest potential compensatory behavioral responses to infant cues, which appear to be dissociated from

decreased neural response in mothers with high CME in anterior insula to own infant in a companion fMRI task. Further analyses in larger samples are warranted and may employ more precise behavioral micro-coding techniques to assess mother-infant interactions in these at-risk dyads.

Keywords: Childhood Maltreatment Exposure, Salience Processing of Infant Cues, Motivated Maternal Approach Behaviors

Disclosure: ThermoFisher Scientific, Employee (Spouse)

W61

Social Stress During Adolescence Followed by Western-Style Diet Leads to Physiological Dysregulation, Depressive Phenotype, and Decreases in Reward Sensitivity in Adulthood

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Background: The prevalence of major depressive disorders (MDD) and obesity in adolescence has steadily increased over the last decade. The comorbidity between these conditions has been widely reported, and a relationship between depression- and anxiety-like states with cardiac and metabolic dysfunction has also been demonstrated in rodents. In addition, western-style high fat/carbohydrate diet (HFD) has been linked to the development of metabolic syndrome and mood dysregulation. While much has been elucidated about the neural basis of depression and obesity, how they converge is unknown. It is unclear whether chronic stress induces physiological and neurobiological changes associated with metabolic dysfunction or vice versa, thus understanding potential mechanism(s) and/or directionality is paramount.

Methods: Adolescent (postnatal day [PD]30) male C57bl/6J mice were exposed to HFD either before or after chronic social defeat stress (CSDS), and then tested for behavioral and physiological dysregulation. Mice were given free access to HFD or normal chow (NC) for 14 days (PD30-44) prior to CSDS exposure (10 days; ~10 minutes/day), and subsequently tested for sucrose preference and social avoidance. Antidepressant response was tested in a separate cohort of mice that were exposed to 21 days of fluoxetine (FLX) in the drinking water (80mg/L). Lastly, a separate group of mice were given 1-week of HFD or low fat/carbohydrate diet (LFD) followed by subthreshold defeat stress (three 5-minute sessions, 15 minutes apart).

Results: Mice exposed to HFD before CSDS did not show changes in caloric intake, but showed significant change in total body weight after 6-weeks of HFD as compared to NC-stressed mice. ($P < 0.05$) Those in the HFD condition showed a significant decrease in preference for sucrose and saccharin (i.e., increased anhedonia) when compared to the NC-exposed mice. ($P < 0.001$) The group of mice exposed to CSDS before HFD showed no change in calories consumed, but a significant increase in body weight after only 10 days of HFD consumption. ($P < 0.05$) HFD-exposed mice treated with FLX showed an attenuated antidepressant response (i.e., increased social avoidance) as compared to NC controls. ($P < 0.05$) Mice exposed to 1-week of HFD showed increased social avoidance after a subthreshold defeat. ($P < 0.05$) All mice in the HFD condition showed a susceptible phenotype to subthreshold stress.

Conclusions: Together, these findings indicate that HFD before stress during adolescence blunts reward sensitivity and increases susceptibility to future stressors. Also, stress exposure followed by HFD induces rapid physiological/neurobiological changes resulting in mood-related deficits and attenuated antidepressant response. The concurrent exposure to stress and western-style high fat/carbohydrate diet during adolescence can lead to the development of maladaptive behaviors and negative health outcomes in adulthood.

Keywords: Comorbidity Depression and CVD, Adolescent Depression, Social Defeat Stress, Neuro-Inflammation, High Fat Diet

Disclosure: Nothing to disclose.

W62

Greater Monoamine Oxidase B Distribution Volume in the Prefrontal Cortex in Traumatic Brain Injury With Persistent Symptoms: An [11C]SL2511.88 Positron Emission Tomography Study

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Background: Traumatic brain injury (TBI) is an important public health problem affecting 1 to 2% of the general population, with approximately half of affected individuals having a poor outcome. Despite the high prevalence, there are no pharmacological intervention strategies for persistent TBI symptoms. Development of neuroimaging markers associated with pathological changes in TBI could aid the development of therapeutics. Neuroimaging markers include decreased fraction anisotropy and lower mean diffusivity measures with magnetic resonance imaging, greater translocator protein and increased tau binding with positron emission tomography. While effect sizes are substantial for some of these methods in severe repeated injury such as in professional athletes, substantial effect sizes of neuroimaging markers are lacking in TBI from community-based samples.

Reactive astrocytosis occurs in humans with Chronic Traumatic Encephalopathy (CTE) and animal studies of TBI. Reactive astrocytosis is also associated with greater expression of monoamine oxidase B (MAO-B). Recently [11C]SL25.1188, a new PET MAO-B radiotracer was validated with outstanding characteristics including standardized uptake values exceeding 5, high selectivity, a ratio of specific binding to free and non-specific binding exceeding 7 for many brain regions, and a lack of brain penetrant radioactive metabolites. It was hypothesized that given the association of astrocytosis with TBI, greater MAO-B VT, a PET index of MAO-B density would be present in TBI.

Methods: Fifteen participants with TBI in the last 5 years with persistent symptoms and 20 healthy controls underwent [11C]SL2511.88 PET scanning. None smoked cigarettes nor had a substance abuse disorder. A two-tissue compartment model was applied and analyses are completed for 7 TBI and 20 healthy. Independent sample t-tests were applied to compare MAO-B VT between TBI and healthy in the Prefrontal Cortex (PFC) and several subregions of the PFC.

Results: Patients with TBI had significantly greater MAO-B VT in the PFC ($t25=3.44$, $P = .002$), Dorsolateral Prefrontal Cortex ($t25=2.76$, $P = .011$), Ventrolateral Prefrontal cortex ($t25=2.73$, $P = .011$), Medial Prefrontal Cortex ($t25=2.94$, $P = .007$), Orbitofrontal Cortex ($t25=2.27$, $P = .032$). Effect sizes of differences in MAO-B VT between TBI and healthy were robust (~1.4).

Conclusions: Given the effect size of differentiating TBI from healthy in the sample analyzed to date, MAO-B imaging with [11C]SL2511.88 PET is showing promise as a biomarker in TBI. Inferring from studies of astrocytosis and MAO-B, this imaging approach may be considered for monitoring astrocytosis in TBI. As the study progresses, the analysis sample will be increased to include all of those scanned plus additional subjects recruited.

There are also pathophysiological implications of robustly elevated MAO-B VT in the PFC. In cell lines, rodent transgenic models of overexpression, and postmortem human brain, greater levels of MAO-B are associated with increased MAO-B activity, which is implicated in the impairment of mitochondrial function, synthesis of neurotoxic products, and dysregulation of non-serotonergic monoamines. In addition, in a transgenic mouse model of globally increased MAO-B in astrocytes, abnormal behaviors compatible with several psychiatric syndromes were found during open field assessment, such as reduced total movement, less distance traveled, lower movement speed, and decreased duration of time spent moving.

Keywords: Traumatic Brain Injury, Positron Emission Tomography (PET), Monoamine Oxidase B

Disclosure: Nothing to disclose.

W63

Insulin Sensitivity and Glucose Metabolism of Olanzapine and a Combination of Olanzapine and Samidorphan: A Phase 1 Exploratory Study in Healthy Volunteers

Abstract not included.

W64

Dose–Dependent Reduction of Palatable Food Consumption in Binge Prone Versus Binge Resistant Rats After Orexin Receptor-1 Antagonism

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Background: According to the World Mental Health Surveys performed by the World Health Organization, binge eating impacts females significantly more than males. Binge eating is defined by the consumption of an unusually large amount of palatable food (within a specific period) that is greater than an amount consumed by most individuals, and may occur 2 or more times a week.

We seek to gain a better understanding of this behavior by attempting to identify cellular and/or molecular mechanisms that may be responsible for its occurrence. Orexin (or hypocretins) hypothalamic peptides are involved in the regulation of food intake and may play a critical role in the development of binge eating behavior. The purpose of the study was to determine whether there were dose-dependent differences in the consumption of palatable food pellets (45% fat/35%carbohydrates/25%protein) in binge-eating prone (BEP) and binge-eating resistant (BER) rats after SB-334867 administration (an orexin receptor-1 antagonist).

Methods: Female Sprague-Dawley rats (freely cycling, 200-250g, $n = 8$/group) were individually housed and underwent intermittent feeding tests to identify BEP and BER phenotypes. Vaginal lavages were taken for each rat every day and smears were stained to determine the estrous cycle. The BEP and BER phenotypes were based on the consumption of palatable food pellets. BER rats were those that consistently (> 50% of the time) consumed within the bottom tertile of palatable food across a minimum of nine testing days. BEP rats were those that consistently (> 50% of the time) consumed within the top tertile of palatable food across a minimum of nine testing days. After phenotypes were identified, animals received either vehicle, 5, 10 or 20mg/kg SB-334867 (i.p.). The two-way ANOVA was used to determine statistically significant differences in palatable food consumption in BEP versus BER rats before and after the varying doses of SB-334867.

Results: Preliminary analyses showed that all three doses of SB-334867 reduce palatable food consumption in BEP and BER rats versus vehicle ($p < 0.05$). There was a significant, dose-dependent reduction in palatable food consumption in BEP rats when comparing 5mg/kg versus 20mg/kg SB-334867 ($p < 0.05$). However, this reduction was not observed in BER rats.

Conclusions: These data show that antagonism of the orexin system (specifically, orexin receptor-1) is more effective in reducing consumption of palatable food in animals that demonstrate binge eating prone behavior. Increased consumption of a high fat, high sugar diet may alter orexin receptor responsiveness and lead to heightened orexin-mediated reward sensitivity in female rats that are vulnerable to binge eating prone behavior.

Keywords: Orexin Receptor Antagonist, Binge Eating, Rodents, Maladaptive Feeding

Disclosure: Nothing to disclose.

W65

Selective Deletion of the Melanocortin-4 Receptor in the Prefrontal Cortex Alters Feeding and Executive Function-Like Behavior

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Background: Mutations of the melanocortin 4 receptor (MC4R) are strongly linked to obesity in humans, and globally removing MC4R in the mouse brain induces obesity. MC4R is strongly expressed in the hypothalamus, but deletion of MC4R within this region does not entirely account for the obesity induced by global knockout, which suggests MC4R in other regions influence this phenotype. Neurons of the arcuate hypothalamus produce the peptides that activate (α-MSH) and inhibit (AgRP) the MC4R. The MC4R is highly expressed in the medial prefrontal cortex (mPFC), which is implicated in human feeding behavior, obesity, and eating disorders. We hypothesized that manipulation of the MC4R in the mPFC (mPFCMC4R) would affect feeding and executive function-like behavior.

Methods: We examined how pharmacologic manipulation of the MC4R affects neuronal dynamics in the mPFC using MC4R-2a-cre mice. For behavioral testing, we injected viral-mediated cre-recombinase into the IL-mPFC of male MC4Rlox/lox mice to selectively delete mPFCMC4R. Following this manipulation, we examined metabolic and behavioral changes including food intake, appetitive and aversive reversal learning, and novelty-suppressed feeding.

Results: MC4R agonism depolarized the membrane and increased action potential frequency of mPFCMC4R neurons. Selective deletion of mPFCMC4R increased food consumption and induced weight gain. This manipulation did not affect baseline exploratory behavior, but it increased the latency to feed in a novel context. Additionally, mPFCMC4R deletion impaired reversal learning by inducing perseverative behavior in a cognitive flexibility test without affecting initial learning.

Conclusions: Our data highlight a novel pathway from the arcuate nucleus of the hypothalamus to the medial prefrontal cortex that regulates food intake and other feeding behaviors. These findings contribute to our understanding of the mechanisms that govern feeding behavior, especially in the context of

decision-making and cognitive rigidity, which are aberrant in individuals with eating disorders. We plan to investigate the downstream targets of mPFCMC4R cells to further integrate this pathway into the neural circuitry of feeding behavior as well as examine this circuitry in females.

Keywords: Feeding Behavior, Medial Prefrontal Cortex, Melanocortin

Disclosure: Nothing to disclose.

W66

Evaluation of [C-11]7-Chloro-2-[4-Methoxy-3-(2-(4-Methylpiperidin-1-Yl)ethoxy)phenyl]isoindolin-1-One as a PET Imaging Agent for 5-HT2C Receptors in the Nonhuman Primate Brain

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Background: The serotonin 5-HT2C receptor (5-HT2CR) is abundantly expressed throughout the central nervous system, and is involved in a variety of neuroendocrine and neurobehavioral processes. With the availability of selective agonists and antagonists, 5-HT2CR has been indicated as a novel pharmacotherapeutic target for the treatment of depression, schizophrenia, anxiety, drug abuse, obesity, and Parkinson's disease. However, a direct relationship between 5-HT2CR physiology and brain diseases has proven difficult to establish due to an inability to accurately quantify 5-HT2CR density and functional status in vivo. Therefore, development of a selective radioligand that will enable in vivo imaging and quantification of 5-HT2CR densities represents a significant technological advancement in understanding both the normal function and pathophysiology of the 5-HT2CR. Furthermore, by enabling functional imaging studies to determine dose-receptor occupancy, its application will provide an excellent tool to facilitate the discovery of therapeutic agents targeting 5-HT2CR. However, the radioligands developed so far are not ideal for in vivo brain imaging. We developed a novel 11C-labeled radioligand, [C-11] 7-chloro-2-[4-methoxy-3-(2-(4-methylpiperidin-1-yl)ethoxy)phenyl]isoindolin-1-one (1) . We report here the radiosynthesis and microPET evaluation of [C-11]1 in nonhuman primate brain as a promising PET imaging antagonist for 5-HT2CR.

Methods: (1) and its corresponding normethyl precursor (2) were synthesized via multi-step synthetic approaches. In vitro competition binding assays of (1) were conducted by NIMH Psychoactive Drug Screening Program (PDSP). [C-11]1 was prepared via O-methylation of (2) with [C-11]CH3I in the presence of 0.1 M Bu4NOH in DMF followed by HPLC purification. Log P of 1 was measured between 1-octanol and phosphate buffer at PH 7.4. [C-11]1 was intravenously administered to anesthetized rhesus monkeys weighing 7-10 kg for dynamic microPET imaging to assess in vivo regional brain uptake on a Siemens MicroPET Focus 220 scanner. Baseline study was initially performed to determine the extent of brain uptake in vivo. To test for specific binding with [C-11]1, a second imaging study was conducted in which [C-11]1 was administered 30 min after a dose of 0.3 mg/kg SB-242084, an antagonist commonly used for 5-HT2C blockade.

Results: (1) displayed a high affinity for 5-HT2CR (Ki = 2.4 nM) and a high selectivity over 5-HT2AR (Ki > 1000 nM) and 5-HT2BR (Ki = 194 nM). [C-11]1 was obtained in an average 44% ± 4% decay-corrected radiochemical yield (n = 8) with a radiochemical purity of >98% and a specific activity of 0.5-1.2 Ci/umol. (1) displays moderate lipophilicity with a log P7.4 of 2.81. In the baseline study, [C-11]1 exhibited excellent brain blood barrier penetration and showed high uptake in the choroid plexus (the region with the highest density of 5-HT2CR), hippocampus, and amygdala, and low uptake in the cerebellum, a region known to be bereft of 5-HT2CR. The corresponding time-activity curves (TACs) of [C-11]1 showed that the uptake of radioactivity in the choroid plexus peaked between 20-30 min after injection with SUV value of 5.6, and the peak uptakes in the hippocampus, amygdala, frontal cortex, and cerebellum were achieved at 9.5-22.5 min post-injection. Ratios of uptake in choroid plexus, hippocampus, amygdala, frontal cortex to that in cerebellum peaked at 2.7, 1.6, 1.3, and 1.1, respectively. Administration of a dose of SB-242084 resulted in a marked reduction of radioactivity at the choroid plexus, hippocampus, and amygdala suggesting that brain regional uptake of [C-11]1 reflected specific 5-HT2CR binding.

Conclusions: We have developed a novel C-11-labeled radioligand [C-11]1 for 5-HT2CR. [C-11]1 exhibited high brain uptake with high specific binding to 5-HT2CR in nonhuman primate brain. [C-11]1 is the first antagonist suitable for in vivo 5-HT2CR PET imaging. This work was funded by a grant from the National Institute of Health (1R21MH108928).

Keywords: 5-HT2CR, PET Imaging, Non-Human Primate

Disclosure: Nothing to disclose.

W67

Intranasal Insulin Decreases Palatable Food Intake, Increases Satiety and Enhances Positive Mood in Women With Obesity: Potential Implications for the Treatment of Obesity

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Background: Appetite control in humans involves complex interactions between homeostatic, reward, cognitive and emotional processes. The hormone insulin has been proposed to play a role in appetite control via modulation of homeostatic and reward systems and has also been shown to improve cognitive function and mood. In particular, insulin acting at receptors in the brain has been shown to reduce food intake and enhance memory. There is also preliminary evidence that central insulin may improve aspects of mood and reduce responses to stress. However, it is unclear whether the effects of central insulin on food intake and mood are dependent upon Body Mass Index (BMI). This is an important question because of the potential for individuals with obesity to show resistance to some central actions of insulin. An effective method to deliver insulin to the brain in humans, in the absence of relevant systemic absorption, is the intra-nasal route of administration. The aim of the present study was to examine the effects of intra-nasal insulin (INI) on appetite and mood in female participants with and without obesity.

Methods: Thirty-five lean (BMI = 22.21 kg/m2 ±1.9; age = 23.7 ±4.8) and seventeen obese women (BMI = 34.0 kg/m2 ±3.38; age = 26.0 ±7.9) participated in the study. Using a randomised, crossover, double-blind, placebo-controlled design, participants received either 160 IU/1.6 mL insulin via a nasal spray (Actrapid®, Novo Nordisk, Bagsværd, Denmark) or placebo in a counterbalanced order. The placebo consisted of water, 2.7 mg/ml m-cresol/ml and 16 mg/ml glycerol. Upon arrival at the laboratory, participants completed visual analogue scale (VAS) ratings of appetite and mood and the Positive and Negative Affect Scale (PANAS). Further appetite and mood ratings were taken throughout the test day. In line with previous findings that INI decreases food consumption in a postprandial state, participants were provided with a sandwich lunch that comprised 40% of daily

energy expenditure and were asked to consume all of it. After lunch, participants self-administered insulin or placebo and 30 minutes later were scanned using fMRI (fMRI scanning results are currently being analysed and therefore are not reported here). Following the fMRI scan, participants were offered a palatable snack of chocolate chip cookies and told they could eat as much as they liked. They then completed various cognitive tests including tasks from the P1vital® Oxford Emotional Test Battery (ETB). The ETB suite used comprised the following tasks: The Emotional Categorisation Task (ECAT) displayed positive and negative self-referent personality descriptors (e.g. "cheerful" versus "hostile") and participants indicated whether they would like or dislike to be referred to as such. In the Emotional Recall Task (EREC) participants were asked to recall as many words as they could remember from the ECAT. Finally, in the Emotional Recognition Memory Task (EMEM) words were re-presented from the ECAT along with new distracter words and participants were asked to report if they had previously seen the word.

Results: There was a main effect of drug administration on cookie intake whereby INI significantly decreased consumption of the cookies ($p < 0.05$) and this effect was more pronounced for the participants with obesity. Analysis of the VAS mood and appetite ratings showed that INI significantly increased ratings of fullness and happiness ($p < 0.05$). INI also significantly increased PANAS positive, but not negative, mood ratings and this effect was greater for the participants with obesity ($p < 0.05$). All participants were more accurate in the ECAT test after administration of INI regardless of the valence of the descriptors ($p < 0.05$).

Conclusions: INI decreased the intake of a palatable cookie snack eaten in the absence of hunger and induced positive mood. Most interestingly, these effects of INI were more pronounced in women with obesity than in lean women. Both women with obesity and lean women experienced higher levels of fullness and happiness after INI and were more accurate in the self-categorisation of emotional words. These data demonstrate for the first time that women with obesity may be hypersensitive to the beneficial effects of insulin on appetite and mood. Taken together these novel results suggest that further investigation of the potential therapeutic utility of INI for appetite control in people with obesity is warranted.

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Keywords: Insulin, Appetite, Mood, Obesity, Cognition

Disclosure: P1vital, P1vital Products, Board Member; P1vital, Employee; P1vital, P1vital Products, Stock / Equity

W68

Effects of a 12-Week Exercise or Diet Intervention on the Neuronal Response to Visual Food Cues in Adults With Overweight/Obesity

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Background: Obesity rates are rapidly rising in the United States, giving rise to myriad health and quality-of-life concerns. Weight loss is associated with a reduction in comorbid conditions, such as cardiovascular disease and hypertension. Weight loss can be difficult, however, and preventing weight regain is even more challenging. Understanding the neuronal mechanisms underlying the regulation of energy balance could help to identify strategies for successful weight-loss maintenance. Towards this end, the current study used functional magnetic resonance imaging (fMRI) to examine the neuronal effects of two core lifestyle modification interventions for weight loss: exercise and diet. Specifically, the study aims were to determine the effects of exercise and diet on the neuronal response to visual food cues in adults with overweight/obesity, and how this relates to weight loss.

Methods: Thirty-nine adults with overweight/obesity completed the study. Inclusion criteria included being 21-55 years old and sedentary (< 2 hours planned physical activity per week), with a BMI between 27-40 and weight stability within 5% in the previous six months. After enrolling in the study, measures of body composition were taken, after which participants completed a 3-day, macronutrient-controlled, eucaloric run-in diet immediately prior to completing fMRI scanning while viewing visual stimuli in both fasted and fed states. Visual stimuli consisted of high-calorie foods, low-calorie foods, or non-food objects. The primary contrast of interest compared responses to high-calorie foods vs. non-food objects. After completing these baseline measures, participants were randomized to either a 12-week exercise intervention (treadmill walking, targeted intensity of 75% VO2max, 5 days per week) or a 12-week diet intervention (reduction in energy intake by ~2000 kcal/week to match the energy deficit produced by the exercise intervention). Measures taken at baseline were repeated post-intervention.

Results: Seventeen participants completed the diet intervention (13 female, 4 male; mean +/- SD: age = 40.04 +/- 10.25; baseline BMI = 30.56 +/- 4.0; percent weight lost = 4.26 +/-3.31) and 22 completed the exercise intervention (18 female, 4 male; mean +/- SD: age = 36.79 +/- 9.37; baseline BMI = 29.55 +/- 2.87; percent weight lost = 2.53 +/- 2.92). There were no significant differences between groups in age or baseline BMI ($p > 0.05$), with a trend towards greater percent weight lost in the diet group ($p = .097$). Following the intervention, a significant reduction in response to a meal (fasted > fed) was observed in the diet group in right somatosensory cortex, compared to baseline ($p < .001$, FDR-corrected). This response was not observed in the exercise group, such that the interaction effect was significant ($p = .022$, FDR-corrected). There was no significant correlation between intervention-associated change in neuronal response to a meal and percent weight lost ($p > 0.05$).

Conclusions: Following a 12-week intervention, a greater food-cue-related reduction in response to a meal was observed in a diet group, compared to an exercise group, in the right somatosensory cortex. In previous studies, we have frequently observed increased response to high-calorie foods compared to non-food objects in this region, in both normal-weight and overweight/obese individuals, and have observed blunted response to a meal in individuals with overweight/obesity compared to lean individuals (i.e., less change in response from fasted to fed states). As such, this increased response to a meal following the diet intervention could potentially reflect a "normalization" of function in the context of diet, but not exercise. The observation that effects seem unrelated to weight-loss points to an interesting topic of future study.

Keywords: fMRI, Obesity, Exercise, Diet, Food Cues

Disclosure: Nothing to disclose.

W69

Dopamine D2 Receptor Overexpression in the Nucleus Accumbens Core Indirect Pathway Induces Dramatic Weight Loss During Scheduled Fasting Only in Females

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Background: Anorexia nervosa (AN) is an eating disorder observed predominantly in females that is characterized by hypophagia, weight loss, and compulsive exercise. Increased dopamine D2/D3 receptor binding in the anteroventral striatum has been previously reported in recovered AN patients. Activity-based anorexia (ABA) refers to the extreme hyperactivity, weight loss, and hypophagia exhibited by rodents exposed simultaneously to running wheels and scheduled fasting, and provides a rodent model for maladaptive behaviors in AN.

Methods: We virally overexpressed either dopamine D2 receptors (D2Rs) or EGFP on indirect striatal pathway neurons in the nucleus accumbens (NAc) core (D2R-OENacInd), which endogenously express D2Rs, and tested both sexes in the open field and the ABA paradigm. For the ABA paradigm, all mice underwent 2 days of acclimatization to single housing and constant running wheel access. Then, mice entered the baseline phase (4 days) in which food and running wheels were available continuously. Finally, mice entered the restriction phase (14 days) in which running wheels were continuously available, but food was only available 7 hours daily starting at 0900h. Bodyweight, food intake, and running distance were measured daily. During restriction, mice were removed from the study and sacrificed (termed dropout) after losing 25% of their baseline bodyweight. The number of days until dropout provided a measure of survival. Daily food anticipatory activity was measured as the total distance run on wheels during the 4 hours before food delivery. Additionally, and identical experiment was conducted, but wheels were locked so mice were unable to run.

Results: In the open field, D2R-OENacInd mice showed increased distance traveled. During the baseline phase of ABA, D2R-OENacInd did not alter bodyweight, but increased food intake, and increased wheel running only on the first day of baseline. However, when food was available only 7 hours a day during the restriction phase, female, but not male, D2R-OENacInd mice showed robust reductions in survival and bodyweight irrespective of wheel access. Female D2R-OENacInd mice also showed reductions in food intake during restriction with, but not without, wheel access. During restriction, female D2R-OENacInd mice also showed increased total wheel running activity and food anticipatory activity. The only effect of D2R-OENacInd on male mice during restriction was a small increase in total wheel running, which began late in the restriction phase.

Conclusions: While D2R-OENacInd has little effect on male mice in the ABA paradigm, female D2R-OENacInd mice show dramatic reductions in survival and bodyweight with or without wheel access, and hypophagia during the wheel condition. Our findings reveal a dramatic sex difference in the ability of D2R-OENacInd mice to maintain a sustainable bodyweight when exposed to a fasting schedule.

Keywords: Activity-based Anorexia, Anorexia Nervosa, Food Restriction, D2 Dopamine Receptor, Nucleus Accumbens Core

Disclosure: Nothing to disclose.

W70

Optogenetic Dissection of Contributions of the Nucleus Accumbens Shell to Decision Making Under Risk of Punishment

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Background: The nucleus accumbens shell (NAcSh) is an important component of the neural circuitry mediating some forms of cost/benefit decision making, but its role in decision making involving risk of punishment is unclear. The current experiments addressed this question using circuit- and cell type-specific optogenetic approaches in rats performing a decision-making task involving risk of punishment. Previous work from our labs used an optogenetic approach to show that activity in the basolateral amygdala (BLA) makes different contributions to choice behavior during distinct components of the decision process. Here we used a similar approach to evaluate 1) the roles of BLA-nucleus accumbens shell (NAcSh) projections, and 2) the roles of NAcSh neurons that express D2 dopamine receptors (D2Rs).

Methods: Rats were trained in a risky decision-making task in which they made discrete trial choices between two response levers, one that produced a small, "safe" food reward and the other that produced a large food reward accompanied by varying probabilities of footshock punishment. BLA-NAcSh projections (Experiment 1) or D2R-expressing NAcSh neurons (Experiment 2) were optogenetically inhibited during discrete phases of the trials: deliberation (the time between trial initiation and reward choice); delivery of the 3 possible outcomes (small safe; large punished; large unpunished); and the intertrial interval. In Experiment 1, inhibition was accomplished via transduction of BLA with AAV-halorhodopsin and an optic fiber implanted in NAcSh. In Experiment 2, D2-cre transgenic rats were used in combination with transduction of NAcSh with floxed AAV-halorhodopsin and an optic fiber implanted in NAcSh.

Results: Inhibition of BLA-NAcSh projections during either deliberation or the large, punished outcome increased choice of the large, risky reward (increased risk taking), suggesting that, during both deliberation and evaluation of the large, punished outcome, BLA input to NAcSh is required to bias subsequent choices away from risky options. Inhibition of D2R-expressing neurons during deliberation also increased risk taking, but unlike BLA-NAcSh projections, inhibition during the large, punished outcome had no effect on choice behavior. Inhibition during all of the other trial phases had no effect on choice performance.

Conclusions: These data provide insight into contributions of NAcSh activity to risky decision making. First, the NAcSh receives risk- and reward-related information from the BLA to bias choices toward safer options during deliberation, and to provide negative feedback about punished choices. Further, activity of NAcSh D2R-expressing neurons may signal this information during deliberative processing, the integration of which biases choices toward safer options. Future experiments will investigate whether BLA inputs specifically onto D2R-expressing neurons are critical for this latter process.

Keywords: Optogenetics, Nucleus Accumbens Shell, Basolateral Amygdala, Risky Decision-Making, Punishment

Disclosure: Nothing to disclose.

W71

Specificity and Coordination of Dopamine Release in Different Functional Domains of the Striatum During Habit Formation

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Background: Release of the neuromodulator dopamine into the striatum is implicated in reward learning and formation of habits surrounding rewards. Diverse cortical and thalamic projections

to the striatum delineate several domains that are assumed to have different functions: 1) The ventromedial nucleus accumbens core (NacCore) and shell (NacShell) receive predominantly "limbic" input and govern motivation and early stages of habit learning. 2) The dorsomedial striatum (DMS) receives "associative" input and regulates flexible, goal-directed behavior. 3) The dorsolateral striatum (DLS) receives "sensorimotor" input and regulates inflexible, stimulus-response habits. In addition, a projection from midbrain to striatum releases dopamine at high concentrations and plays a prominent role in shaping the function of these striatal domains. However, the precise information conveyed by striatal dopamine signals, their regional specificity, their temporal stability, and their coordination are under active debate.

Methods: We characterized dopamine release using chronically implantable microelectrodes for fast-scan cyclic voltammetry simultaneously in NacCore, NacShell, DMS, and DLS of rats performing a novel habit-formation paradigm. In this task, animals are "overtrained" to respond for food pellets on a chained (seeking-taking) reinforcement schedule, designed to bias towards habitual responding. Most importantly, the task enables repeated testing of such habit development throughout behavioral training. Thus, this new approach permitted us to investigate dopamine dynamics in real-time resolution across trials, daily sessions, and weeks of behavioral training throughout the evolution from flexible, goal-directed behavior to inflexible, habitual behavior. Furthermore, we assessed coordination of trial-by-trial dopamine release between regional domains of the striatum.

Results: Our findings demonstrate that the different functional domains of the striatum receive highly distinct dopamine signals. These signals were stable throughout behavioral training. Dopamine release in NacCore and NacShell was consistent with a reward-prediction error (RPE) signal, whereas DMS and DLS dopamine signals were less RPE-related. Using our novel task, we were able to reliably track the gradual development of habitual responding across weeks of behavioral training. In rats that developed a food-seeking habit, dopamine release in NacShell and DMS was increased during the onset of food seeking ("distal" responses), whereas dopamine was decreased towards the end of food seeking ("proximal" responses), compared to non-habitual rats. In contrast, NacCore and DLS dopamine did not differ between habitual and non-habitual rats. Trial-by-trial correlation of regional dopamine signals revealed strong coordination between NacCore/NacShell and DMS, but not DLS.

Conclusions: Although we found that all of the sampled striatal domains exhibited reward-related dopamine release, substantial heterogeneity existed across different functional domains with regards to signal features and the encoded information. With regards to the traditionally assumed functions mentioned above, we found that: 1) NacCore and NacShell dopamine signals are prominent and consistent throughout behavioral training and, thus, are by no means restricted to the early stages of habit learning. 2) DMS (and NacShell) dopamine is thought to contribute to flexible behavior, yet, these signals were associated with habit formation. 3) In contrast, DLS dopamine appeared to not contribute to habit formation, thus, strongly deviating from what is generally assumed regarding DLS function. Furthermore, dopamine release in limbic (NacCore/NacShell) and associative (DMS) striatal domains was more coordinated with another one on a trial-by-trial basis compared to coordination between limbic (NacCore/NacShell) and sensorimotor regions (DLS). It is noteworthy, that none of the measured dopamine signals encoded the habit per se, as group differences were found much prior to habit development. Instead, seeking-related variations in NACShell and DMS dopamine signaling appear to be a predisposition that alter individuals' bias to develop habits. Taken together, these findings demand careful future investigation of such heterogeneous dopamine release dynamics and their coordination, specifically also their dysregulation, as these circuits are heavily implicated in a variety of disorders.

Keywords: Dopamine, Striatum, Habit Formation, Fast Scan Cyclic Voltammetry

Disclosure: Nothing to disclose.

W72

Contributions of Gonadal Hormones to Intertemporal Choice in Male and Female Rats

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Background: Intertemporal choice involves decisions among options that differ in both reward magnitude and delay to reward delivery. All else being equal, individuals prefer large over small rewards; however, individuals tend to more readily choose small over large rewards the longer they must wait for the large reward (i.e., the value of the large reward is "discounted" by the delay to its delivery). Individual differences in intertemporal choice can predict life outcomes such as educational attainment and socioeconomic status. Moreover, extreme preferences for either small, immediate rewards (greater "impulsive choice") or large, delayed rewards (reduced impulsive choice) associate with psychiatric disorders. Gonadal hormones have been proposed to contribute to individual differences in intertemporal choice both within and between sexes, but relatively few studies have directly tested this hypothesis. The current study evaluated the contributions of gonadal hormones to intertemporal choice in male and female young adult rats.

Methods: Young adult (4 mo.) male and female Fischer 344 x Brown Norway F1 hybrid rats were trained in an intertemporal choice (delay discounting) task in which they made discrete trial choices between levers that yielded a small, immediate reward (1 food pellet) vs. a large reward (3 food pellets) delivered after a delay period. The delays to large reward delivery increased in blocks of trials across each session (0, 10, 20, 40, 60 s delays). Rats were initially trained on the task, then divided into two groups matched for choice behavior that received gonadectomy or sham surgery. After recovery, rats were re-tested on the task, and data compared both between groups and before/after surgery.

Results: Relative to males, female rats displayed greater impulsive choice and delay intolerance. Ovariectomy in females had no effect on choice behavior. In contrast, castration in males caused an increase in choice of the small, immediate reward (increased impulsive choice) compared to both sham controls and pre-surgical baseline performance. Surprisingly, 5 days of subcutaneous injections of a physiological dose of testosterone (125 µg) in castrated rats did not reverse this effect, suggesting that the effects of castration on impulsive choice are not mediated solely by reductions in circulating testosterone.

Conclusions: Considered together, these data demonstrate a role for testicular but not ovarian hormones in maintaining preference for large, delayed rewards, but that testosterone alone (at least under the conditions tested here) is not sufficient to reproduce this effect.

Keywords: Impulsivity, Decision Making, Delay Discounting, Gonadal Hormones, Testosterone

Disclosure: Nothing to disclose.

W73

Serotonin 1B may Influence Impulsive Action Through Effects on Reward Sensitivity

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Background: The serotonin 1B (5-HT1B) receptor is implicated in a number of psychiatric disorders involving dysregulation of impulsive behavior, including ADHD and substance use, gambling, and conduct disorders. Prior work has shown that the absence of 5-HT1B receptor expression in adult mice (tetO1B model) causes increased impulsivity, specifically in the impulsive action domain.

Methods: Our studies here examine the effects of 5-HT1B on other phenotypes theoretically related to impulsive action, in order to understand the behavioral and neural systems mechanisms of the influence of serotonin on impulsivity. We examined the behavioral effects of 5-HT1B knockdown in both males and females using the tetO1B mouse line in paradigms designed to test motivation, habitual-like behavior, effort-based choice, and reward sensitivity.

Results: Mice lacking 5-HT1B expression in adulthood showed increased motivation as assessed in several operant behavior tasks, including random ratio (about 50% higher than controls) and progressive ratio (about 141% higher than controls) schedules of reinforcement. To test the effect of 5-HT1B on effort-based choices, we used a concurrent choice paradigm in which mice could freely consume reward provided in a dish, or lever press on a random ratio 20 schedule for the same reward. An absence of 5-HT1B receptor expression resulted in increased lever pressing compared to controls. This did not seem to be the result of changes to the formation of habit-like responding because these mice showed normal decreases in lever pressing following satiety-induced devaluation, as well as normal contingency degradation. However, during free feeding, mice lacking 5-HT1B receptors consumed more reward (evaporated milk) but not more chow, suggesting 5-HT1B may influence reward sensitivity. Our current work begins to explore the influences of 5-HT1B on reward sensitivity by measuring intake of freely available evaporated milk, and hedonic responses to sucrose with an automated lickometer apparatus. Preliminary data suggests that mice lacking 5-HT1B show increased reward sensitivity compared to controls. Taken together, these data point to a hypothesis that 5-HT1B may influence behavioral phenotypes such as motivation and impulsivity via changes in reward sensitivity. Finally, to explore whether impulsive action could be increased by increasing reward value on a trial by trial basis, we developed a novel behavioral paradigm based on the classic Go/No-Go paradigm, but altered to include cued trials with high and low reward payouts. Interestingly we find that mice act more impulsively on high-reward trials compared to low-reward trials – responding with faster latencies on Go trials, and more false alarms on No-Go trials when the reward is larger.

Conclusions: Overall, we find that a lack of 5-HT1B receptor expression results in increased reward sensitivity and increased impulsive action. Additionally, increasing reward value results in increases in impulsive action.

Keywords: Impulsivity, Serotonin 1b Receptor, Reward Sensitivity, Incentive Salience, Motivation

Disclosure: Nothing to disclose.

W74

Transcranial Magnetic Stimulation as a Potential Translational Biomarker for Modulation of AMPA Receptor Function

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Background: Translational biomarkers that capture modulation of specific neural circuitry are critical for drug development in neuroscience. To assess the impact of glutamate receptor modulators, assays that capture the result of activated neural circuits are required. However, there has been little progress in identifying and validating neurocircuitry modulation biomarkers for glutamate-based pharmacology. Metrics that reflect glutamate-mediated activation of neural circuits can be obtained by motor cortex transcranial magnetic stimulation (TMS) in rodents and in humans. We used TMS to activate a simple motor cortical circuit as a tool to assess whether TAK-653, an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor positive allosteric modulator (PAM), enhances TMS-evoked motor responses in rodents and humans.

Methods: For the rodent studies, 51 adult male Sprague Dawley rats were administered TAK-653. Blood and brain levels of TAK-653 were assessed in 20 rats at 0.1, 0.3, 1, 8, and 50 mg/kg. In 31 rats, TAK-653 effects on corticospinal excitability were assessed using single-pulse TMS (spTMS) under pentobarbital (25 + 15 mg/kg, i.p., 30 minutes apart) anesthesia. After appropriate depth of anesthesia was confirmed, animals were positioned beneath a figure-of-8 stimulating coil centered on the interocular line to stimulate the bilateral motor cortex, and 3-axis accelerometers were attached to the soles of the hind limbs to record amplitude of motor evoked responses in the form of a mechanomyogram (MMG). TMS took place 75-135 minutes after TAK-653 administration via oral gavage (7 time points, 10 minutes apart). Ten single pulses at 80% maximum stimulator output intensity were applied at each time point. The resultant MMGs were converted to voltage values. For each hind limb, three-dimensional vector amplitudes were calculated post hoc [$\sqrt{(x2+y2+z2)}$] and averaged to generate one MMG value per spTMS.

In humans, spTMS at 120% of baseline resting motor threshold (rMT) was applied to the primary motor cortex to assess the effect of TAK-653 on corticospinal excitability in 24 healthy volunteers. This double-blind, placebo-controlled, three-way crossover study assessed two doses of TAK-653 (0.5 mg and 6 mg). TMS assessments were performed at baseline, as well as 0.5 and 2.5 hours post dosing in all subjects. An a priori criterion for success was set as a statistically significant difference in change from baseline with either dose compared to placebo at 2.5 hours post-dosing for either of two primary endpoints, which were the peak-to-peak amplitude of MEPs and rMT.

Results: The animal study showed a similar increase in MMG amplitude at TAK-653 doses of 0.3 mg/kg and higher (1, 8, and 50 mg/kg) but not at 0.1 mg/kg. The plasma levels of TAK-653 at the effective doses at 2-hour post dose were 5.74 ng/mL or higher, within the range of previous positive behavioral assays. MMG amplitudes at the 125-minute post-dosing time were 587 ± 176 μV (mean + SD) for the vehicle group, 610 ± 334 μV for 0.1 mg/kg, 1167 ± 280 μV for 0.3 mg/kg, 887 ± 133 for 1 mg/kg, 1193 ± 525 μV for 8 mg/kg, and 1086 ± 135 μV for 50 mg/kg. A repeated measures ANOVA revealed an overall significant effect of treatment ($P=0.005$).

The human study showed a statistically significant increase in average peak-to peak MEP amplitude from baseline compared to

placebo at the higher dose of TAK-653 (6 mg) and a smaller, non-significant increase from baseline at the lower dose (0.5 mg) compared to placebo. The placebo group showed a MEP amplitude of 899 ± 693 μV at baseline and 760 ± 537 μV 2.5h post dosing; the 0.5-mg dose showed baseline MEP amplitude of 841 ± 591 μV and post-dosing amplitude of 858 ± 517 μV. The 6-mg dose changed MEP amplitude from 1004 ± 575 μV at baseline to 1102 ± 840 μV post dosing. Compared to placebo, the change from baseline at 0.5 mg did not show a significant difference (least square means; P=0.4328), but was significant for the 6 mg dose (least square means; P=0.0286). There were no significant differences in rMT for any of the groups. Mean (SD) TAK-653 plasma concentration was 46.0 (8.8) ng/mL at 2.5 h postdose of 6 mg. All adverse events were mild to moderate in intensity and no serious adverse events occurred.

Conclusions: TMS studies both in rats and humans showed that corticospinal excitability increased after TAK-653 administration. The lowest effective TAK-653 dose in rats was 0.3 mg/kg at exposures of 5.74 ng/mL and higher. Beyond a threshold exposure required to see a response, we could not demonstrate any continued exposure-response effect with these current data. The human data revealed a dose-dependent effect, as 0.5 mg TAK-653 showed a non-significant increase in MEP amplitude and 6 mg showed a significant increase compared to placebo. This study is the first demonstration of a translational physiological assessment of neural circuitry responses to an AMPA receptor modulator. The data show that TAK-653 has a pharmacodynamic effect downstream to target engagement at similar exposures in rodents and humans. This study validates the use of neurocircuitry biomarkers to capture the impact of pharmacological manipulation of glutamatergic synapses.

Keywords: TMS, AMPA Receptor Positive Allosteric Modulator, Translational Biomarker Development, Biomarker

Disclosure: Takeda, Employee.

W75

Dissecting the Role of Non-Canonical Ventral Pallidal Neurons in Reward Processing

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Background: Impairments in reward processing and related behavior is a core symptom of addiction, chronic pain, and mood disorders. Dysfunction of the ventral basal ganglia, which is comprised of the ventral pallidum (VP) and nucleus accumbens shell (NAcSh) has been implicated in the etiology of affective symptoms in each of these disorders. Canonical basal ganglia models posit that the VP is exclusively an output of the NAc. However, a subpopulation of VP neurons project to the NAcSh, and reward-related neural activity in the VP precedes reward-related activity in the NAcSh. It is completely unknown whether VP terminals in the NAcSh form functional synapses, or whether this pathway modulates reward-related neural activity in the NAcSh or reward behavior. To understand how the basal ganglia coordinates reward behavior in health and disease, it is crucial to elucidate the functional role of the VP-NAcSh pathway.

Methods: To establish the post-synaptic targets and neurochemical identity of the VP to NAc pathway, we first used rabies tracing to identify monosynaptic inputs from the VP originating from genetically-defined spiny projection neurons (SPNs) and interneuron populations in the NAc (n = 6/geneotype; D1-Cre, A2a-Cre, PV-Cre and ChAT-Cre). The VP was then transduced with channelrhodopsin (ChR2) and Patch clamp electrophysiology was used to determine the synaptic connectivity onto genetically defined populations of NAc neurons (n = 68-116 cells/geneotype).

We then used fiber photometry to measure activity of NAc-projecting VP neurons during a reward-consumption task. Briefly, retro-AAV was injected into the NAcShell, Cre-dependent gCamp was injected to the VP and an optic fiber was implanted over the VP (n = 9 gCamp, 9 GFP controls). Photons were collected via a photodetector and aligned to seeking and consumption of a highly palatable reward.

Finally, to determine the effect of NAc-projecting VP neuron activity on reward processing and NAc activity in vivo, we transduced the VP with ChR2, and implanted optic fibers coupled to 16-channel recording electrodes into the NAcSh (n = 12 ChR2, 12 GFP controls). Stimulation of VP terminals in the NAc was paired with reward consumption, or delivered randomly throughout the behavioral session (scrambled control); microstructure of reward consumption and orofacial taste reactivity was analyzed.

Results: We found that NAc-projecting VP neurons preferentially targeted SPNs in the NAcSh. Rabies tracing revealed that seeding from SPNs labeled significantly more VP neurons than seeding from interneurons ($p < 0.0001$). With patch clamp electrophysiology 83.7% of SPNs received monosynaptic GABAergic innervation from the VP, while only 36.5% of interneurons received innervation.

Photometry analysis revealed that NAc-projecting VP neurons were active during the reward consumption (1.8 ± 0.27 z-scores above baseline), but not during reward seeking. Stimulation of the VP terminals in the NAc inhibited firing of MSNs (baseline: 2.2 ± 0.7 Hz, post light-stim: 0.9 ± 0.4 Hz). Finally, stimulation of the VP to NAc pathway prolonged reward consumption (F(3)= 4.77, p -0.016) and increased orofacial hedonic reactions (F(3)=4.49, p = 0.019) to palatable reward. Stimulation did not alter locomotor activity, support self-stimulation or induce a real time place preference.

Conclusions: A pause in firing of NAcSh SPNs is necessary for reward consumption, but the substrate of this pause is unknown. The VP send a sparse projection to the NAcSh, where it preferentially innervates SPNs and inhibits their firing in vivo. Activity of NAc-projecting neurons persists during reward consumption, and stimulation of this pathway increases reward consumption and hedonic value of rewards. Our data suggests that the VP inhibits NAcSh SPNs to promote reward consumption, challenging the canonical view of information flow in the basal ganglia.

Keywords: Basal Ganglia, Synaptic Function, Reward and Aversion, Electrophysiology, Neuromodulation

Disclosure: Nothing to disclose.

W76

Self-Medication With Psychedelic Microdoses Amongst Microdosers With Disorders

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Background: A substantial number of people worldwide suffer from mental health problems during their lifetime. Although standard therapy and prescriptions are often effective, it is known that treatment is not a "one-size-fits-all" cure; many patients experience unbearable side effects, and some never reach remission. In light of this, there has been renewed interest in the therapeutic potential of psychedelic drugs, with preliminary clinical trials using 'regular' full psychedelic doses demonstrating promising results.

Over the last couple of years, the use of low 'micro' doses of psychedelics for health-related purposes has received increased visibility and interest to reduce symptoms of anxiety, depression or pain. Anecdotal evidence suggests that next to regular psychedelic doses, low doses are also effective, and may be more suitable for certain conditions. Nonetheless, empirical evidence regarding the efficacy of microdosing for symptomatic relief is lacking and no scientific effort is made to inquire on a larger scale for which conditions people use psychedelic microdoses and whether they deem this to be more effective than standard treatments (ST) or 'regular' high psychedelic doses (HPD). The present study therefore investigated the self-rated effectiveness (SRE) of microdosing with psychedelics (MDP), compared to ST and HDP.

Methods: An online questionnaire was launched on several websites and fora for four months. In total 63% (N = 3'590) of the people who initially clicked the link consented and were 18 years of age or older and completed the survey. Of them, 7.2% (N = 410) had experience with microdosing and was diagnosed by a medical doctor or therapist with at least one mental or physical disorder. This group was included in the analyses.

Respondents were asked which psychedelics they used to self-medicate with, for which condition and whether they experienced it as effective. The latter was assessed with three questions: 'Do you feel the treatment worked' (Q1), 'did the symptoms disappear' (Q2), and 'did your quality of life improve' (Q3). Binary logistic regression (odds ratio, OR) was conducted to compare the SRE with psychedelic microdoses with ST, and HDP, for the mental and physical disorder diagnoses for each of the three effectiveness questions.

When cell count was less than 10 events per independent variable, no regression was conducted. For each OR, 95% confidence intervals (CIs) are given and statistical significance was set at $p = 0.05$. An OR of 1.5 is defined as small, two as medium, and three as large.

Results: Odds ratio showed that SRE of MDP was statistically higher ($p < 0.01$) compared to that of ST for both mental (OR(Q1) = 2.3; OR(Q2) = 2.48; OR(Q3) = 2.77) and physical (OR(Q1) = 6.14; OR(Q2) = 7.74; OR(Q3) = 4.36) diagnoses. These effects were specific for neurodevelopmental and anxiety disorders. In contrast, SRE of MDP was lower compared to that of higher psychedelic doses for mental disorders such as anxiety and depression (OR (Q1) = 0.15; OR(Q2) = 0.31; OR(Q3) = 0.13; p(OR Q1,2,3) < 0.01), while for physical disorders no difference was shown (OR(Q1) = 0.45, p = 0.27; OR(Q2) = 0.79, p = 0.79; OR(Q3) = 0.25, p = 0.09).

Conclusions: This study demonstrates that SRE of MDP to alleviate symptoms of a range of mental or physical diagnoses is higher compared to ST and lower than HDP. Findings provide a rationale to assess indications of the therapeutic potential of psychedelics using randomized clinical trials in patient populations, and support assessment of effectivity claims of psychedelics, and whether these are dose-related, disorder-specific and superior to ST.

Keywords: Psychedelics, Mental Disorders, Self-Medication with Psychedelics, Microdosing with Psychedelics

Disclosure: Nothing to disclose.

W77

A Randomized, Double-Blind, Controlled, 6-Week Trial to Assess a Novel Digital Intervention Designed to Improve Cognitive Dysfunction as Adjunct Therapy to Antidepressant Medication in Adults With Major Depressive Disorder

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Background: Systematic reviews have demonstrated that a majority of patients with major depressive disorder (MDD) have cognitive deficits on neuropsychological tests and/or report subjective experience of cognitive impairment (Fava et al., 2018), yet few treatment options are available for improving cognition in MDD. Thus, alternative treatments for depression are needed, specifically for those suffering from cognitive impairment. AKL-T03 is an investigational digital therapeutic delivered through a video game-like interface that presents sensory and motor stimuli designed to target the prefrontal-cortex–based cognitive control system. It uses the adaptive algorithm Selective Stimulus Management Engine (SSMETM) to administer a personalized treatment regimen specific to the cognitive deficiency of each individual patient (Anguera et al., 2016).

Methods: Adults aged 25-55 with mild to moderate MDD (HAM-D-17 score ≥14 and ≤22), as defined by the DSM-V, with an objective impairment in cognition as measured with the Brief Assessment of Cognition in Schizophrenia (BACS) - Symbol Coding Test (z-score < 50), enrolled in a double-blind, randomized, controlled study of AKL-T03. The control condition was a digital word game developed to match AKL-T03 for engagement, time on task, and expectation of benefit. Participants were assessed at baseline and then began their assigned treatment for ~25 minutes/day, 5 days/week over six weeks at home, after which they returned to the clinical site for post-treatment assessment. The primary outcome measure was sustained attention as measured by the mean reaction time during the first Half of the Test of Variables of Attention (T.O.V.A. ®) (RTmean-H1) an FDA-cleared objective measure of attention and inhibitory control. Additional measures of cognition (subjective and objective) were evaluated as secondary outcomes.

Results: AKL-T03 (N = 37) showed a significantly greater improvement in RTmean-H1, compared to the control group (N = 37); p =0.005. No further between-group differences were found, however several of the secondary objective measures showed within-group change: Symbol Coding Test (AKL-T03: $p < 0.001$; Control: $p < 0.001$), Trail Making Test A (AKL-T03: $p = 0.001$; Control: $p = 0.005$), Trail Making Test B (AKL-T03: $p = 0.006$). Additional objective (Letter Number Span, Stroop) and subjective (CPFQ, PDQ) measures of cognition did not reach significant difference from baseline. Only two treatment-related AEs were reported in the AKL-T03 group (headache) and no AEs were reported in the control group.

Conclusions: The results of the study suggest that AKL-T03 may be a safe and effective novel digital intervention for the treatment of cognitive dysfunction associated with MDD. AKL-T03 treatment showed significantly greater improvement in sustained attention compared to a control condition, and showed statistically significant improvement from baseline in multiple additional measures of cognition. Further analyses exploring dose effects as well as responder analysis may provide more details regarding the clinical relevance of these results.

Keywords: Neuroplasticity, Cognition, Major Depression, Brain Training

Disclosure: Akili, Consultant, Akili, Advisory Board, VeraSci, Employee

W78

Modeling a Schizophrenia Risk Variant in Human Induced Pluripotent Stem Cell Derived Neurons: SCN2A V1282F Neurons Have Attenuated NA Currents

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Background: Drug discovery, development and translation for central nervous system disorders pose many unique challenges. Neuropsychiatric disorders have been difficult to treat due to the complexity of the disorders and lack of knowledge that we have of the fundamental biology and pathophysiological underpinnings. We aim to better model neuropsychiatric disorders utilizing human genetic sequencing studies and human induced pluripotent stem cells (hiPSCs). Whole exome and genome sequencing studies have identified disease-causing rare risk variants in patients with high effect size. By introducing the risk variants into hiPSCs using CRISPR/CAS9 genome editing tools and differentiating the cells into neurons, we can study how the variant and gene may be contributing to neuropsychiatric disorders in a human system. We chose to investigate variants in the voltage-gated sodium channel SCN2A (Nav1.2) because it harbors the highest number of risk variants identified in patients with autism, intellectual disorder, and schizophrenia. Variants identified in these patients are predicted to be loss of function. We selected the risk variant V1282F because it has been identified in two unrelated individuals with schizophrenia and is a coding variant. We hypothesize V1282F neurons will have attenuated sodium currents and are interested in uncovering the secondary effects this variant.

Methods: CRISPR/CAS9 genome editing tools were used to introduce disorder-associated risk variants into hiPSCs to create risk and protective variant isogenic human cell lines. hiPSCs were differentiated into neurons and matured for two months. Na currents were measured by whole-cell patch clamp.

Results: We generated two hiPSC lines for each genotype: control homozygous protective (G/G), heterozygous (G/T), and V1282F homozygous risk (T/T) and confirmed the hiPSC lines express the pluripotent stem cell transcript NANOG. The hiPSCs were then differentiated into neurons and all lines expressed the neuronal protein MAP2. First, we wanted to determine whether the variant altered expression of SCN2A. We found SCN2A transcript levels were not altered in V1282F neurons compared to control neurons, but we did discover a 2-fold increase in transcript levels of the sodium channel family member SCN8A (Nav1.6) in V1282F neurons compared to control neurons. Next, we wanted to analyze the function of the variant SCN2A protein. We observed a 40% decrease in Na channel current density in the V1282F neurons compared to control neurons, which suggests the variant is altering the function of the channel. Additional morphological, transcriptional, and functional assays are underway to further characterize the V1282F neurons.

Conclusions: By using this approach and model system, we aim to gain a new understanding of how risk variants identified through genome sequencing studies may contribute to neuropsychiatric disorders and gain new knowledge about the genes and mechanisms involved in a human system. Through this work we identified a novel phenotype in SCN2A V1282F risk variant neurons. We observed a significant decrease in Na current in the V1282F neurons compared to control neurons. We predict that SCN8A may be compensating for the decreased activity of the SCN2A V1282F protein due to the increased level of SCN8A RNA observed in the V1282F neurons when compared to control neurons. Overall, we confirmed that a schizophrenia risk variant that was identified through whole exome sequencing does display a phenotype that may contribute to disease. We intend to utilize this model system to investigate how other disease-causing risk variants may contribute to neuropsychiatric disorders as a means to identify potential new therapeutic drug targets and as a platform for drug screenings.

Keywords: Induced Pluripotent Stem Cells (iPSCs), Disease Modeling, SCN2A, CRISPR/Cas9, Schizophrenia

Disclosure: Nothing to disclose.

W79

Evaluation of Depression and Anxiety in a Phase 3, Double-Blind, Placebo-Controlled Trial of the Neuroactive Steroid GABAA Receptor Positive Allosteric Modulator SAGE-217 in Postpartum Depression

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Background: Postpartum depression (PPD) can have short- and long-term impacts on the mother and her family. PPD is one of the most common complications during and after pregnancy, with an average of 11.5% of new mothers across the United States experiencing symptoms of PPD. Dysregulation of GABA, the major inhibitory neurotransmitter of the central nervous system, has been implicated in PPD etiology in animal models and human studies, supporting the examination of positive allosteric modulators (PAMs) of GABA-A receptors (GABA-A-Rs) as potential PPD therapies. In the GABA-A-R δ subunit deficient mouse model of PPD, SGE-516, a synthetic neuroactive steroid GABA-A-R PAM, decreased depression-like behaviors. Brexanolone injection, an intravenous formulation of the neuroactive steroid GABA-A-R PAM allopregnanolone, was recently approved by the FDA for the treatment of adults with PPD, further supporting the relevance of the GABAergic mechanism in PPD. SAGE-217, an investigational, oral neuroactive steroid GABA-A-R PAM with a pharmacology distinct from benzodiazepines was previously examined in a pivotal trial in major depressive disorder, where it achieved clinically meaningful and statistically significant improvements in depressive symptoms versus placebo. SAGE-217 was further examined in this double-blind, randomized, placebo-controlled Phase 3 trial (NCT02978326) in women with PPD.

Methods: This outpatient study was conducted with women, ages 18-45, ≤6 months postpartum, diagnosed with PPD (defined as a major depressive episode with onset in the 3rd trimester or ≤4 weeks postpartum), and a Hamilton Rating Scale for Depression (HAM-D) total score ≥26 at baseline, who were randomized 1:1 to receive either SAGE-217 or placebo capsules for 14 days, with follow-up through Day 45. Day 15 change from baseline in HAM-D total score was the primary endpoint. Change from baseline in HAM-D total score at all other time points, Montgomery-Åsberg Depression Rating Scale (MADRS) scores, and Hamilton Rating Scale for Anxiety (HAM-A) scores throughout the trial were secondary endpoints. MADRS response (decrease in score of ≥50%) and remission (score ≤10) rates were assessed. The difference between treatment groups in change from baseline to post-baseline time points in HAM-D total score was evaluated by an mixed-effects model for repeated measures (MMRM) with treatment, baseline HAM-D total score, baseline antidepressant use, assessment time point, and time point-by-treatment interaction as fixed effects. An unstructured covariance structure was used to model the within-subject errors. The Kenward-Roger approximation was used to estimate denominator degrees of freedom. MADRS and HAM-A scores were evaluated by a similar MMRM, and categorical measures of response and remission were assessed by a general estimating equation model. P-values for secondary endpoints were not adjusted for multiplicity. Adverse event (AE) reporting, standard clinical measures, and the Columbia-Suicide Severity Rating Scale were used to assess safety and tolerability.

Results: Study drug (SAGE-217 or placebo) was administered to 151 women. SAGE-217 achieved a statistically significant decrease in Day 15 (primary endpoint) least-squares (LS) mean HAM-D total score versus placebo (-17.8 vs. -13.6, $p = 0.0028$). Statistically significant improvements in the SAGE-217 group versus the placebo group were observed at Day 3 (unadjusted $p = 0.0252$) and were sustained after study drug cessation through Day 45 (unadjusted $p = 0.0027$). MADRS scores also improved significantly for SAGE-217 compared with placebo from Day 8 (-20.3 vs. -16.3, unadjusted $p = 0.0322$) through Day 45 (-24.8 vs. -19.0, unadjusted $p = 0.0018$). At Days 15 and 21, the MADRS response rate was significantly higher for SAGE-217 versus placebo (Day 15: 73% vs. 48%, unadjusted $p = 0.0045$; Day 21: 70% vs. 52%, unadjusted $p = 0.0421$) and was numerically greater throughout the trial. The MADRS remission rate in the SAGE-217 group was significantly greater from Day 15 (54% vs. 30%, unadjusted $p = 0.0087$) through Day 45 (59% vs. 38%, unadjusted $p = 0.0339$). SAGE-217 demonstrated significant reductions in anxiety versus placebo as assessed by HAM-A from Day 3 (-12.0 vs. -8.9, unadjusted $p = 0.0169$) through Day 45 (-18.6 vs. -13.6, unadjusted $p = 0.0002$). The most common (≥5%) AEs in the SAGE-217 group were somnolence, headache, dizziness, upper respiratory tract infection, diarrhea, and sedation. No increase in suicide ideation signal was detected.

Conclusions: SAGE-217 achieved the primary endpoint of a reduction in depressive symptoms versus placebo as assessed by HAM-D total score change from baseline at Day 15. SAGE-217 treatment resulted in rapid (by Day 3), clinically meaningful, statistically significant, and sustained (beyond study drug dosing through Day 45) reductions in depressive symptoms as assessed by HAM-D total score in this Phase 3, double-blind, randomized, placebo-controlled trial in women with PPD. SAGE-217 was well tolerated, and the HAM-D results were supported by secondary assessments of depression and anxiety favoring SAGE-217 compared with placebo, including MADRS total score, MADRS response rate, MADRS remission rate, and HAM-A. This trial, in conjunction with clinical trials of brexanolone injection and animal studies of neuroactive steroid GABA-A-R PAMs, provides further support for the concept of positive allosteric modulation of GABA-A-Rs as a mechanism for rapid-acting therapy in PPD.

Keywords: SAGE-217, GABA, Postpartum Depression

Disclosure: Sage Therapeutics, Inc., Consultant

W80

Transcranial Magnetic Stimulation (TMS) in Bipolar Depression: An Open Label Safety and Efficacy Trial

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Background: Treatment options are limited for patients with bipolar depression. Data from the STEP-BD study support the notion that antidepressants added to mood stabilizers provide no improvement in outcome but may carry the risk of precipitating a mixed or manic episode. Neurostimulation methods such as transcranial magnetic stimulation (TMS) which can target treatments to depressive episodes may provide an option for bipolar depressed patients.

Methods: An open label 23 patient study (12 women and 11 men) (15 with BP I and 8 with BP II) of the safety and efficacy of TMS in bipolar depression was conducted at Sheppard Pratt Health System and the Mayo Clinic. All subjects met the Mini-International Neuropsychiatric Interview criteria for either bipolar I or II depression. In order to qualify for TMS treatment, subjects had to be on at least one mood stabilizing agent and off all antidepressant medication for at least two weeks. Subjects were followed during their participation using the Montgomery Asberg Depression Rating Scale (MADRS) as the primary outcome measure with secondary outcomes provided by the 17 item Hamilton Depression Rating Scale (HamD-17) and the Clinical Global Impression-Severity and Improvement Scales (CGI-S and CGI-I). Each were done at weekly visits. TMS treatment sessions followed the standard protocol for unipolar depression of 5 days a week treatment at 10Hz for 3000 pulses for up to 7 weeks or until remission as determined by MADRS < 10.

Results: Of the 23 patients who began treatment, 22 completed a course of treatment as defined by at least 30 treatments or meeting remission criteria. One patient with BP I stopped after one week of treatment due to increasing agitation. Of the 22 completers, 20 (91%) met response criteria (50% decrease in baseline MADRS score) and 15 (68%) met remission criteria. Patients with BP I were more likely to meet remission criteria than BP II patients (73% vs. 63%). Of the remitters, 11 of 15 (73%) met remission criteria by week 5 (usual treatment for unipolar depression is 6 to 7 weeks). Including the one patient who stopped treatment, the Cohen's d effect size comparing pretreatment MADRS scores to post treatment is 3.29.

There were no significant adverse events except for the one patient with increased agitation who dropped out after one week and another patient also with bipolar I who paused treatment for 1 week to increase her lithium due to worsening sleep. She completed 7 weeks of treatment as a responder.

Conclusions: The remarkable positive results at two sites suggests that TMS may well be an effective treatment for bipolar depression. The higher response rates than seen in unipolar depression suggest that as bipolar disorder is more likely to be a better biologic target than unipolar depression as unipolar depression likely includes a more heterogenous group of patients with stressful environmental circumstances, personality disorders and psst trauma. This study is being continued to determine duration of response and we hope will lead to interest in a randomized controlled trial to get FDA approval for TMS as a routine intervention for bipolar depression.

Keywords: Bipolar Depression, Repetitive Transcranial Magnetic Stimulation, Clinical trial, Efficacy and Safety

Disclosure: LivaNova, Neuronetics, Consultant, Neuronetics, Compass Pathways, Grant, Sage Therapeutics, Consultant, Sunovion, Honoraria, Janssen, Consultant, Genomind, Advisory Board

W81

A Role for Serotonin in Reward and Punishment: A Pharmacological fMRI Study

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Background: Psychological and neurocognitive approaches to major depressive disorder (MDD) consider negative cognitive biases to play a key role in the development of depressive symptoms, i.e. depressed mood and loss of interest or pleasure (anhedonia). Selective serotonin reuptake inhibitors (SSRIs) are considered first line treatment for MDD and acute effects of SSRIs in the modulation of negative bias in emotional processing have been described (Harmer et al., 2008). A blunted hedonic response to rewards and an enhanced sensitivity to punishment similarly describe a negative bias in reward processing (Eshel &

Roiser, 2010). Whether and how acute SSRI administration affects reward and punishment processing, however, has not been clarified yet. Here, we investigate whether an acute increase in serotonergic signaling affects reward and punishment sensitivity on a neural level.

Methods: We administered a single oral dose of 20 mg escitalopram (SSRI), or placebo to healthy participants (n = 22; 11 female; mean±SD = 24±2 years) in a double-blind, placebo-controlled, crossover design. We then used functional magnetic resonance imaging (fMRI) during performance of a well-established monetary reward task, which elicits reliable activation in neural reward circuitry (Forbes et al., 2009; Forbes et al., 2010) and was designed to index the neural response towards feedback about win (reward) and loss (punishment). Data were acquired using a Siemens Verio 3 Tesla scanner, equipped with a 32-channel head coil. We pre-processed data with SPM12 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, UCL, London, UK) and Matlab© (The MathWorks Inc., Natick, MA). Pre-processing comprised realignment, slice-time correction, co-registration with the mean anatomical image, normalization to the Montreal Neurological Institute (MNI) space based on the unified segmentation approach (Ashburner & Friston, 2005) and spatial filtering using a Gaussian kernel with 8 mm full width at half maximum. Pre-processed data were analysed using an event-related design in a general linear model (GLM) in order to analyse the neural hemodynamic response to reward and punishment feedback at peak plasma concentration. For each participant and scan, the main effect of task at each voxel was calculated using a t-statistic, generating 3D statistical images for the contrasts of interest (win-neutral and loss-neutral). These first level contrast images were then included in second level whole brain analyses for the feedback phase in a flexible factorial model including the factors subject and drug. Thereafter, statistical analyses were computed using each contrast of interest (win−neutral and loss−neutral) in order to investigate differences between the SSRI and the placebo administration. After using an initial voxel threshold of $p < 0.001$, significant results were obtained with family-wise error (FWE) correction at cluster and peak level with $p < 0.05$.

Results: Mean plasma levels of escitalopram were in the expected range, mean±SD = 26±13 ng/ml. Following placebo administration, we found greater BOLD response in the thalamus (cluster-size k = 273 voxels, Tmax = 7.4, pFWE = 0.014 at peak-level, MNI coordinates: x = 12, y = -26, z = 4) and the anterior caudate (k = 300 voxels, Tmax = 7.4, pFWE = 0.014 at peak-level, MNI coordinates: x=12, y=22, z=2) compared to SSRI administration during punishment feedback (placebo > SSRI, loss-neutral). The reversed analysis (SSRI > placebo) of the loss-neutral contrast did not yield any significant voxels, even without correction for multiple comparisons. Analysis of reward feedback (win-neutral) did not reveal any significant drug effects.

Conclusions: Here, we show evidence of an acute increase in serotonergic signaling to attenuate the neural response to punishment feedback. More specifically, our results suggest that the thalamus and the anterior caudate may play an important role in the serotonergic modulation of aversive signals in reward-related regions. The observed link between acute serotonin transporter blockade and a lower hemodynamic response during punishment feedback indicates potential for this paradigm to be tested as a potential early biomarker for SSRI-response in disorders typically associated with an enhanced sensitivity to punishment, such as MDD, and MDD-associated substance use disorders.

Keywords: Selective Serotonin Reuptake Inhibitors (SSRIs), Reward, fMRI, Serotonin Transporter, Punishment

Disclosure: Nothing to disclose.

W82

Sex Differences in Innate and Adaptive Neural Oscillatory Patterns Predict Resilience and Susceptibility to Chronic Stress in Rats

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Background: Major depressive disorder (MDD) is currently the leading cause of disability worldwide and is two times more prevalent in women than in men. The mechanisms associated with the increased female vulnerability to depression is unknown. Aberrant neural oscillatory activity within the putative depression network is an emerging mechanism underlying MDD. However, sex differences in neural oscillatory activity and its contribution to depression susceptibility remains poorly characterized. We therefore sought to evaluate sex differences in innate and stress-induced neural circuit dysfunction within regions of the depression network and determine whether temporal changes in oscillatory patterns are predictive of the subsequent manifestation of depression-like behaviour.

Methods: Baseline behaviours in the forced swim test (FST), elevated plus maze (EPM), and sucrose preference test were first collected from male and female Wistar rats, after which rats were stereotactically implanted bilaterally with stainless steel electrodes into the prefrontal cortex (PFC), cingulate cortex (Cg), nucleus accumbens (NAc), and dorsal hippocampus (dHIP). Following recovery, baseline local field potential (LFP) recordings were taken for 30 minutes. Rats were then exposed to mild chronic unpredictable stress (CUS), comprised of various non-debilitating and uncontrollable physical and psychological stressors. To elucidate stress-induced changes in circuit function and behaviour LFP recordings were taken three times a week, with FST and EPM behaviours re-assessed weekly. The CUS procedure was stopped once half of the animals (within each sex) exhibited a depression-like phenotype. Stress susceptible animals were characterized by a minimum 60% increase in FST immobility and 20% decrease in sucrose preference. Conversely, animals that did not show more than a 10% increase or 10% decrease in FST immobility and sucrose preference, respectively, from baseline were labeled stress resilient. Females were staged prior to all recordings and behavioural tests with vaginal lavage. Chronux software for MATLAB was used to evaluate the spectral power and coherence at each frequency band within each region with the behavioural outcomes and the stage of the estrous cycle.

Results: At baseline, the majority of sex differences in spectral power were found in the dHIP, with female rats displaying higher theta and reduced high gamma power compared to male rats. Female rats also had higher baseline coherence in the low frequency bands in all regional connections, except the Cg-NAc and PFC-dHIP. Within sex, females subsequently categorized as resilient exhibited higher theta (6-10 Hz) coherence selectively within the dHIP connections, namely the PFC-dHIP (P=0.001), Cg-dHIP ($P < 0.0001$) and NAc-dHIP (P=0.002), compared to females categorized as susceptible. Consistent with the known enhanced female responses to stress, a shorter CUS exposure was sufficient to induce depressive-like behaviour in stress-susceptible females (3 weeks) compared to stress-susceptible males (5 weeks). Both susceptible males and females exhibited a CUS-induced reduction in theta (6-10 Hz) power in the PFC, Cg and dHIP compared to baseline, with only the susceptible females showing reduced NAc theta power. CUS also induced a reduction in theta (6-10 Hz) coherence between the PFC-NAc (P=0.002), PFC-Cg (P=0.028) and Cg-NAc (P=0.03) selectively in susceptible female rats. Resilient females showed increased theta power in dHIP and gamma power in PFC, Cg and NAc, whereas resilient males exhibited a

stress-associated increase in theta (6-10 Hz) coherence between the PFC-NAc (P=0.046) and Cg-NAc (P=0.038). A system-wide increase in high gamma coherence (PFC-NAc: P=0.028, PFC-Cg: P=0.003, PFC-dHIP: P < 0.0001, Cg-NAc: P=0.004, Cg-dHIP: P=0.001, NAc-dHIP: P < 0.0001) was also evident in resilient males only. In females, theta power in all regions and gamma power in the PFC and NAc were negatively correlated to FST immobility time. In males a negative correlation between dHIP high gamma power, as well as PFC-NAc and NAc-dHIP gamma coherence, and FST immobility was seen following stress exposure. These CUS-induced oscillatory changes were time-dependent with female resilient animals exhibiting a baseline elevation in theta coherence within the dHIP pathways, followed by early adaptive resiliency responses to increase theta (dHIP) and gamma (PFC, Cg, NAc) power. Conversely, male resilient animals showed early elevations in global high gamma coherence in response to stress that were followed by region-dependent increases in low frequency power.

Conclusions: These findings identify potential key oscillatory markers of resilience and susceptibility to stress and, further, show that stress exposure induces the sex-dependent temporal recruitment of oscillatory changes within circuits that predict the manifestation of depression-like behaviours in susceptible animals. As oscillatory markers of resiliency in both sexes occurred earlier with stress exposure than the changes observed in susceptible animals, we posit that the presence or absence of innate or stress-induced resilience-signatures is the most critical determinant of an animal's subsequent stress response. Our results highlight that the inclusion of sex as an experimental factor in clinical and preclinical studies should be made a priority if we are to fully elucidate the neuropathology of MDD and develop more effective treatments.

Keywords: Depression, Neural Oscillations, Chronic Unpredictable Mild Stress, Sex Differences

Disclosure: Nothing to disclose.

W83

Ketamine Improves Negative Attitudes Toward Self in Treatment-Resistant Depression

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Background: Major depressive episodes in the course of mood disorders represent a major public health problem and the leading cause of years lived with disability. Preclinical and clinical studies have demonstrated that ketamine has rapid antidepressant effects. In this study, we examined response rate, response predictors and duration of ketamine's antidepressant effect in a sample of highly treatment-resistant patients in a naturalistic setting. In addition, we examined symptom improvements that were most closely related to treatment response.

Methods: We included 26 patients with treatment-resistant major depressive episodes. After clinical assessment, patients received a single infusion of ketamine (0.5mg/kg over 40 minutes). Depression rating scores (MADRS, BDI) were obtained at baseline, after 24 hours and after 7 days.

Results: After 24 hours, the mean MADRS score dropped by 37%. Nine of the 26 patients (35%) displayed a clinical response, defined as an improvement of 50% or more from baseline MADRS score. After 7 days, eight of the nine responders still fulfilled response criteria (89%). Importantly, MADRS score percent change from baseline after 24 hours was highly correlated with MADRS score percent change from baseline after 7 days ($r = 0.81$, $p < 0.01$, two-tailed, $n = 25$). Higher body mass index predicted a better response after 24 hours ($r = 0.4$, $p < 0.05$, two-tailed). Responders and non-responders did not differ significantly in baseline depression severity. Responders improved significantly more than non-responders after 24hours and 7 days on the following BDI-II items: Sadness, Self-Dislike, Self-Criticalness, and Loss of Energy.

Conclusions: In a naturalistic setting, a single infusion of ketamine was less effective than would be expected based on data from randomised clinical trials. This may be due to the extreme treatment resistance of the patients in this study, who were treated at a specialised centre for treatment resistant depression. In 89% of responders, treatment response was maintained after 7 days. This finding argues against high frequency ketamine treatment with several infusions per week. Treatment response was associated with improvements in negative attitudes towards self, suggesting that ketamine has important therapeutic effects on self-attitude.

Keywords: Predictor of Treatment Response, Ketamine, Treatment-Resistant Depression

Disclosure: Ricordati, Lundbeck, Advisory Board, Eli Lilly, Honoraria, Servier, Grant, Vifor, Advisory Board, Sunovion, Drossapharm, Honoraria, Janssen, Advisory Board

W84

Mindfulness Meditation-Based Intervention Modulates Large-Scale Neural Functional Interactions in Major Depression

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Background: Major depressive disorder (MDD) is associated with the largest global burden of any disease. MDD is common, debilitating, and difficult to treat. Mindfulness meditation-based interventions target the development of present moment awareness and non-judgmental acceptance of mental phenomena. Meta-analyses of prior randomized control trials have shown that mindfulness meditation-based interventions reduce depressive symptoms and the likelihood of depressive relapse, and are as effective as front-line evidence-based treatments including cognitive behavior therapy and antidepressant medications. Understanding the biological mechanisms that underlie the therapeutic effects of mindfulness meditation-based interventions for MDD promises to inform the development of treatment biomarkers that track and predict clinical outcomes and thereby reduce the massive burden associated with mental illness. Here, for the first time, we investigated the mindfulness meditation-based modulation of fMRI-measured large-scale functional brain systems in MDD. Our investigation targeted brain systems previously implicated in MDD, that is, the frontoparietal, default, and salience brain systems.

Methods: Adults diagnosed with MDD were randomized to either two weeks of structurally equivalent mindfulness meditation-based training or relaxation control training. The mindfulness training program included three 1.5-hour individual sessions in addition to intensive daily home practice (50 min/day) based on mindfulness-based cognitive therapy (MBCT) that targeted the development of awareness and acceptance of difficult experiences. Relaxation control training included in-person psychoeducational sessions and 50 min/day of "rest" periods in which participants were to deliberately retreat from activities of the day and listen to ambient music. Before and after treatment participants completed the Beck Depression

Inventory-II (BDI-II) to measure severity of depressive symptoms. Participants also completed 8 min of resting-state functional MRI (fMRI). The final dataset included 14 individuals in the mindfulness and 17 in the control group, respectively. FMRI data were preprocessed using common procedures with subsequent seed-based functional interaction analyses. Functional interactions were computed between each seed and every other voxel using Pearson correlations. Seeds included the dorsolateral prefrontal cortex (DLPFC), anterior insular (aINS), and posterior cingulate cortex (PCC) in order to characterize the frontoparietal, default, and salience brain systems, respectively. The treatment-specific modulation of functional interactions by mindfulness meditation was statistically evaluated using a whole-brain-corrected spreading interaction analysis. The spreading interaction analyses specifically tested for significant pre- to post-treatment brain change in the mindfulness group while the control group did not change.

Results: The mindfulness meditation-based intervention was associated with a significant decrease in depressive symptoms over and above the control intervention. Whole-brain spreading interaction analysis indicated that the mindfulness group specifically modulated functional interactions of the DLPFC in bilateral fusiform gyrus (right: 140 voxels, peak voxel MNI coordinates [24, -51, -12]; left: 69 voxels, [-24, -63, -15]) and right angular gyrus (248 voxels, [36, -78, 21]). The interactions were characterized by decreased DLPFC functional interactions from pre- to post-treatment in the mindfulness group while the control group did not change. Whole-brain spreading interactions related to the aINS and PCC were not statistically meaningful.

Conclusions: The current study provides evidence that mindfulness meditation-based interventions for MDD specifically modulate functional interactions of the frontoparietal brain system. Specifically, we found decreases in DLPFC-related functional interactions with several regions involved in the higher-order processing of sensory information and attention, including bilateral fusiform gyri and right angular gyrus. These regions span visual, frontoparietal, and dorsal attention brain systems and are involved in social and emotional cue and attentional processes that are implicated in depressive psychopathology. Moreover, these results are consistent with prior studies of long-term meditators that also showed decreased functional interactions between DLPFC and the visual brain system. In conclusion, results of the current randomized active-control treatment study indicate that mindfulness meditation-based interventions modulate large-scale functional brain systems in MDD. Our findings contribute to a brain-based mechanistic treatment model of mindfulness meditation for MDD.

Keywords: Mindfulness Meditation, Major Depressive Disorder, Functional MRI (fMRI), Resting State Functional Connectivity, Treatment Mechanisms

Disclosure: Vorso Corporation, Consultant

W85

Glucocorticoid Receptors (GR) and its Chaperone Proteins and Target Genes in Prefrontal Cortex of Depressed Suicide Subjects and Normal Control

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Background: Abnormality of HPA function has been implicated in the pathophysiology of suicidal behavior. This is primarily based on the observation of abnormal Dexamethasone suppression test (DST) in patients with depression. Some studies also observe increased CRF in the CSF of depressed patients. Whereas not all depressed patients have abnormal DST, almost all patients who commit suicide have an abnormal DST and it could even be a predictor of suicidal behavior. This abnormality in DST has been related to an abnormal feedback mechanism mediated by glucocorticoid and mineralocorticoid receptors in the brain. Some studies show that GR and MR receptors in the postmortem brain of depressed suicide subjects (DS) are decreased. Teenage suicide is an important public health concern, as about 40,000 teenagers die of suicide in the United States. In an earlier study, we determined the protein and gene expression of glucocorticoid receptors and one of its target genes known as GILZ in the postmortem brain of teenage suicide victims and normal control subjects.

We found that GR mRNA and protein expression was significantly reduced in the prefrontal cortex and amygdala of teenage suicide victims compared to normal control (NC). We also found that target gene GILZ mRNA and protein expression was also significantly reduced in these two areas in DS compared to NC. To further understand and examine the role of GR in suicide we have now studied the mRNA expression of some chaperone proteins such as FKBP5, FKBP4, HSP90, and target genes P23, P11 and Bag 1 in the prefrontal cortex of 24 adult depressed suicide subjects and matched 24 normal subjects.

Methods: Prefrontal cortex (Broadman area 10) was obtained from the brain collection program of Maryland Psychiatric Research Center Baltimore in collaboration with the office of the Chief Medical examiner of the state of Maryland. The subjects were diagnosed using the structural clinical interview for DSM 4. The mRNA expressions of the GR, proteins and target genes, FKBP4, FKBP5, HSP70, HSP90, P23, and P11 were determined in depressed suicide subjects and normal control using the qPCR technique.

Results: We found that one of the isoforms of GR known as Pan-Gr was reduced in the prefrontal cortex of depressed suicide subjects as compared to normal controls in the PFC. We also found that the gene expression of the chaperone proteins FKBP4, FKBP5, Bag 1 and HSP90 was significantly increased in the prefrontal cortex of depressed suicide subjects as compared to normal control subjects. On the other hand, when we compared the gene expression of the target genes, we found that the mRNA expression of P11 and P23 was not significantly changed in the prefrontal cortex of depressed suicide subjects as compared to normal control.

Conclusions: Our results suggest that the mRNA expression of Pan-GR is decreased in the prefrontal cortex of depressed suicide subjects as compared to normal control subjects and the gene expression of the chaperone proteins FKBP4, FKBP5, and HSP90 appear to be significantly changed in PFC of depressed suicide subjects. This study thus suggests that the chaperones, which play an important role in the function of GR, are altered in the PFC of depressed suicide subjects.

Keywords: Suicide, Glucocorticoid Receptor, Prefrontal Cortex, GR Chaperone

Disclosure: Nothing to disclose.

W86

Predicting Relapse After Antidepressant Discontinuation

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Background: The prevention of relapse after achieving remission is an important component in the long-term management of Major Depressive Disorder. Relapse risk is particularly high after

antidepressant medication (ADM) discontinuation, yet no clinical or other predictors are established. Neuroimaging, behavioural and clinical measures may shed light on the mechanisms of relapse and be useful in predicting relapses in advance.

Methods: Two-centre randomized observational study recruited 123 patients who had remitted while taking antidepressant medication and were now intent on discontinuing their medication and 57 healthy control participants. All participants underwent clinical, behavioural and neuroimaging assessment. Patients were then randomized to either discontinue their medication prior to a second assessment or to undergo a second assessment and then discontinue medication. All patients were followed up for 6 months to assess relapses.

Results: Although clinical treatment variables correlated with relapse, and were sensitive to the discontinuation, they did not have out-of-sample predictive power. Resting-state fMRI indicated that abnormalities in functional connectivity may have been normalized by prolonged treatment and remission. However, amongst patients who remained well, discontinuation resulted in an increased functional connectivity between the right dorsolateral prefrontal cortex and the parietal cortex, whereas this decreased in patients who went on to relapse ($Z=5.2$, $p < .001$). An EEG-derived measure of emotional reactivity did not differentiate patients from controls, but strongly differentiated future relapsers from non-relapsers ($d'=0.95$, $p = .004$).

Conclusions: Standard clinical features appear to provide little information to guide decisions about safe antidepressant medication discontinuation. Neuroimaging may provide additional information that may potentially be clinically useful.

Keywords: Relapse Biomarkers, Depression, Computational Psychiatry

Disclosure: Nothing to disclose.

W87

The Gut Microbiome and Psychiatric Disorders: Examining Effects of Antibiotics on Emotional Behavior in Rodents and Humans

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Background: Gut microbiota are essential for healthy gastrointestinal function, but also broadly influence organismal health through effects on the immune and central nervous systems. Several environmental factors can influence microbiome composition, including stress exposure and treatment with drugs such as antibiotics. Stress has well-known negative impacts on emotional behavior and recent evidence suggests that antibiotics, although widely prescribed, may also negatively affect mental health.

Methods: To investigate the potential relationship between antibiotic use and altered emotional behavior, our first study utilized experimental rats that were bred for emotional behavior differences. High Novelty Responder (HR) / Low Novelty Responder (LR) rats were selectively bred based on their locomotor response in a novel environment. Breeding for this trait behavior leads to consistent behavioral differences in rodent emotionality. Here we treated adult male HR and LR rats with an antibiotic cocktail ($n = 8$/treatment/strain) for two weeks prior to behavioral testing and microbiome analysis. Because it was unknown whether HR/LR rats exhibit differences in microbe communities, we used 16S rRNA sequencing to compare the microbiome of HR and LR rats with and without treatment. Our second study is an ongoing retrospective case-control study of patient electronic medical records in the TriNetX database to determine if antibiotics were associated with an increased risk of new mental illness diagnoses. We hypothesized that antibiotic treatment may lead to an increased risk of new mental illness diagnoses regardless of sex. Cohorts were balanced based age, demographics, and past history of medications, procedures, and diagnoses ($N = 70{,}000$-$800{,}000$ before balancing). Outcome measures include new diagnoses of MDD, anxiety disorders, Bipolar Disorder, and Schizophrenia more than one year after the index event. Patients were excluded if they had a history of psychoactive substance abuse or any noncontagious enteritis / colitis. A control comparison of treatment with other anticommensals and risk of mental health diagnoses was also conducted to determine if any observed effects are unique to antibiotics or a result of any disruption of gut microbe communities. Sexes are being analyzed separately to identify sex effects.

Results: In the first study, we found adult HR and LR rats did not exhibit microbiome differences; however, treating them with broad-spectrum antibiotic cocktails reduced microbe community diversity. Antibiotic treatment exacerbated some behaviors, including increasing anxiety-like behaviors in LRs and active coping in both rat strains. Results of the second study are still underway. Preliminary results indicate treatment with antibiotics or anti-commensals are associated with an increased risk for new depression or anxiety diagnoses in females. Females also exhibit a higher risk of sleep disorders following treatment.

Conclusions: The results of this study will help elucidate the relationship between antibiotic-induced dysbiosis and mental health. Findings in the animal model may shed light on how antibiotics alter normal brain function and emotional behavior. The case-control study will be among the first of its kind evaluating the risk of new mental illnesses following antibiotic treatment in a population-based study.

Keywords: Gut Microbiome, Anxiety, Depression, Antibiotics

Disclosure: Nothing to disclose.

W88

Of Mice and Wo/Men: Translating Sex Differences in Cytokine Responses Between Treatment Resistant Depression and Animal Models

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Background: There is a bi-directional relationship between the immune system and major depressive disorder (MDD). Autoimmune diseases and MDD are both more common in women than men. Little is known about mechanisms contributing to the higher incidence of inflammatory and stress-related illness in females.

Methods: We used multiplex ELISA to quantify plasma cytokine protein expression regulated by treatment resistant ($n = 27$) and non-treatment resistant depression ($n = 23$) in men and women compared to age matched (21-55 years) healthy controls ($n = 28$). Depression symptoms were evaluated using the quick inventory of depression symptoms (QIDS) and both factorial analysis and clustering of correlations between cytokines and individual QIDs questions were analyzed. In mice we examined circulating levels of cytokines in male ($n = 34$) and female mice ($n = 28$) exposed to repeated social defeat stress or to 6-day variable stress (male $n = 17$/ female $n = 16$) and 28-day variable stress (male $n = 19$ /female $n = 19$). We examined the relationship between clustering of cytokine profiles to individualized behavior and overall stress susceptibility scores in animal models along with factorial analysis.

Results: We observed greater immune regulation by stress of females compared to males in all animal models. In humans, women with treatment resistant depression had the strongest immune activation and a profile of T cell related cytokines indicative of an autoimmune response ($p < 0.05$). Both women with treatment resistant depression and men with non-treatment resistant depression demonstrated activation an innate immune response suggestive of M1 polarization ($p < 0.05$). We found that patterns of immune activation correlated with specific subsets of symptoms identified by QIDS. We were able to recapitulate a subset of immune activation induced by depression using stress in mice. We identified cytokines that were significantly altered by stress (GM-CSF; $p < 0.01$, IL-6; $p < 0.001$, TNF-a, $p < 0.01$, IP-10; $p < 0.5$, IL-12; $p < 0.05$) and that overlapped with sex specific patterns found in human participants.

Conclusions: There are sex differences in the peripheral immune response to MDD or stress that transcend species. Women with treatment resistant MDD have a sex specific profile of T-cell related immune response suggestive of an autoimmune disease. Sex specific immune responses can be partially modeled using stress paradigms in rodents. Both women with treatment resistant depression and men that are not treatment resistant show activation of cytokines released by the innate immune system. Rodent stress paradigms produced some variation in their regulation of peripheral cytokines. Across both types of stress tested, females produced larger immune responses to stress than males and often drove the main effects of stress. These data demonstrate that not all stressors have the same effect on circulating levels of cytokines. Different animal models for depression may be needed to examine specific immune mechanisms of mood disorders in humans.

Keywords: Sex Differences, Stress, Depression, Cytokines, Behavior

Disclosure: Nothing to disclose.

W89

Novel Mechanisms of Brain-Insulin Resistance in Patients With Major Depression and Acetylcarnitine Deficiency: An in-Vivo Study With the Exosomes Nanotechnology

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Background: The insulin signaling is critical for neuroplasticity and cerebral metabolism in addition to systemic energy metabolism. Unlike the role of systemic insulin action, the understanding of molecular mechanisms of brain insulin signaling in the neurobiology of major depressive disorder (MDD) has been limited to postmortem brain studies. Exosomes are extracellular nanovesicles, secreted from all cells and carrying biological material important for physiological functions, including neuroplasticity and neurogenesis. A key feature of brain-derived exosomes is that they cross the blood brain barrier and express the membrane surface marker, cell adhesion molecule-1 (LCAM-1), which allows their identification and isolation from the plasma of a subject. This is a very new technology that has opened up the possibility to investigate in-vivo molecular mechanisms otherwise inaccessible in humans, overcoming the limitations of postmortem brain studies. Most insights on the role of brain-derived exosomes come from studies on brain tumor and cognitive disorders, however their role in MDD remains to be fully explored.

Methods: 93 subjects participated in this study (64 subjects suffering from MDD and 29 age- and sex- matched controls) and were recruited at the Department of Psychiatry & Behavioral Sciences at Stanford University and the Mood and Anxiety Disorders Program at the Icahn School of Medicine at Mount Sinai. All patients with MDD were in an acute episode during study participation. At both study sites, the psychiatric examination included the Structured Clinical Interview for DSM-IV (SCID) and the psychiatric scale HDRS-21. Brain-derived exosomes were isolated as described in previous papers. Briefly, total circulating exosomes were isolated from plasma by using a precipitation technology and subsequently enriched for brain origin by using of magnetic beads conjuncted with the L1-CAM protein. Assessment of numbers of both total and brain-derived exosomes was performed by using ExoCet based upon a series of standards calibrated at NanoSight. Protein expression in brain-derived exosomes was measured by ELISA. All groups were evenly divided between the experimental plates to account for any inter-plate variability. LAC levels and systemic insulin resistance as assessed by the Homeostatic Model Assessment of Insulin Resistance (HOMA) were measured as we described in previously papers. Two-tailed t-tests, chi-square, Pearson correlations and multiple regression were used as appropriate to specific analyses. We also developed an algorithm in R to test whether integrated measures of central and systemic insulin signaling predicted depression diagnosis (i.e.: discriminate between subjects with MDD and controls).

Results: By using the novel nanotechnology of exosomes, we show an aberrant secretion ($p = 0.036$, effect size=0.62) and cargo of brain-derived exosomes (i.e. LCAM+ exosomes) in subjects suffering from MDD as compared to age- and sex-matched controls. Our new data show an increased in-vivo expression of the insulin receptor substrate-1 (IRS-1) in LCAM+ exosomes ($p = 0.002$) and sex-specific increase in serine phosphorylation of IRS-1 (Men $p = 0.3$, Women $p = 0.02$), independently of psychotropic drug treatment. The degree of phosphorylation of IRS1 reflected the severity of depressive symptoms ($p = 0.02$, $r = 0.4$). In addition, utilizing machine learning, we show that both central and systemic IR predicted depression diagnosis in near 80% of cases. Furthermore, we replicated in this new study cohort the recent discovery of a deficiency of the glutamatergic modulator of brain plasticity and insulin-sensitizing agent acetyl-L-carnitine (LAC) in subjects suffering from MDD.

Conclusions: We report in-vivo evidence for brain insulin resistance (IR) as a possible biological determinant of MDD with sex-specific relationships with severity of depressive symptoms. Furthermore, integrated measures of both central and systemic IR can serve to predict depression diagnosis with a precision of near 80% as showed by machine learning. The current study also shows the utility of exosomes harvested from the blood of a patient in depressive episode and enriched for brain origin as a tool to study in-vivo molecular mechanisms otherwise inaccessible in the human brain. Together with previous findings of decreased levels of the glutamatergic modulator of brain plasticity and insulin-sensitizing agent acetyl-L-carnitine (LAC) in subjects suffering from MDD (a finding replicated in this study cohort), our translational framework suggest that further mechanistic exploration of the link between LAC deficiency and IR (both central and systemic IR) will aim to develop a novel framework of regulation of brain plasticity and personalized medicine strategies to treat MDD.

Keywords: Brain-enriched exosomes, biomarkers, insulin resistance, Acetyl-L-carnitine LAC, glutamate

Disclosure: Alfasigma, Advisory Board.

W90

The Effect of rTMS on the Connectivity Fingerprint

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Background: Repetitive Transcranial Magnetic Stimulation (rTMS) is an FDA approved treatment for depression, yet its mechanism of action remains unknown and treatment response is variable. Studies from the last ten years indicate that it is not local properties of the brain at the stimulation site which matter primarily, but the more distant distribution of its effect via functional or structural connectivities. Our work focuses on understanding this interaction between rTMS stimulation and neural networks. More specifically, we investigated how temporal correlation patterns across different neural components, known as the connectivity fingerprint (Finn et al., 2015), are perturbated by the neuromodulation.

Methods: We acquired structural and resting-state fMRI data at three timepoints in 25 rTMS-treated patients (age: 42 ± 16, 17 F). The first was at baseline, while the second and third were after two and five weeks of treatment. Inclusion criteria were: (1) between 18 and 70 years of age; (2) DSM-IV diagnosis of Major Depression, unipolar without psychotic features, or Bipolar I or Bipolar II Depression without psychotic features; (3) pretreatment 24-item Hamilton Rating Scale for Depression (HAM-D) score$\geq$21. During the study, patients received 3000 stimuli with 4 seconds train of 10 Hz stimulations with 120% of motor threshold over the LDLPFC with Localite Neuronavigation and Magventure Stimulator every weekday for five weeks. The patients' symptoms were assessed with the MADRS at baseline and every week. To model the slope of change, we used hierarchical linear modeling and used this slope as a more robust estimate of the clinical change. MRI scans were collected in a Siemens Prisma 3T scanner utilizing multi-band accelerated echo-planar (EPI) sequence developed at the University of Minnesota and described in detail in the Human Connectome Project (van Essen et al., 2012) (resting state: TR = 720ms, TE = 33.1ms, matrix = 104x90, FOV = 208mm, slice thickness=2mm, voxel = 2x2x2mm, multi-band acceleration factor = 8, two 7-minute 12-second runs with RL and LR phase encoding respectively). To obtain connectivity fingerprints, we first estimated inter-network connectivity across 20 predefined neural networks determined via dual-regression methods (Biswal et al., 2010). This resulted in 190 connectivity pairs across all subjects and timepoints (62 sessions, 17 patients had all three timepoints) and used hierarchical clustering with Euclidean distance and Ward's minimum variance method. In a second step, we subtracted the individual's average connectivity profile (averaged across all timepoints) from each individual's connectivity profile and investigated if this residual was associated with any treatment-related effect.

Results: Hierarchical clustering of the connectivity fingerprint clustered individuals strongly together (Adjusted Rand's index (ARI) = 0.65 with N = 25 groups ($p < 0.001$)). However, this original fingerprint could not separate responders from non-responders or treatment status (ARI < 0.01). In the second analysis we analyzed the residuals. As expected, we could not cluster the individuals, but clustering revealed that TMS-treated timepoints could be separated from baseline (ARI = 0.69 with N = 2 was higher than random labeling; $p < 0.001$). These residual connectivity profiles still correlated more with a general control profile in TP1 than in TP2 or TP3, and progressively became different over time ($p < 0.009$). The greater the difference from baseline, the better the clinical response ($r = 0.55$, $p = 0.02$).

Conclusions: Connectivity fingerprint is highly specific for an individual, and rTMS will not alter its main profile characteristics. However, residual connectivity indicates that TMS alters the connectivity profile that is usually masked by the major individual variance. These treatment-specific connectivity modifications correlated with the clinical response. Future research should determine which neuronal connections drive these changes.

Keywords: Resting State Functional Connectivity, Repetitive Transcranial Magnetic Stimulation (rTMS), Depression

Disclosure: Nothing to disclose.

W91

Amygdala and Caudate Connectivity With the Anterior Cingulate During a First-Manic Episode in Bipolar Disorder: Distinguishing Remitters and Nonremitters Following Eight Weeks of Treatment

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Background: Participants with bipolar disorder, during their first manic episode, showed altered activity in the prefrontal cortex (PFC), including the ventrolateral PFC and the anterior cingulate cortex (ACC), and PFC subcortical and amygdala projection sites, with differences in activity associated with treatment response. To extend these findings to better understand the functional neuroanatomy of bipolar disorder, we investigated functional connectivity among these regions in first-episode manic participants who remitted, compared to those that did not remit, after eight weeks of treatment.

Methods: Participants with bipolar disorder during their first manic episode were recruited (n = 42, Agemean+stdev = 19+5 years, 60% females) and pseudo-randomized to open-label lithium or quetiapine. Participants completed fMRI scans, at baseline and following eight weeks of treatment, while performing a continuous performance task with emotional and neutral distractors. A healthy comparison group (n = 41, Agemean+stdev = 22+6 years, 51% females) received fMRI scans at the same intervals. Participants with bipolar disorder were stratified into those who remitted after eight weeks of treatment (n = 21; total scores on both Young Mania Rating Scale and Hamilton Depression Rating Scales <10 for at least one week at week eight visit) compared to those who did not (n = 21). The amygdala and caudate were defined as seeds and functional connectivity among seeds and the ACC and ventrolateral PFC to emotional distractors was calculated at baseline and following eight weeks of treatment. A 3-group (healthy, remitter, nonremitter) by seed hemisphere (left, right) analysis of covariance was conducted, covarying age and sex, with hemisphere as a repeated within-subject factor and baseline connectivity between the ACC and the amygdala or caudate as the dependent variables. Parallel models were conducted with baseline connectivity among seed regions and the ventrolateral PFC as the dependent variable. Significance was defined as $p < 0.05$, corrected (standard Bonferroni correction for four models). Change overtime in connectivity (week eight minus baseline) was also calculated among seed regions and the vlPFC and ACC, with parallel models repeated with change in connectivity as the dependent variable to investigate connectivity trajectories association with treatment response.

Results: At baseline, nonremitters showed a loss of negative connectivity between the right ACC and bilateral amygdala seed regions and increased positive connectivity between the right ACC

and bilateral caudate seed regions, compared to both the remitters and healthy participants. Remitters did not significantly differ from healthy participants. Changes in ACC connectivity following treatment was not observed between groups.

Conclusions: These results provide evidence of alterations in ACC-caudate and ACC-amygdala functional connectivity in people with bipolar disorder during a first manic episode, and specifically in those who do not remit following eight weeks of treatment.

Keywords: Bipolar Disorder, fMRI Functional Connectivity, Predictor of Treatment Response

Disclosure: Nothing to disclose.

W92

A Molecular Rationale for n-3 PUFA Augmentation of Antidepressant Action

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Background: Epidemiological, biomarker, and some clinical trials suggest that eicosapentaenoic acid (EPA)-enriched n-3 fatty acids ameliorate depressive symptoms for some patients with major depressive disorder (MDD). However, we do not know who will benefit from n-3 treatment, which symptoms might improve, nor what dose is required for a clinical response. We hypothesized that overweight-obese patients with depression and high inflammatory markers would be most likely to benefit from n-3 therapy.

Methods: This 2-site (MGH-Emory), UG3-funded study recruited subjects with MDD, IDS-C scores ≥ 25, body mass index (BMI) > 25 and hs-CRP levels > 3 mg/L. Sixty-one subjects were randomized to oral placebo, 1 g, 2 g, or 4 g of EPA daily for 12 weeks. Capsules contained a total of approximately 1000 mg n-3 fatty acids, with an EPA:DHA ratio of 4:1. The primary endpoints were a sustained (weeks 8 &12) decrease in level of plasma IL-6 or LPS-stimulated peripheral blood mononuclear cell TNF-α levels (effect size [ES] > 0.4) for n-3 vs. placebo. Secondary endpoints were a decrease in mean IDS-C scores with ES > 0.35 for any dose of EPA vs. placebo and a sustained response with ES > 0.35 for any dose of EPA vs. placebo. Exploratory measures included changes in hs-CRP, gene expression of IL-6 & TNF-α, LPS-stimulated IL-6 production and plasma TNF-α levels.

Results: Neither reductions in plasma IL-6 nor LPS-stimulated TNF-α levels met our pre-specified response criteria. The IDS-C total score reduction also did not meet the pre-specified response criteria; however, subjects randomized to 2 g and 4 g EPA daily were more likely than placebo to achieve sustained response (at both week 8 and 12) on the IDS-C, with odds ratios (OR) of 2.3 and 3.4 respectively (exceeding the hypothesized ES >0.35). Exploratory analysis of hs-CRP levels demonstrated a statistically significant decrease over time with the 4 g dose and a a consistent pattern of decrease in CRP with increasing EPA dosage; at treatment week 12, the OR for having at least 25% decrease in hs-CRP exceeded the criterion 0.40 ES level with 2g or 4g daily EPA vs placebo. At treatment week 12, the OR of having at least a 25% decrease in LPS-stimulated IL-6 or in gene expression of IL-6 was above the criterion 0.40 ES level for all 3 doses of EPA relative to placebo.

Conclusions: While we did not find that EPA-enriched n-3 fatty acid supplements met our prespecified response criteria in plasma IL-6 or stimulated TNF-α levels, exploratory analysis indicate that n-3 may modulate specific inflammatory biomarkers in MDD, and higher doses of n-3 may be related to a sustained clinical response in a subset of patients with inflammatory depression.

Keywords: Inflammation, Lipids, Antidepressants

Disclosure: Nothing to disclose.

W93

Cardiac-Related Brain Pulsatility in Adolescents With Bipolar Disorder is Elevated in White Matter and Under-Responsive to Acute Aerobic Exercise

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Background: The increased risk and premature onset of cardiovascular disease (CVD) among adults with bipolar disorder (BD) is not fully explained by traditional CVD risk factors. Studying adolescents with BD can inform on early factors that underlie a possible brain-heart link. We investigated cardiac-related brain pulsatility, a proxy of arterial stiffness, assessed from resting state functional magnetic resonance imaging (MRI) at baseline and following a session of acute aerobic exercise.

Methods: 54 adolescents (27 BD; 27 controls) underwent MRI scanning before and 20 minutes after moderate-intensity recumbent cycling. Five minutes of task-free blood oxygenation level dependent (BOLD) images were collected pre- and post-exercise. Images were retrospectively sorted based on the position in the cardiac cycle. A Fourier series modeled pulsatility in each voxel using a non-parametric statistic. We tested for group differences in the proportion of grey and white matter regions that showed BOLD pulsatility. Subsequent group differences were also performed by voxel-wise analysis.

Results: BD adolescents had a significantly higher proportion of pulsatile voxels in both grey and white matter ($p < 0.022$) relative to controls and after controlling for pulse pressure and body mass index. There were no voxel-wise group differences in BOLD pulsatility when considering baseline-only data; however, post-exercise, there were marked pulsatility differences in grey and white matter clusters, notably the base of the brain where larger arteries tend to reside, the basal ganglia, and parietal-occipital white matter regions.

Conclusions: Cardiac-related BOLD pulsatility was more prominent in BD adolescent than controls at rest and post-exercise. At a voxel-wise level the group differences were only apparent when considering the post-exercise session, as the exercise challenge tended to elicit changes in controls that were muted for BD. The localization of these differences were predominantly subcortical grey and white matter regions. Intracranial pulsatility may be indicate of vascular dysfunction that contribute to BD and thus this metric could serve as a target for interventions designed to bring about cerebrovascular changes.

Keywords: Bipolar Disorder, Cardiovascular Physiology, Adolescent

Disclosure: Nothing to disclose.

W94

Functional Connectivity Biomarkers of Emotion Regulation That Distinguish Risk for Bipolar Versus Unipolar Depression in Clinically Asymptomatic High-Risk Youth

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Background: Although major depressive disorder (MDD) and bipolar disorder (BD) have distinct interventions and outcomes, they are clinically indistinguishable during the depressive phase of illness. We lack neurobiological measures that reliably differentiate MDD from BD or that predict the development of emotional dysfunction in youth at risk for these disorders; this has often led to misdiagnosis and ineffective treatment. Indeed, the most common initial presentation for BD in childhood is a depressive episode which, if misdiagnosed or improperly treated, may induce a switch to a manic episode, cycle acceleration, or increased likelihood of a mixed state. Thus, there is a compelling need for clinicians to have precise biomarkers that differentially predict vulnerability for the development of BD versus MDD before illness onset. To advance our knowledge of differential biomarkers for BD versus MDD, we examined longitudinally functional neuroimaging differences in emotion-regulation brain circuitry among never-disordered youth at familial risk for BD, youth at familial risk for MDD, and healthy controls, and examined whether functional connectivity in emotion-regulation circuitry predicted the onset of a mood or other psychiatric disorder.

Methods: 139 adolescents participated in this longitudinal investigation of familial risk for mood disorders. Participants were youth at high familial risk for BD (BD-risk) (n = 43), at high familial risk for MDD (MDD-risk) (n = 46), and low-risk control adolescents with no personal or family history of psychopathology (CTL) (n = 50). At the time of entry into the study, participants were 12.9 +/- 2.7 years old and had no current or lifetime personal history of any psychiatric disorder. Participants were followed longitudinally for 4.3 +/- 2.3 years and completed clinical assessments at baseline and at follow-up. A seed-based resting state fMRI (rsfMRI) approach with rigorous motion correction was conducted in SPM12 using the Conn Toolbox to compare connectivity profiles in the BD-risk relative to MDD-risk and CTL groups in emotion-regulation circuitry, with functionally defined bilateral amygdala and dorsal striatal seeds using peak coordinates from the literature with statistical thresholding of $p < 0.001$ voxel-level and $p < 0.05$ cluster-level FDR-correction and Bonferroni correction for multiple seeds. We then used logistic regression to examine whether functional connectivity in emotion-regulation circuitry predicts psychopathology at the follow-up assessment in at-risk youth.

Results: Youth at risk for BD had significantly greater connectivity between the bilateral amygdala and middle frontal gyrus, between the left amygdala and right ventrolateral prefrontal cortex (VLPFC), and between the right dorsal striatum and right middle frontal gyrus than did youth at risk for MDD and CTL youth. Using logistic regression analyses, baseline functional connectivity between the amygdala and middle frontal gyrus predicted the subsequent onset of a DSM-5 diagnosis of a mood or anxiety disorder in the combined at-risk sample ($p = 0.026$, ß = -11.01). Though assessed, no other psychiatric disorders were present on 4-6 year follow-up.

Conclusions: The present study identified unique brain-based signatures of familial risk for BD and for MDD in never-disordered youth of parents with these disorders. Moreover, functional connectivity between the amygdala and the middle frontal gyrus, a region implicated in both emotion regulation and cognitive control, appears to be an early predictive biomarker of future onset of internalizing psychopathology in youth at familial risk for mood disorders. Thus, study findings highlight key brain regions within emotion regulation circuitry that may serve as targets for novel prevention, early identification. and treatment approaches for at-risk youth.

Keywords: Early Identification of Risk, Children and Adolescents, Mood Disorders, Emotional Regulation, Resting State Functional Connectivity

Disclosure: Nothing to disclose.

W95

(R)-Ketamine Rapidly Ameliorates Decreased Spine Density in the Medial Prefrontal Cortex and Hippocampus of Susceptible Mice After Chronic Social Defeat Stress

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Background: The N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine is one of the most attractive antidepressants since this drug can produce rapid-onset and sustained antidepressant effects in treatment-resistant patients with major depression and bipolar disorder. However, the precise molecular mechanisms underlying ketamine's antidepressant actions remain to be elucidated. Chronic stress paradigms such as chronic social defeat stress (CSDS) and chronic unpredicted mild stress (CUMS) are known to profoundly reduce dendritic spine density and functions in the prefrontal cortex (PFC) and hippocampus; which could contribute to the morphological and functional alterations observed in depressed patients. Notably, ketamine (10 mg/kg, 24 hours post treatment) rapidly ameliorated decreased spine synapse number in the medial PFC (mPFC) in mice after CUMS (Li et al., Biol. Psychiatry 2011). Furthermore, we reported that ketamine (10 mg/kg, 8 days post treatment) and its two enantiomers, (R)-ketamine and (S)-ketamine (10 mg/kg, 8 days post treatment) significantly ameliorated decreased spine density in the mPFC and hippocampus of CSDS susceptible mice (Yang et al., Transl. Psychiatry 2015; Dong et al., Int. J. Neuropsychopharmacol. 2017). Importantly, (R)-ketamine elicited a more potent beneficial effect on decreased dendritic spine density in the PFC and hippocampus of CSDS susceptible mice compared with (S)-ketamine (Yang et al., 2015).

A recent study using single-cell two-photon calcium imaging in awake mice showed that dendritic spine formation in the PFC was required for the sustained antidepressant effects of ketamine but not for its acute antidepressant effects (Moda-Sava et al., Science 2019). At present, there are no reports showing acute (i.e., 3 hours post treatment) effects of ketamine in the decreased spine density in the PFC after CSDS or CUMS. The present study was, therefore, undertaken to examine whether (R)-ketamine can ameliorate the decreased spine density in the mPFC and hippocampus of CSDS susceptible mice.

Methods: Eight-week-old adult male C57BL/6 mice (weight, 20–25 g; Japan SLC, Inc., Hamamatsu, Japan) and male adult CD1 (ICR) mice (n = 30), aged 13-15 weeks (body weight >40g, Japan SLC, Inc., Hamamatsu, Japan) were used. CSDS were performed as previously reported (Yang et al., 2015; Dong et al., 2017; Qu et al., Acta Neuropsychiatr. 2018). Saline (10 ml/kg), or (R)-ketamine (10 mg/kg) was administered intraperitoneally (i.p.) into CSDS susceptible mice. Furthermore, saline (10 ml/kg) was administered i.p. into control (no CSDS) mice. Brain samples 3 hours after a single administration of saline or (R)-ketamine were collected for Golgi-Cox staining. The data shown are the mean ± standard error of the mean (S.E.M., n = 8). Data were analyzed using one-way analysis of variance (ANOVA), followed post-hoc Tukey test.

Results: Spine density in the prelimbic (PrL) region of mPFC, CA3 and dentate gyrus (DG) of hippocampus of CSDS susceptible mice was significantly lower than that of control mice, consistent with previous reports (Yang et al., 2015; Dong et al., 2017; Qu et al., Acta Neuropsychiatr. 2018). In contrast, spine density in the infralimbic (IL) region of mPFC and CA1 of hippocampus of CSDS susceptible mice was not different from control mice. A single injection of (R)-ketamine (10 mg/kg) significantly ameliorated the decreased spine density in the PrL area of mPFC, CA3 and DG of hippocampus of CSDS susceptible mice.

Conclusions: The present study suggests that (R)-ketamine rapidly (<3 hrs) can ameliorate the decreased spine density in the PrL region of mPFC, CA3 and DG of hippocampus of susceptible mice after CSDS, suggesting that rapid-effects of (R)-ketamine on dendritic spine formation are associated with its rapid-antidepressant effects. Further detailed study using single-cell two-photon calcium imaging is needed to confirm the acute effects of ketamine and its enantiomers for spine formation in rodents with depression-like phenotype.

Keywords: Ketamine, Dendritic Spines, Fast-Acting Antidepressant, Medial Prefrontal Cortex, Hippocampus

Disclosure: Patent on R-ketamine, Patent, Self

W96

Reduced Baseline Resting-State Alpha Power Reverses Following P2X7 Inhibition in Major Depression

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Background: Resting state electroencephalography (EEG) studies report abnormalities in alpha power in patients with major depressive disorder (MDD). While alpha (8-13Hz) oscillations are broadly implicated in depression, the direction of the effect has been variable when comparing MDD relative to healthy control (HC) subjects1,2.

Alpha oscillations are dominant under rest conditions and decrease when the brain is engaged in processing (i.e. reflecting desynchronization), thus lower levels of alpha indicate a state of higher excitation. Functionally, alpha reflects a balance between excitatory-inhibitory (E-I) inputs and underlies a mechanism that supports relative stimulus processing (e.g. suppression of irrelevant stimulus processing) and thus supports attention/cognitive functions3,4.

JNJ-54175446 is a selective, brain penetrant P2X7 receptor antagonist being assessed for use in the treatment of mood disorders. Previously we observed effects of JNJ-54175446 on resting state alpha power in healthy volunteers (unpublished finding). In the current study we evaluate resting state alpha power in MDD patients at baseline and post JNJ-54175446.

Methods: Patients with MDD who met DSM-IV or V diagnostic criteria participated in a placebo-controlled 10-day trial using JNJ-54175446. Participants were randomized into treatment group A [10M/16F; Mean age = 36(+SD12); Mean HAMD = 19 (+4); Day 1-600mg JNJ-54175446; Day 2-10- 150mg/day], treatment group B [9M/17F; Mean age = 39(+14); Mean HAMD = 16(+5); Day 1-3-placebo; day 4- JNJ-54175446 600mg; day 5-10- 150mg] or placebo [7M/10F; Mean age = 42(+12); Mean HAMD = 21(+4); Day 1-10- placebo]. A separate P2X7 PET study suggests 80% target engagement at this dose. Resting state EEG data were recorded using the Stat X24 wireless system (ABM) under eyes open (EO) and eyes closed (EC) conditions (5-min each) during each of 3 study timepoints including baseline, day 3 and day 10. HC subjects (n = 138) provided baseline data (EO and EC) for comparison with patients. Absolute and relative power spectral densities (PSDs) were calculated for each 1 second epoch (1-59Hz bins) and were grouped into EEG bandwidths for each sensor site. Alpha power was compared between MDD and HC groups at baseline, and within and between patient groups before and after treatment. Baseline alpha within the MDD groups also was correlated with (1) baseline clinical measures of depression severity (Inventory of Depressive Symptomatology, IDS-30), and (2) with a previously defined emotion processing bias (larger N170 evoked response to sad versus neutral faces5) in the same patient cohort. Statistical analyses were conducted using within- and between- group ANOVA and t-tests, and with correlation analyses. Significance levels are provided in the results section.

Results: Alpha power was higher in the healthy volunteer group compared to MDD patients at baseline in the EO condition but not in the EC condition, and these differences reached nominal statistical significance in prefrontal and central regions ($p < 0.05$, uncorrected). In the MDD group, baseline EO alpha in prefrontal sites was correlated with the magnitude of the emotion processing bias ($r = 0.41$, $p < 0.01$, uncorrected), and negatively correlated with depression severity as assessed by the IDS-30 ($r > -0.32$, $p < 0.01$, uncorrected). The latter finding is consistent with Jiang et al. 20161, where alpha in posterior sites was shown to correlate negatively with depression severity. Within treatment group A, significant increases in EO alpha power were observed following JNJ-54175446 throughout the brain at day 3 and at day 10 ($p < 0.05$, corrected). In treatment group B, no significant change was observed in alpha power on day 3 (i.e. prior to receiving treatment) while increases were observed throughout the brain by day 10 ($p < 0.05$, corrected). No significant difference was observed in the placebo group across the 3 sessions ($p > 0.15$). No between patient group comparisons of alpha power reached statistical significance.

Conclusions: In the MDD patients, alpha power at baseline was lower than alpha power in healthy volunteers in prefrontal and left temporal brain regions. Baseline alpha power correlated with illness severity and with baseline emotion processing biases in overlapping prefrontal areas. Lower alpha power at baseline and correlations with illness severity replicate findings in the literature2,3. The correlation between alpha power and the emotion processing bias is consistent with a role for alpha in the balance between E-I mechanisms that underlie stimulus processing and cognitive functions (i.e. attention). The increase in resting state EO alpha power post-JNJ-54175446 suggests that the reduced levels of alpha seen at baseline are sensitive to P2X7 receptor antagonism. Moreover, together with the baseline correlation between alpha power and the emotion processing bias, the post JNJ-54175446 increase in alpha power may be consistent with the post-treatment reduction in emotion processing bias previously reported in this patient cohort5.

References:

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2. Koo et al., J Neural Trans, 2017
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4. Foster et al., Curr Opin Psych, 2018
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Keywords: P2X7, Clinical trial, Major Depression Disorder, EEG

Disclosure: Janssen Research and Development, Employee

W97

Childhood Adversity in Bipolar Depression is Associated With Higher Serotonin 1A Receptor Binding in the Hippocampus

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Background: Reported childhood adversity (CA) in bipolar disorder (BD) is three times more common than in the general population and predicts a more severe course of BD. Both CA and BD are associated with serotonergic (5-HT) dysfunction,

specifically, upregulation of the serotonin 1A receptor (5-HT1AR). However, there has been no Positron Emission Tomography (PET) study of 5-HT1AR binding in BD patients reporting CA. We hypothesized higher 5-HT1AR binding in BD patients with CA relative to healthy volunteers in the hippocampus, a region sensitive to stress during development, and in the raphe nuclei, since 5-HT1AR in this region are ligand gated ion channel autoreceptors that regulate 5-HT neuron firing and release. Since severity of CA is associated with degree of psychopathology, our secondary hypothesis was that 5-HT1AR binding would be highest in patients reporting greatest severity of CA.

Methods: Thirty-one BD and 29 healthy volunteers underwent [11C]CUMI-101 PET scans to measure 5-HT1AR binding and completed the Childhood Trauma Questionnaire (CTQ) to assess CA. Subjects were defined as healthy ($n = 29$), BD with mild CA ($n = 11$) and BD with severe CA ($n = 20$), based on the CTQ.

Results: 5-HT1AR binding was greater in BD with CA relative to the healthy group in hippocampus ($t56 = 2.86$, $p = 0.006$), primarily driven by higher binding in BD with severe CA relative to healthy volunteers ($t56 = 3.22$, $p = 0.002$). In raphe nuclei, there was no observed difference in 5-HT1AR binding across groups.

Conclusions: This elevated hippocampal 5-HT1AR binding represents a promising biomarker for investigating the potential neurobiological link between CA and BD.

Keywords: Bipolar Disorder, Childhood Adversity, Positron Emission Tomography Imaging

Disclosure: Nothing to disclose.

W98

The In Vitro Effects of Lithium on Telomere Length and Epigenetic Age in Lymphoblastoid Cell Lines From Patients With Bipolar Disorder and Controls

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Background: Bipolar disorder (BD) has been associated with markers of accelerated aging in different tissues, including telomere shortening and epigenetic aging in peripheral blood and post-mortem brains. While the clinical implications of such findings are continuously being identified, the potential anti-aging effects of known mood stabilizers used for the treatment of BD have begun to be explored. Lithium, as one of the most effective and commonly used medications, has been previously shown to alter telomere length under certain conditions. However, its potential effects on epigenetic aging have never been investigated. Moreover, although ex vivo assessments of aging biomarkers in patients' samples are the standard, the use of in vitro models for the mechanistic study of aging in BD has yet to be explored. Based on this, this pilot study aimed to investigate the effects of lithium treatment on telomere length and epigenetic aging in lymphoblastoid cell lines (LCLs) from patients with BD and controls.

Methods: LCLs from 14 patients with BD and 14 healthy controls matched for age, sex, and ethnicity were generated from lymphocytes through Epstein-Barr virus transformation. After 72 hours of stabilization in culture, 0.5 x 106 cells were treated with RPMI-1640 medium containing either 1mM lithium acetoacetate or vehicle for 7 days, with a change of medium after 96 hours in culture. DNA was then isolated from all cells, followed by the assessment of telomere length by real-time quantitative PCR using a relative quantification method and of genome-wide DNA methylation levels using the Infinium MethylationEPIC BeadChip (Illumina). Epigenetic age was estimated using the Horvath method, and a measure of epigenetic aging acceleration was estimated by regressing the predicted epigenetic age on the chronological age of subjects. After confirming normal distribution of all variables with the Shapiro-Wilk's test, data were analyzed by Pearson's correlation tests, paired t-tests (pre- vs. post-lithium treatment), and independent t-tests (BD vs. controls), whenever appropriate.

Results: Pre-treatment telomere length was significantly correlated with the chronological age of all subjects ($r = -0.69$, $p < 0.001$), and was found to be significantly shorter in cells from BD patients compared to controls ($t(22) = 3.006$, $p = 0.007$). While no difference was detected in cells from controls ($p = 0.906$), lithium treatment significantly increased telomere length in cells from patients ($t(11) = -4.43$, $p = 0.001$). As an exploratory analysis, we found no significant effects of lithium on the expression of genes related to telomere length (TERC, TERF1, TERF2, and TERF2IP) in either patients or controls using pre-existing microarray data performed in the same samples (PMID: 28939162). Pre-treatment epigenetic age did not significantly correlate with chronological age in the LCLs ($r = -0.159$, $p = 0.420$) and did not show any differences between both groups ($t(26) = -1.495$, $p = 0.147$). Moreover, epigenetic aging acceleration was not significantly different between patients and controls either pre- ($t(26) = -1.869$, $p = 0.073$) or post-treatment ($t(26) = -1.843$, $p = 0.077$). Lithium treatment had no effect on epigenetic age or epigenetic aging acceleration in cells from BD patients or controls ($p > 0.05$ for both comparisons), although it decreased the expression of the DNA methyltransferase 1 (DNMT1) in controls ($t(13) = 2.508$, $p = 0.026$). No further lithium-induced gene expression differences were found for other enzymes related to the DNA methylation system (DNMT3A, DNMT3B, DNMT3L, TET1, TET2, TET3, TDG, and MECP2). Finally, no correlation was detected between telomere length and epigenetic age or epigenetic aging acceleration in LCLs.

Conclusions: This pilot in vitro study supports the hypothesis of an anti-aging property of lithium based on its effects on telomere length, especially in cells from patients with BD. Our results also suggest that epigenetic age is not an ideal aging marker in LCLs, possibly due to widespread methylation changes induced by the viral transformation process. Since growing evidence suggests an important role for epigenetic aging acceleration in BD, future studies should explore the effects of lithium on this marker in other cell models (preferably untransformed primary cultures), as well as replicate our findings in larger samples sizes and in BD patients divided by their clinical responsiveness to lithium. Finally, the search for novel drugs that can target either telomere length or epigenetic aging and their potential mood stabilizing properties is warranted.

Keywords: Bipolar Disorder, Aging, Telomeres, DNA Methylation, Epigenetics

Disclosure: Nothing to disclose.

W99

Sparse Latent Space Regression (SELSER) for Treatment Prediction in Major Depressive Disorder

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Background: Predicting individual response to an antidepressive therapy allows us to determine whether the therapy would be effective for a particular patient. Here, we designed a sparse latent-space treatment prediction algorithm based on resting-

state electroencephalography (rsEEG). The algorithm was validated on the rsEEG data set from a large placebo-controlled antidepressant treatment prediction study.

Methods: The developed algorithm, referred to as Sparse EEG Latent SpacE Regression (SELSER), optimizes a latent space model that maps the pretreatment rsEEG to the treatment outcome. A set of spatial filters were optimized to linearly transform the multichannel EEG signals in the sensor space to low-dimensional latent signals. A linear regression model was then built to relate the band powers of the latent signals to the treatment outcome. We derived a computationally efficient algorithm for optimizing all the model parameters by directly minimizing the prediction error while preventing the number of latent signals from getting too large to guard against overfitting.

Results: SELSER was applied to data from the Establishing Moderators and Biosignatures of Antidepressant Response in Clinic Care (EMBARC) study. Treatment outcome was pre- minus post-treatment difference in Hamilton Depression Rating Scale (HAMD17) scores. Model performance was tested using 10-fold cross-validation. For the sertraline arm, only alpha signals from the resting eyes open condition were significantly predictive of the observed treatment score changes during cross-validation ($r = 0.60$, root mean square error (RMSE) $= 5.68$, p< 10^-12; permutation test-verified using 1000 permutations $p < 0.001$). When the sertraline-trained model was applied to the placebo arm, however, outcome could not be predicted ($r = -0.03$, RMSE $=$ 9.77, $p = 0.63$), thus demonstrating the specificity of this model for sertraline prediction (Fisher's z test: $z = 4.94$, $p = 8x10^{-7}$).

Conclusions: A novel rsEEG-based latent-space machine learning algorithm was developed for treatment prediction in depression. Results showed that symptom change was robustly predicted in a manner specific for sertraline (versus placebo).

Keywords: Electroencephalography, Depression, Treatment Outcome Prediction, Antidepressant

Disclosure: Nothing to disclose.

W100

Meta-Analysis Identification and Modeling of a Bipolar Disorder Risk Mutation in LRP8 Using Human Induced Pluripotent Stem Cells

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Background: Transcriptomics technologies such as high-throughput next-generation sequencing and microarray platforms provide exciting opportunities to assess the expression of all genes in a genome. But results in individual studies may not be reproducible due to such factors as study design, sample size, technology platform and bioinformatics method. Very large number of studies across disease areas have be published in the past decades. Integrating these studies to provide a unified picture and panoramic view of complex diseases is critical to understand gene function, disease mechanism, and treatment response. Among the 5000+ studies integrated in Janssen disease BodyMap database, we selected 31 bipolar disorder studies of human postmortem brain samples, applied meta-analysis to identify bipolar disease signature genes that are consistently induced or suppressed across the studies. Following identification of disease associate alleles, they can be studied in gene edited human iPS cells; allowing us to gain an understanding the functional consequences of these alleles and their relevance to the cause of disease.

Methods: Since 2012, we have been gathering high-quality transcriptomics studies from both public and internal sources, applying consistent preprocessing, QC, normalization and statistical inference procedures to all studies of the same technology platforms, then integrating them into the Janssen BodyMap database. We have implemented various tools to comprehensively profile targets in individual tissues and cells at baselines, changes in diseases, responses to treatments in human animal models. To understand the effect of disease-linked mutations at the cellular level, Cripsr-Cas9 gene editing was performed to introduce the desired base pair change to produce isogenic cell line containing either the protective or risk alleles.

Results: In a subset of 15 studies using prefrontal cortex samples, we identified a disease signature of 204 genes by r-th ordered p-value (rOP) and random effect model (REM). Further analysis of the signature genes and their enriched pathways revealed strong disease association. In Reelin signaling pathway, both the Reelin ligand and its receptor ApoER2(LRP8) are suppressed. GWAS study revealed strong association of LPR8 rs5174 (R952Q) SNP with bipolar disorder. To determine the effect of this mutation, isogenic iPS cell lines were generated and neurons produced from these lines were characterized. Neurons homozygous for the R952Q allele had significantly increased total Dab1 levels, as has been seen in Reelin and Reelin receptor knockout mice. Response to Reelin treatment in iPS neurons yielded a similar amount of active, phosphorylated Dab1 and Akt, suggesting signaling downstream of Dab1 is unaffected by the mutation, yet ratios of active to total Dab1 are reduced in R952Q lines after Reelin treatment, due to the high expression of unphosphorylated Dab1. Further studies are being conducted to understand gene expression and functional consequences of these changes in total Dab1 in R952Q containing iPS neurons.

Conclusions: Here, through a meta-analysis, we have identified the Reelin pathway and the R952Q mutation in LRP8 as associated with Bipolar Disorder. Modeling of this allele in human iPS neurons has allowed us to understand the consequences of the R952Q mutation at the molecular and cellular level. Future studies will shed light on gene expression differences and functional effects of this LRP8 mutation in human iPS neurons.

Keywords: Reelin, Bipolar Disorder, Induced pluripotent Stem Cells (iPSCs)

Disclosure: Janssen, Employee

W101

Severity of Psychomotor Retardation in Depression is Associated With mRNA Enrichment of Inflammatory and Metabolic Signaling Pathways

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Background: Inflammation affects basal ganglia motor circuits in association with psychomotor retardation, a key symptom of major depressive disorder (MDD). We previously reported associations between plasma concentrations of protein inflammatory biomarkers and motor slowing as measured by a battery of neuropsychological tests probing psychomotor speed in medically-stable, unmedicated outpatients with MDD. Furthermore, a composite score for psychomotor retardation comprised of assessments of both pure motor and cognitive-motor processing speed was correlated with plasma interleukin (IL)-6 concentrations.

Methods: To confirm and extend these relationships between inflammation and motor slowing using gene expression, and to

discover potential novel pathways associated with motor speed, we examined Illumina microarray data in whole-blood from patients with MDD (n = 88).

Results: Linear regression adjusting for age, sex, race, body mass index (BMI), education and depression severity (excluding psychomotor retardation) revealed associations between the psychomotor composite score and 360 gene probes (|r|>0.30, p < 0.01), which were significantly overrepresented in transcriptional networks regulated by nuclear factor (NF)-k-B (35/129 genes, p = 8.34e-98) and the metabolism-related hepatocyte nuclear factor (HNF)-4alpha (54/148 genes, p = 1.62e-153). Immune-related pathways relevant to Toll-like receptor, IL-1, IL-17 and tumor necrosis factor (TNF) signaling, as well as cytoskeleton remodeling and glycolysis-related pathways, were significantly enriched (all p < 0.01) in genes positively associated with motor speed (n = 122/360 transcripts). Transcript origin analysis showed that gene transcripts positively associated with motor slowing were derived primarily from plasmacytoid dendritic cells, natural killer cells, and B cells, whereas transcripts negatively associated with motor slowing were predominantly expressed by T cells. Finally, Gene Set Enrichment Analysis (GSEA), which queries the entire gene set, found that mammalian target of rapamycin (mTOR) signaling pathways were enriched in the slowest compared to the fastest quartile of patients (FDR q<0.25).

Conclusions: These results demonstrate that inflammatory and metabolic signaling and transcriptional control networks in peripheral blood immune cells are involved in the systemic inflammatory responses that impact motor circuits to contribute to behavioral deficits in patients with MDD.

Keywords: Psychomotor Speed, Depression, Inflammation, Cytokines, Gene Expression

Disclosure: Nothing to disclose.

W102

Psilocybin-Assisted Treatment of Major Depressive Disorder: Results From a Randomized Trial

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Background: Major Depressive Disorder (MDD) is a prevalent condition that confers substantial public health burden. Current approved treatments are limited in effectiveness and adherence. Recent evidence suggests that one or two administrations of psilocybin with psychological support produces antidepressant effects in cancer and treatment-resistant depression populations.

Methods: This is a randomized waitlist control trial investigating the immediate and enduring antidepressant effects of two psilocybin administration sessions (20 and 30mg/70kg on sessions 1 and 2, respectively) with nondirective psychological support in patients diagnosed with MDD. Outcome measures include the GRID-Hamilton Depression Rating Scale (GRID-HAMD) scores at Baseline (>17 required for enrollment) and 1- and 4-weeks post psilocybin sessions.

Results: Twenty-one (of 24) participants have completed the intervention and the 1- and 4-week post psilocybin assessments (Mean age=40, SD = 12; female=67%; Mean GRID-HAMD=23, SD = 3.6; Mean Years w/Depression = 21.5, SD = 12.2). There were no differences in mean GRID-HAMD scores between immediate treatment (IT) and delayed treatment (DT) conditions at baseline (MIT=22.9, SD = 3.6; MDT=23.0, SD = 5.6) but there were significant differences between conditions at 1-wk (MIT=7.9, SD = 7.2; MDT=23.8, SD = 5.4; effect size: d=2.5) and 4-wk (MIT=8.4, SD = 5.7; MDT=23.9, SD = 5.6; effect size: d=2.7). The effect sizes for this intervention are more than three-times over the threshold needed to be considered a "large" treatment effect. The proportion of participants in the delayed condition meeting criteria for clinically significant response (>50% decrease in depression scores) or remission (<7 GRID-HAMD) from depression during the delay was 0% compared to those in the immediate treatment condition at 1-wk (62% response, p<.01; 39% remission, p<.05) and 4-wk (62% response, p<.01; 39% remission, p<.05). No serious adverse events were reported within 24 hours of psilocybin administration. Non-serious adverse events (within 24 hours) included headache (n = 18), chest pressure (n = 1), dizziness (n = 1), visual distortion (n = 1), stiffness/soreness (n = 1), and mild controllable repetitive muscle motion (n = 1).

Conclusions: These preliminary data extend previous studies in depressed cancer patients and patients with treatment-resistant depression by suggesting that psilocybin may be efficacious for treatment of MDD in the general population. Future analyses will include long-term follow-up assessment at 3, 6, and 12-months.

Keywords: Psychedelics, Psilocybin, Major Depressive Disorder

Disclosure: Heffter Research Institute, Advisory Board, Usona Research Institute, Grant

W103

Predicting Future Pathology Using ARIMA to Model Longitudinal Time Series Data and to Mark Neural Dysfunction to Emotional Faces in Adults With Pediatric Onset Bipolar Disorder

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Background: Autoregressive Integrated Moving Average (ARIMA) models have been regularly used to forecast longitudinal time series data such as the stock market and the weather for many years and may be useful to model longitudinal clinical data. Patients with Bipolar Disorder (BD) show longitudinal fluctuations in depression and mania over time that may be studied using ARIMA models, which assume that the outcome variable depends on its previous values in the time series. Clustering these autoregressive parameters may provide more nuanced clustering than standard linear models.

The Course and Outcome of Bipolar Disorder in Youth (COBY) study has followed a group of participants with pediatric onset BD for up to 18 years and has amassed demographic data and weekly assessments of clinical risk factors of BD over that time. This study seeks to evaluate the use and clustering of ARIMA models of longitudinal clinical data to identify neural differences that predict future clinical outcomes.

Methods: 64 adults with pediatric onset Bipolar Disorder were used, with mean age (standard deviation) = 26.3 (3.8), 36 female. Weekly assessments of depressive symptoms were collected for up to 18 years prior to and up to 20 months following fMRI scanning, using the Psychiatric Status Rating scale (range 0-6), part of the Adolescent Longitudinal Interval Follow-Up Evaluation (ALIFE). We first used the “forecast” package in R to model weekly self-reported depression severity prior to the scan for each participant individually. Second, using k-means clustering, the autoregressive parameters from the first step were clustered to identify subgroups within the sample. Clustering of the autoregressive parameters was done by comparing the results of 2, 3, 4, and 5 subgroups.

We thirdly evaluated and extracted in SPM 12 at p<.001, k>30 threshold, using a large region of interest (ROI), neural activity to

the main effect of cluster contrast, during an emotional face processing task. This task includes emotional face distractors of fear, happy, sad, and angry. The ROI included dorsolateral, medial, and orbital prefrontal cortex, amygdala, caudate head and body, precuneus, and anterior cingulate cortex. This extracted significant neural activity was then used along with scan day clinical severity, cluster groups, and demographic measures to predict clinical outcome up to 20 months post scan using regularized regression and followed with standard regression for inference.

On scan day, clinical severity measures were collected including the Hamilton Depression Rating Scale (HAMD), Young Mania Rating Scale (YMRS), Mood and Anxiety Symptom Questionnaire (MASQ), Sensation Seeking Scale (SSS), Affective Lability Scale (ALS), Barratt Impulsiveness Scale (BIS), Positive and Negative Affect Scale (PANAS), and State-Trait Anxiety Inventory (STAI).

Results: First, ARIMA parameters were standardized for all participants to (AR=4, I=0, MA=2). Second, the four autoregressive functions optimally produced three groups with each autoregressive function p<.001. Cluster 1 showed consistency in self-reported depression scores over time (n = 28), cluster 2 showed moderate self-reported depression scores (n = 15), and cluster 3 showed severe self-reported depression scores near to scan (n = 16).

Third, the three clusters defined above showed differential activity to the main effect of cluster during the emotional face processing task across all emotions in bilateral precuneus (left: F(2,220)=13.01, p<.001, k=47 and right: F(2,220)=10.41, p<.001, k=37) and bilateral caudate (left: F(2,220)=11.50, p<.001, k=32 and right: F(2,220)=12.44, p<.001, k=58). Cluster 1 generally showing the lowest activity and cluster 3 showing the greatest activity in all four regions.

Finally, the regularized regression analysis showed that SSS, ALS, and right precuneus activity to angry faces were non-zero predictors of the slope of the trajectory of post-scan weekly self-reported depression scores. Together these variables explained 46.8% of the variance in the slope of depression scores after the scan. The three clinical variables explained 36.7% (F(2, 29)= 8.42, p = .001) and right precuneus activity to angry faces added 10.1% (F(3,28)=8.22, p<.001). SSS showed a positive relationship and ALS and right precuneus activity to angry faces showed negative relationships to the outcome.

Conclusions: Our findings, after using and clustering ARIMA models to identify subgroups of participants with pediatric onset BD, suggest that higher precuneus and caudate activity to emotional faces may be scarred effects of recurrent depressions over time in pediatric onset BD. In addition, neural activity specifically to angry faces in right precuneus, along with clinical variables of sensation seeking and affective lability explained a large portion of the variance and may be useful for predicting future depression outcomes in BD. Precuneus activity to angry faces may represent a novel predictive feature of BD depression. These data suggest that ARIMA models are useful for identifying nuanced clinical subgroups that show differential neural activity. Additional testing of ARIMA models in independent longitudinal samples is needed.

Keywords: Childhood Onset Bipolar Disorder, ARIMA Model, Functional Neuroimaging, Facial Emotion Processing, Precuneus

Disclosure: Nothing to disclose.

W104

Resting-State EEG Waveform Complexity as a Predictive Biomarker for Repetitive Transcranial Magnetic Stimulation Treatment Outcomes in Major Depressive Disorder

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Background: Major depressive disorder (MDD) affects approximately 5-6% of the population worldwide. Around one third of patients with major depressive disorder are resistant to pharmacotherapy. Repetitive transcranial magnetic stimulation (rTMS) is a safe and effective treatment for pharmaco-resistant depression. Biomarkers that predict clinical outcomes in depression are essential for increasing the precision of treatments and clinical outcomes. The electroencephalogram (EEG) is a non-invasive neurophysiological test that has promise as a biomarker sensitive to treatment effects. Pilot studies have identified EEG biomarkers with potential predictive values for rTMS treatment outcomes using power spectrum indices (linear) and non-linear metrics (including entropy, complexity, fractal dimension). Several studies suggest nonlinear EEG metrics may characterize specific features underlying the pathophysiology of depression of relevance to rTMS treatment outcomes. Waveform complexity (Cw) is a newly-developed measure of the diversity of waveform patterns found in the signal traced from a single EEG channel. In healthy subjects, greater Cw was better than several other complexity metrics for predicting performance on cognitive tasks. A role for Cw in predicting clinical treatment outcomes has not yet been investigated. We explored the relationship of this novel EEG biomarker (Cw measured at the site of stimulation) to rTMS treatment outcomes for MDD.

Methods: Data from MDD patients who had EEG records and received rTMS treatment at Butler Hospital (N = 79) were analyzed. Resting-state (eyes closed) EEG was collected with an 8-channel montage (FP1, FP2, FPz, F3, Fz, Cz, Pz, Oz), before the first (N = 72) and after final (N = 48) rTMS session. Treatment was delivered as a series of daily sessions (10-Hz or 5-Hz) over 4-9 weeks to the left DLPFC. Waveform complexity (Cw) was calculated on baseline and post-treatment EEG data processed and calculated as described by Parameshwaran et al. Higher Cw values indicate greater diversity of waveforms. Inventory of Depressive Symptomatology [self-report] (IDS-SR) and Patient Health Questionnaire (PHQ-9) scales were used to quantify treatment outcomes. T-tests, pearson correlation matrices, and logistic regressions were used to examine associations of the EEG metric measured at channel F3 (Cw immediately beneath the rTMS delivery site) and treatment outcomes, including percent change from baseline to LOCF scores, categorical responders (LOCF score is <= 50% of baseline score) and remitters (LOCF score < = 14 on IDS-SR or < = 4 on PHQ-9). Statistical significance was determined by two-sided test with p-value < 0.05.

Results: There were no significant baseline F3 Cw differences between sexes or in relation to age. Baseline F3 Cw had no significant correlation with baseline depression severity measured by either scale.

Baseline F3 Cw was positively correlated with PHQ-9 percent change (r = 0.48, p < 0.001) and negatively correlated with post-treatment PHQ-9 score (Pearson correlation r = -0.39, p = 0.001). Mean (+/- SD) baseline F3 Cw was significantly higher among PHQ-9 responders than non-responders (79.2 +/- 10.2 vs 71.2 +/- 17.1, p = 0.03). Categorical PHQ-9 remitters had also significantly higher baseline F3 Cw compared to non-remitters (81.6 +/- 8.0 vs. 73.5 +/- 15.3, p = 0.005). Baseline F3 Cw remained associated with PHQ-9 response (p = 0.01) and remission (p = 0.006) status in logistic regression models controlling sex, age, baseline PHQ-9 score and total number of TMS sessions. Change in F3 Cw from baseline to endpoint (endpoint - baseline) was negatively correlated with change in severity as measured by percent change on PHQ-9 (r = -0.32, p =0.03) and positively correlated with post-treatment PHQ-9 score (r = 0.30, p = 0.04).

Baseline F3 Cw was also a predictor of improvement on the IDS-SR scale (percent change) (r = 0.24, p = 0.04); other tests

(described above) with IDS-SR yielded trend level results in the same direction as those reported for PHQ-9.

Conclusions: It has been hypothesized that temporal structure or waveform in EEG signal may reflect underlying spatiotemporal patterns of neuronal activity or reflect features of the internal state that predict outward behaviors. Cw is a novel EEG complexity metric, greater values of which have been associated with better performance on cognitive tests of pattern recognition ability and deductive reasoning. In a naturalistic MDD treatment sample, we found Cw in channel F3 was not associated with depression severity at pre-treatment baseline, but higher baseline values significantly predicted better rTMS treatment outcomes. Decrease in Cw over time during rTMS significantly correlated with improvement in depressive symptoms. Replication and cross-validation are required to verify this clinical significance.

Keywords: Depression, EEG Biomarkers, TMS, Neural Complexity

Disclosure: Nothing to disclose.

W105

Open Board

W106

Abnormal Hippocampal Macro- and Microstructure in Offspring at High Familial Risk for Depression: Associations With Parental Bonding

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Background: The hippocampus is known to be involved in mood disorders, but it is unclear if hippocampal abnormalities are risk factors for, or consequences of major depressive disorder (MDD). Studying offspring of individuals with MDD who themselves are threefold more likely to develop the disease [1] can elucidate whether hippocampal abnormalities are true risk factors, that is, present before disease development. Furthermore, offspring of individuals with depression often experience more early life adversity (ELA). ELA is associated with poorer hippocampal morphology and function, as well as the development of mood disorders, but it is not always accounted for in studies of the hippocampus and MDD (risk).

We test the contributions of being at risk for depression and ELA on hippocampal morphology, using a longitudinal 30+ year three generational study of offspring at high- and low- familial risk for MDD [1]. The study design and longitudinal battery of clinical, structural and diffusion MRI, and developmental data together allow us to test: (1) whether familial risk for depression is associated with hippocampal macro- (grey matter volume) and microstructural (mean diffusivity) differences; (2) which hippocampal sub-regions contribute most to these differences; (3) whether these abnormalities are observed in at risk individuals regardless of depression status (i.e., are not compensatory developments); and (4) whether measures of ELA mediate associations between being at high risk and hippocampal abnormalities.

Methods: Sample: The sample consists of 118 children, G2, and grandchildren, G3, of probands (G1) with and without major depressive disorder (MDD). G2 and G3 offspring of G1 probands with MDD were classified as high-risk for MDD; those of probands without MDD as low risk [1]. (Gen 2 mean ± SE age at MRI scan: 46.8± 0.89yrs, 53% female; Gen 3, 22.8 ± 0.72 yrs, 39% female).

Imaging: MRI scanning was performed using a GE Signa 3 Tesla whole-body scanner with an 8-channel, phased array head coil. To evaluate volumetric differences, Freesurfer 6.0 was used on T1-weighted structural scans for (para)hippocampal segmentation "FS60" into 12 subfields, of which we evaluated CA1 and CA4 (dentate gyrus) regions. To investigate hippocampal microstructural difference, mean diffusivity (MD), a measure that is thought to reflect cell density, was assessed in CA1 and CA4 subfields. Hippocampal MD was assessed using diffusion MRI with MRtrix analytic pipeline [2].

Measures: Diagnoses were assessed at each wave using the Schedule for Affective Disorders and Schizophrenia (SADS). Parental bonding was measured with the parental bonding index, in which offspring report degrees of care and overprotection received by their mother and father. Using standard cutoffs parenting style for each parent is characterized into one of four groups: affectionate constraint, affectionless control, optimal parenting, neglectful parenting.

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

Results: All analyses were adjusted for sex and age at scan. Bilateral hippocampal volume (left: $\beta = -122.9$ (std. err.: 48.3), $p = 0.01$; right $\beta = -139.5$ (66.0), $p = 0.03$) and bilateral CA1 volumes (left: $\beta =$ -27.3 (std. err.: 11.0), $p = 0.0132$; right: $\beta =$ -31.3 (15.1), $p = 0.038$) were smaller in the high-risk group. When controlling for lifetime MDD, bilateral hippocampal volume (left: $\beta = -100.2$ (std. err.: 51.0), $p = 0.049$; right $\beta =$ -140.9 (69.7), $p = 0.04$) and right CA1 (right: $\beta =$ -33.9 (15.7), $p = 0.03$) volumes remained significantly associated with risk status. However, ever having had depression (lifetime MDD) was not associated with volumes.

Mean diffusivity was significantly increased in the right CA4 of high-risk participants controlling lifetime MDD status (right: $\beta =$ 7.98 x 10-5 (2.83 x 10-5), $p = 0.005$) and marginally increased in the left CA4 (right: $\beta = 6.23$ x 10-5 (3.26 x 10-5), $p = 0.056$).

To investigate the effects of early life adversity, we assessed the association between maternal and paternal bonding and hippocampal structure. Maternal and paternal bonding were each associated with increased right CA4 mean diffusivity (maternal: $\beta =$ -6.8*10-5 (std. err.: 3.3 x 10-5), $p = 0.04$; paternal $\beta =$ -6.5 x 10-5 (2.90 x10-5), $p = 0.02$). Specifically, neglectful parenting was associated with higher mean diffusivity in the CA4, bilaterally.

The association between being at high familial risk for depression and higher CA4 MD was not mediated by parental bonding.

Conclusions: High familial risk for depression is associated with hippocampal differences, namely reduced volume and increased diffusivity, regardless of whether the individual develops the disorder themselves. Poor parental bonding is associated with higher mean diffusivity in CA4, suggesting lower cellular density, but does not mediate the association between risk and CA4 microstructure. These findings indicate that hippocampal differences observed in individuals with depression are not due to atrophy but are a trait and exist even in those high risk offspring without MDD.

References:

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Keywords: Hippocampus, Early Life Adversity, Major Depressive Disorder (MDD), Structural MRI, Translational Research

Disclosure: Nothing to disclose.

W107

Neurotrophic and Neurogenic Effects of an Alpha5-GABAA Receptor-Preferring Positive Allosteric Modulator

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Background: Reduced GABAergic function is a well-replicated finding in depression, schizophrenia, other brain diseases such as Alzheimer's, and during normal aging, with evidence spanning multiple biological scales in live subject and post-mortem brain studies (PMID:28697889). Further studies have shown that GABAergic deficits occur ahead of glutamatergic changes, and that the somatostatin (SST)-positive interneuron subtype is selectively vulnerable (PMID:30449530). SST+ GABAergic interneurons inhibit the distal dendrites of pyramidal neurons, hence gating excitatory input, largely through ionotropic alpha5-containing GABAA receptors (a5-GABAA-R) exclusively located in this cellular compartment in cortical layers and hippocampus. Hence, GL-II-73, a novel imidazobenzodiazepine molecule with preferential efficacy at augmenting a5-GABAA-R function, displays anxiolytic, antidepressant and procognitive efficacies in adult mice, stress models or during normal aging (PMID:31192221). The proposed mechanism is through regulating defective excitation-inhibition balance. In these studies, we began testing whether these behavioral effects extend to restoring neuroplasticity and neurogenic deficiencies observed in stress conditions or during aging.

Methods: Neuroplasticity studies. Young (2 month) and old (22 months) C57B6 mice received water or GL-II-73 treatment in drinking water (30mg/kg) for 2 months. Mice were assessed for working memory deficits using the Y-maze test. In the aging cohort, brains were harvested after chronic treatment (2months) and stained using Golgi-Cox technique ($n = 4$ mice/group; $n =$ ~40 cells/group, 8+ cell/mouse). Dendritic length and spine density were quantified in the prefrontal cortex and hippocampus using a stereological approach (NeuroLucida).

Neurogenic studies. 129S6/SvEvTac mice were chosen for these initial studies, due to their known low neurogenesis levels. 30 8-week old male mice were given GL-II-73 (30mg/kg, p.o.) for 28 days and its effects on all adult hippocampal neurogenesis steps (proliferation of newborn cells, survival and maturation of newborn neurons) were assessed using standard immuno assays against BrdU, KI-67 and DCX. The effects of GL-II-73 were compared to vehicle-treated mice and to fluoxetine-treated animals (18mg/kg/d, p.o.; $n = 10$/group).

Results: Chronic GL-II-73 treatment in adult mice reversed age-related impairment in working memory as well as the age-induced reductions in dendritic length and spine number in both prefrontal cortex and hippocampus ($p<0.05$ vs young; $p<0.05$ vs old-vehicle treated group). Results were mostly observed in apical dendrites where a5-GABAA-R are located.

For neurogenesis, GLII73 increased proliferation of progenitor cells, increased survival of newborn cells, and accelerated maturation of newborn cells in both dorsal and ventral hippocampus ($p<0.05$ versus vehicle treated). Fluoxetine increased proliferation, survival and maturation, as previously shown (PMID: 28218311), but variability in response limited comparative analyses across all groups.

Conclusions: Chronic treatment with GLII73, a novel molecule augmenting a5-GABAA-R function, (1) reverses neuronal atrophy naturally occurring during aging and (2) increases all step of hippocampal neurogenesis in adult mice. Current studies are expending these effects to chronic stress models in male and female mice. These results show that GL-II-73 has potential disease-modifying efficacies in addition to its previously-reported therapeutic efficacies (pro-cognitive efficacy, antidepressant and anxiolytic properties without sedation).

Keywords: Cortical Inhibition, GABA, Neurogenesis, Neurotrophic

Disclosure: Inventor, Patent

W108

Discovery of Rare Variants Associated With Risk for Major Depressive Disorder in 1,748 Veteran Twins by Whole-Genome-Sequencing

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Background: Major Depressive Disorder (MDD) is a complex neuropsychiatric disease with known genetic associations but without known links to rare variation in the human genome. Initial studies, based on SNP-arrays of common alleles, have identified 47 regions associated with MDD among individuals of European ancestry. Here, we report the sequencing of 1,711 whole genomes at 30X mean depth, including 988 monozygotic twins, 632 dizygotic twins, and 91 singletons sequenced in an effort to identify rare genetic variation associated with MDD.

Methods: Sequencing results were hierarchically clustered using 54 quality metrics to identify and remove bias, resulting in 33 samples being excluded. Population structure was characterized via principle component analysis. Specimen organization within the cohort was also used to observe other potential biases, concluding no batch effect was present as a result of collection date, sequencing date, or sample processing. Kinship among twins was quantified and its impact on statistical significance of associations measured via association trials, finding a moderate inflation of test statistics when including twins. Veterans were classified as having MDD based on psychiatric interview diagnostic criteria.

Results: Using previously published genome-wide association summary statistics from one independent study, we calculated a polygenic risk score (PRS) using 13 loci. In preliminary, unadjusted analyses, we found a modestly higher PRS among Veterans with a history of MDD than among those without a history: -0.101 versus -0.112 (p-value 0.17). An initial look at average rare variant burden across loci was found to be 9,320 rare variants per 0.5Mb window, including an average of 174 exonic rare variants per 0.5Mb window within our patient cohort. A deeper exploration of 47 loci is underway.

Conclusions: These preliminary results may represent a replication of the MDD associations in a new independent cohort and provide support for undertaking a deeper investigation of MDD rare variation. These initial investigations together set the stage for discovering new rare variants in this complex and chronic disease and potentially expanding the genetic risk landscape for MDD.

Keywords: Whole Genome Sequencing, Major Depressive Disorder, Twins

Disclosure: Nothing to disclose.

W109

Molecular Adaptations of the Blood-Brain Barrier Promote Depression vs Stress Resilience

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Background: Preclinical and clinical studies suggest that inflammation and vascular dysfunction contributes to the pathogenesis of major depressive disorder (MDD). Chronic social stress alters blood-brain barrier (BBB) integrity through loss of tight junction protein claudin-5 (cldn5) in mice, promoting passage of circulating proinflammatory cytokines and depression-like behaviors. This effect is specific to the nucleus accumbens, a brain region associated with mood regulation, however the mechanisms involved are unclear. Moreover, compensatory responses leading to proper behavioral coping strategies and active resilience are unknown.

Methods: Here we combined behavioral experiments to cell-specific transcriptome-wide gene-level expression profiling, pharmacological manipulations and morphological analyzes. We identified BBB molecular changes associated with resilience vs stress-induced loss of cldn5 following 10-day chronic social defeat stress in male mice. To add translational value to our mouse findings, we confirmed relevance to human depression and antidepressant treatment.

Results: We show that permissive epigenetic regulation of cldn5 expression and low endothelium expression of repressive cldn5-related transcription factors are associated with stress resilience. Region- and endothelial cell-specific whole transcriptomic analyzes revealed molecular signatures associated with stress vulnerability vs resilience and we identified signaling pathways and relevant genes mediating stress susceptibility. Pharmacological inhibition of stress-induced gene expression rescued cldn5 expression in the NAc promoting social interactions and resilience. Importantly, we confirmed these changes in posmortem NAc samples of depressed patients without antidepressant treatment in line with CLDN5 loss. Conversely, deleterious CLDN5-related molecular changes are dampened in treated MDD.

Conclusions: These findings reinforce the importance of considering stress-induced neurovascular pathology in depression and provide novel therapeutic targets to treat this mood disorder and promote stress resilience.

Keywords: Major Depression, Stress Resilience and Susceptibility, Blood-Brain-Barrier, Neurovascular, Epigenetics

Disclosure: Nothing to disclose.

W110

The Development of a Rodent Flanker Task to Establish Neurophysiological-Based Biomarkers of Cognitive Control

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Background: Deficits in cognitive function, such as reward sensitivity and cognitive control, are a common feature of virtually all neuropsychiatric disorders. While perturbations in cognitive control have been studied extensively in humans, it has been challenging to examine these complex processes in laboratory animals. In turn, stagnation in the development of animal-based tasks to assess these processes has impeded the identification and development of innovative treatments for neuropsychiatric disorders. As part of a larger effort to create reliable and valid cross-species assays of cognitive function, we have developed a cross-species version of the Erikson Flanker Task to determine if human and rodent EEG-based biomarkers of cognitive control share common features.

Methods: Using fading and correction procedures combined with touch-sensitive response technology, we trained male and female Long Evans rats to discriminate between detailed photographic stimuli previously validated for use in the Flanker Task across species. Discrimination was deemed successful when the criterion of 70% response accuracy per stimulus type during the session was recorded on two consecutive days. Following training, rats underwent stereotaxic surgery for implantation of surface and depth electrodes for neurophysiology data collection. Following recovery from surgery and the recovery of stable discrimination performance, we conducted a Flanker Task test session during which continuous EEG and LFP data were collected. In parallel studies, continuous EEG data were collected in healthy male and female human subjects tested in an analogous task using the stimuli validated for use in rats.

Results: In human subjects, the task elicited expected interference effects, with incongruent trials associated with longer response times (T-test; $t = 28.14$, $p < 0.0001$, Cohen's $d = 3.60$) and higher error rates (T-test; $t = 14.57$, $p < 0.001$, Cohen's $d = 1.87$) compared to congruent trials. In the EEG, a robust N200 effect was observed, and was larger for incongruent versus congruent trials (ANOVA; $F = 45.59$, $p < 0.001$, $\eta 2p = .40$). On error trials, a robust error-related negativity (ERN) was also observed over frontocentral recording sites in the 0-100ms post-response time window and was larger for errors than for correct trials (ANOVA; $F = 128.95$, $p < 0.001$, $\eta 2p = .68$). In rodents, the task elicited the expected interference effect on accuracy (T-test; $t = 15.99$, $p < 0.0001$) and a non-statistically significant trend toward the expected interference effect on response time (T-test; $t = 1.98$, $p = 0.105$). In the anterior cingulate cortex, errors elicited a larger negativity than did correct responses in the 175-200ms time window (ANOVA; $F = 5.99$, $p = 0.05$).

Conclusions: We have developed a touchscreen-based rodent Flanker Task that elicits similar behavioral performance to human subjects in an analogous task. In the human task, robust neural signatures of conflict monitoring and error processing emerged. Preliminary electrophysiological recordings in rodents highlight event-related potentials that have qualitative similarities to those observed in humans. Data collection is ongoing for in-depth analysis of neurophysiological correspondence across species in response to similar pharmacological challenges. These efforts may establish novel, cross-species, EEG-based biomarkers of cognitive control that will provide strong predictive power to screen therapeutics for the treatment of neuropsychiatric disorders. Ultimately, this work may enable a more effective use of rodents to predict treatment outcomes in humans.

Keywords: Flanker Task, EEG Biomarkers, Cognitive Control

Disclosure: Nothing to disclose.

W111

Psilocybin Administration to Healthy Participants: Safety and Feasibility in a Placebo-Controlled Study

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Background: Treatment-resistant depression (TRD) remains a significant unmet medical need. From an antidepressant drug development perspective, interest has in recent years been directed at compounds with known pharmacology. Psilocybin belongs to a class of drugs referred to as psychedelics ('mind-manifesting'). Psilocybin was isolated from psilocybe mushrooms in 1957 and synthesised in 1958. It is believed that partial agonism at 5HT2A receptors is a key contributor to its biological effects. The compound was used in psychiatric research and psychodynamic orientated psychotherapy from the early 1960s until it became a Schedule 1 substance in the USA in 1970. In recent years, several small studies have shown indications of efficacy of psilocybin in depressive states related to cancer and in TRD. Psilocybin is now in clinical development for TRD in the USA, Canada and Europe. The first study in this clinical development program, reported here, evaluated safety and feasibility in healthy participants. It also explored simultaneous administration of psilocybin in healthy participants and facilitated experiential training of specialised therapists for subsequent studies. The study's primary objective was to assess the short-term effects of psilocybin on emotional processing and cognitive function at Days 7 and 28; these results will be presented later, once all the data has been analysed.

Methods: This was a phase 1, randomised, double-blind, placebo-controlled study to evaluate the effects of 10 mg and 25 mg psilocybin as compared to placebo, in healthy participants, conducted at the Institute of Psychiatry, Psychology and Neuroscience, London, UK. The following assessment tools were administered: Tellegen Absorption Scale (TAS), NEO-Five Factor Inventory (NEO-FFI), Symptom Checklist-90 item (SCL-90), Positive and Negative Affect Schedule (PANAS), Pictorial Empathy Test (PET), Reading the Mind in the Eyes Test (RMET), Social Value Orientation (SVO), Toronto Empathy Questionnaire (TEQ), Scale of Social Responsibility (SSR), Spatial Working Memory (SWM), Rapid Visual Information Processing (RVP), Paired Associates Learning (PAL). Results from these instruments will be presented later. The study planned to recruit 90 participants, aged 18 plus, with no prior psilocybin experience within 1 year of enrolment. Participants were enrolled in the study for 12 weeks following study drug administration. They completed baseline assessments 1 day prior to study drug administration, including assessments of emotional processing and cognitive function. During this visit, they also took part in a 2-hour preparatory group session with the study psychiatrist, lead therapist and chaperones. On Day 0, participants stratified by sex and age (18-35 years old; > 35 years old) were randomised to the study drug (placebo, 10 mg psilocybin, or 25 mg psilocybin, administered orally, in a 1:1:1 ratio.). The sessions lasted approximately 6 hours and were supported by a trained chaperone and were supervised by the study psychiatrist and a lead therapist. The study drug could be administered simultaneously to up to 6 participants. All participants were assessed for safety and asked to complete the PANAS and 5D-Altered States of Consciousness questionnaire (5D-ASC). After the acute effects of study drug administration had subsided, participants returned home, coming back to the clinic the next morning for safety assessments and a discussion about the subjective experience during the session, conducted by study therapists.

Results: 89 participants were recruited, with first participant first visit date 17 August 2018 and last participant last visit date 19 July 2019. Four strata were populated, (males 18-35) n = 24; (males 35+) n = 24; (females 18-35) n = 23; (females 35+) n = 18. 38% of the participants had prior psilocybin experience. A total of 25 dosing sessions were completed, with up to 6 participants per session (2 sessions with 1 participant, 3 with 2 participants, 5 with 3 participants, 11 with 4, 2 with 5, and, 2 with 6). In the simultaneous administration sessions, each participant was supported by an assisting therapist, overseen in the treatment room by a lead therapist. During this study, 46 therapists received experiential training for subsequent studies. Psilocybin induced expected transient psychedelic experiences. The drug was well tolerated and no serious adverse events were reported.

Conclusions: This study demonstrated the safety and feasibility of psilocybin administration to healthy participants in a controlled setting with trained therapists. Additionally, simultaneous administration to up to 6 volunteers was shown to be feasible in this population.

Keywords: Psilocybin, Psychedelics, Emotional Processing, Cognitive Functioning

Disclosure: Shareholder, Employee

W112

Accounting for Effects of Lifetime, Current, and Community Stressors on Depressive Symptoms in Genetic Studies of Depression

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Background: Depression is a heritable disease, with estimates for h2 from 8.7% to above 40%, supporting a strong genetic basis but underscoring a complex interaction with a larger proportion of environmental causes. This has led to a stress-diathesis model arguing that environmental impact on depressive symptoms are primarily mediated through induced stress. These environmental causes may account for the wide range of heritability estimates or highly variable, difficult to replicate genetic findings. Such environmental stressors are not normally incorporated in most genetic studies of major depression as stress is a multifaceted, complex concept involving, but not limited to, lifetime cumulative stressors, community or culturally specific stressors, and current ongoing stressors. The likely biopsychosocial contributions to depression in humans demand a proper account of such environmental stressors to establish a valid genetic-environmental model for major depression. The Old Order Amish and Mennonite (OOA/M) population is a founder population that is an ideal cohort to explore genetic contribution to disease due to relative genetic uniformity and large, well-documented pedigree structures. Another less discussed advantage in studying complex genetic-environmental contributions is that the OOA/M population is also a culture- and community isolate, where some of the environmental stressors maybe be more readily quantified. There has been great interest in the search for key markers in genetic predisposition, an effort that is now intensified by continued emergence of massively large phenotype datasets and genome-wide approaches. A limitation in the search for broadly penetrative exposures across the population is lack of ability to examine stressors that are unique to specific cultural groups and communities. To that end, we examined lifetime traumatic events, current state stress, and culturally specific community stressors related to the specific social and religious norms as in the OOA/M, and tested whether these three types of stressors independently contribute to depression, and if so, whether they may impact the heritability estimate of depressive symptoms.

Methods: OOA/M with large family pedigrees were recruited as part of the Amish Connectome Project. Structured Clinical Interview for DSM-IV was completed to verify lifetime and current psychiatric diagnoses. Depressive symptom severity was

measured by the Beck Depression Inventory (BDI). Current subjective stress level was assessed by the Perceived Stress Scale (PSS); past lifetime traumatic events were accounted by a Lifetime Stressor Inventory (LSI), and finally potential stress from OOA/M specific cultural and religious lifestyle was assess by a Community Life Survey (CLS), a novel 15-item questionnaire developed to evaluate feelings of belonging vs. nonconformity in this community. Heritability was estimated using the variance components method implemented with SOLAR-Eclipse software (http://www.solar-eclipse-genetics.org/). Heritability was calculated for depressive symptoms based on BDI as well as depression diagnosis. Sex and age were used as covariates.

Results: 451 participants (277 female) were included in this analysis: 117 subjects were found to have a diagnosis of a depressive disorder based on SCID-IV. Potential OOA/M specific cultural and religious lifestyle stressors did not differ significantly between patients with MDD and non-depressed controls and did not correlate with BDI in either patients with MDD or in the non-MDD control group (all $p>0.05$). In comparison, current stress level as measured by PSS correlated positively with BDI in non-depressed controls as well as depressed subjects (all $p < 0.01$). Number of severe past lifetime stressors also significantly correlated with BDI in non-depressed subjects ($p<0.05$) but not in those with a depression diagnosis ($p>0.05$). Heritability for BDI and diagnosis of depression were both statistically significant and comparable to the h2 of depression previously reported in the literature.

Conclusions: We tested heritability along with three distinct stress measures on depressive symptoms in a unique population isolate. Our results highlight that depression is significantly heritable in this population but environmental contributions in the forms of stressors can also be meaningfully quantified. We found that longitudinal and current stress, but not culturally specific community stress, significantly contribute to depressive symptoms in the population. Our findings suggest that accounting for the effects of environmental stressors may increase the precision of heritability estimates of depression and present opportunity for more robust genetic studies.

Keywords: Stress and Depression, Heritability of Depression, Lifetime Stress, Genetics of Depression, Human Genetics

Disclosure: Nothing to disclose.

W113

Emotion-Related Network Reorganization Following Fish Oil Supplementation in Depressed Bipolar Offspring: An fMRI Graph-Based Connectome Analysis

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Background: Although previous resting-state functional connectivity studies have observed aberrant connectivity within fronto-limbic networks of patients with bipolar disorder, pathoetiological mechanisms remain poorly understood. We previously reported that depressed adolescents with a biological parent with bipolar I disorder exhibit robust deficits in omega-3 polyunsaturated fatty acids (n-3 PUFA), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Preclinical evidence suggests that n-3 PUFAs promote synaptic maturation and plasticity in developing brain circuits. A neuroimaging study found that developmental n-3 PUFA insufficiency reduced resting-state functional connectivity between the dorsal anterior insula (seed) and ventromedial prefrontal, insula cortex, and the superior temporal sulcus of adult non-human primates. These and other data support a potential link between n-3 PUFA insufficiency and aberrant network connectivity. Graph-based network analysis is a technique used to interrogate topological properties of functional brain networks. Here we used graph-based network analysis to investigate the effects of n-3 PUFA (fish oil, FO) supplementation on emotion-related 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 Not Otherwise Specified 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/I) 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). After preprocessing, matrices of functional connectivity were calculated in 32 regions associated with emotion regulation. The time points corresponding to negative emotion stimuli of each region of interest (ROI) were extracted and concatenated. Adjacent matrices were obtained by calculating the Pearson's correlation coefficients between each pair of ROIs. Global and nodal topological properties were then estimated, and correlational analysis between clinical and topological measurements were performed.

Results: Patients supplemented with FO, but not placebo, exhibited a significant baseline-endpoint increase in erythrocyte n-3 PUFA (EPA+DHA) levels. At the global level, significant group-by-time interactions were observed for local efficiency ($p = 0.01$) and clustering coefficient ($p = 0.01$). At the nodal level, significant group-by-time interactions were observed for degree centrality ($p = 0.002$) and node efficiency ($p = 0.002$) for the left insula. At the edge level, the strength of nine connections exhibited baseline-endpoint increases in the FO group ($p<0.05$, NBS corrected) but not in placebo group, and there was a significant group-by-time interaction for the sum of the strength of nine connections ($p = 0.0003$). Correlation analysis found that increases of the node degree of the left insula was associated with decreases in manic symptom severity scores in the FO group only ($r = 0.45$, $p = 0.04$).

Conclusions: FO supplementation induces significant structural reorganization and degree of communication changes among an ensemble of connections associated with emotional regulation. These results suggest that FO supplementation increases information transferring efficiency and segregation ability in emotion networks, and adds to a growing body of evidence implicating n-3 PUFAs in cortical circuit plasticity.

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

Disclosure: Nothing to disclose.

W114

Elevated Right Ventrolateral Prefrontal-Amygdala Resting-State Functional Connectivity Distinguish Youth at High-Risk for Bipolar I Disorder: A Cross-Sectional fMRI Study

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Background: Youth with established bipolar I disorder exhibit structural and functional abnormalities in ventrolateral prefrontal

cortex (VLPFC)-amygdala (AMY) connectivity. However, it is unknown whether these alterations are present prior to the initial onset of manic symptoms and what factors may contribute to the development of these brain changes. The initial onset of mania typically occurs during adolescence, a developmental period involving functional and structural maturation of VLPFC-AMY connectivity. Although symptoms of ADHD and having a biological parent with bipolar I disorder are significant risk factors for developing bipolar I disorder, central prodromal features and risk biomarkers relevant to symptom progression are poorly understood. Emerging evidence further suggests that youth with bipolar I disorder exhibit deficits in omega-3 polyunsaturated fatty acids (PUFA) which have been implicated in PFC circuit maturation. In the present cross-sectional study, we compared VLPFC-AMY resting-state functional connectivity (RSFC) in ADHD youth with (high-risk) and without (low-risk) a biological parent with bipolar I disorder and a healthy comparison group, and evaluated relationships with omega-3 PUFA levels and ADHD and mood symptoms.

Methods: Three groups of psychostimulant-free adolescents (10-18 years) were recruited: adolescents with ADHD and a biological parent with bipolar I disorder (high-risk), adolescents with ADHD and no family history of bipolar I disorder (low-risk), and healthy adolescents with no personal or family history of psychiatric illness (healthy controls). RSFC fMRI scans were performed, and red blood cell (RBC) fatty acid composition and ADHD (ADHD-RS), mania (YMRS), and depression (CDRS-R) symptom ratings determined. Resting-State echo-planar images were collected using a Philips 3.0 T MR scanner (TR = 2000 ms, TE = 30 ms, Flip angle = 75°). Bilateral AMY were used as the seeds to estimate connectivity with the VLPFC. A mask of the VLPFC was created using the AAL template. Omnibus ANOVA and post hoc t-tests were conducted to explore differences among the three groups. Regions showing group-wise differences in the ANOVA model were used as masks to extract the VLPFC-AMY functional connectivity strength to assess relationships with omega-3 PUFA levels and clinical ratings.

Results: A total of n = 97 male and female (61% male) adolescents (mean age: 14.5±2.5 years) were included in the analysis (healthy controls, n = 33; low-risk, n = 37; high-risk, n = 27). Using the left AMY as the seed, significant group-wise functional connectivity differences with the right VLPFC were observed (voxel $p<0.05$, cluster size = 97). Both high-risk ($p = 0.0015$) and low-risk ($p = 0.029$) groups exhibited greater left AMY to right VLPFC connectivity compared with healthy controls, and high-risk and low-risk groups did not differ from each other. Using the right AMY as the seed, significant group-wise functional connectivity differences with right VLPFC were observed (voxel $p<0.05$, cluster size = 125). The high-risk group exhibited greater right VLPFC-AMY connectivity compared with both healthy controls ($p < 0.001$) and low-risk ($p = 0.017$) subjects, and low-risk subjects did not differ from healthy controls. Among all subjects (n = 97), but not among ADHD patients only, both left and right VLPFC-AMY connectivity were positively correlated with ADHD-RS total scores (left: $r = 0.25$, $p = 0.01$; right: $r = 0.21$, $p = 0.04$), and neither were correlated with YMRS or CDRS total scores. Among ADHD patients (n = 64), left and right VLPFC-AMY connectivity were inversely correlated with the RBC omega-3 PUFA (EPA, DPA, DHA) levels (left: $r = -0.30$, $p = 0.03$; right: $r = -0.28$, $p = 0.04$), and were positively correlated with the ratio of arachidonic acid to eicosapentaenoic acid (AA/EPA) (left: $r = 0.56$, $p<0.0001$; right: $r = 0.58$, $p<0.0001$).

Conclusions: Both high- and low-risk ADHD youth exhibit greater left AMY to right VLPFC RSFC compared with healthy controls. However, high-risk youth also exhibit greater right AMY to right VLPFC RSFC compared with both healthy controls and low-risk subjects. Greater right and left VLPFC-AMY RSFC were associated with greater ADHD symptom severity and lower omega-3 PUFA levels. Together these findings suggest that right VLPFC-AMY resting state hyper-connectivity distinguish youth at high-risk for bipolar I disorder, and may represent a central prodromal risk biomarker that may be altered through omega-3 PUFA supplementation.

Keywords: Omega-3 Fatty Acids, Bipolar Disorder, ADHD

Disclosure: Nothing to disclose.

W115

Suicidal Behavior, but not Ideation, is Reflected in Structural Brain Changes: Reduction in Frontal and Temporal Cortical Thickness and Thalamic and Hippocampal Volume

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Background: Abnormalities in fronto-parietal and temporal regions, have been linked to suicidal ideation and attempts. However, structural differences in these regions are generally examined in the context of remote lifetime suicidal events. In this study, we aimed to identify the structural differences between individuals with recent and remote suicide attempts. Examination of the neurobiology of suicidal individuals closely after a suicidal event may inform suicide risk and aid in suicide prevention.

Methods: Data were pooled from two studies (total N = 227) and included individuals who recently attempted suicide (within three days), individuals with remote lifetime suicide attempts, individuals with lifetime suicidal ideation but no attempts and depressed patients without lifetime suicidal ideation or attempts. Freesurfer version 5.3.0 was used to extract cortical thickness and volume on T1 MRI weighted structural images.

Results: Lifetime history of suicide attempts (recent and remote) was associated with lower inferior temporal, middle temporal, superior temporal, and lateral orbitofrontal cortical thickness and smaller thalamic volume. Smaller hippocampal and thalamic volumes were observed in recent suicide attempters compared to remote suicide attempters. There were no structural differences associated with lifetime suicidal ideation.

Conclusions: The structural brain differences associated with suicidal behavior but not with suicidal ideation support the notion of qualitative distinct phenomena, with recent suicide attempts associated with considerably more reduction of brain tissue. Changes in hippocampal and thalamic volume in patients may be more related to state vulnerabilities for suicidal behavior. These structures underlie impaired processes associated with acute suicide risk such as decision making, affect regulation and pain processing.

Keywords: Suicide, Structural MRI, Depression

Disclosure: Neuronetics, Grant, GSK, Stock / Equity, Self

W116

The Impact of White Matter Hyperintensities on Structural Connectomics in Late-Life Depression

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Background: Late life depression (LLD) is associated with high incidence of chronic disease, cognitive dysfunction, and poor prognosis. LLD has been associated with structural brain changes, including the appearance of white matter

hyperintensities (WMH). Previous work done in our lab demonstrated that LLD is associated with brain network ("connectome") dysfunction, particularly reduced structural connectome resilience. For the present study, we explored whether WMH had a differential impact on network connectivity in LLD as assessed via graph theory-derived metrics. We further examined the role of depressive symptoms and cognitive reserve on WMH associations with connectome characteristics.

Methods: 59 participants were analyzed from larger neuroimaging study on LLD (34 controls (16 M/18 F), 25 LLD participants (10 M/15 F). Brain MRIs were acquired on a 3.0T Achieva scanner (Philips Medical Systems, Best, The Netherlands) using an eight-channel SENSE (Sensitivity Encoding) head coil. Diffusion tensor images (DTI) were acquired using single-shot spin-echo echo-planar imaging sequence (field of view: 240 mm; voxel size: 0.83 × 0.83 × 2.2 mm; TR/TE: 6,994/71 ms; flip angle: 90 degrees). Sixty-seven contiguous axial slices aligned to the anterior commissure–posterior commissure line were collected in 32 gradient directions with b = 700 s/mm 2 and one acquisition without diffusion sensitization (B 0 image). Connectomes were generated with DTI tractography data and Freesurfer-based parcellation scheme yielding 85 x 85 connectivity matrices. Graph theory based metrics were derived using the Brain Connectivity Toolbox. Associations between WMH and network metrics were obtained with Pearson's correlations. Cognitive reserve was calculated using a latent factor analysis.

Results: WMH burden was only associated with reduce network efficiency in control subjects ($r = -.38$, $p = .03$). However, in participants with LLD, WMH was negatively associated with global efficiency ($r = -.41$, $p = .049$), transitivity ($r = -.42$, $p = .04$), degree ($r = -.43$, $p = .036$), strength ($-.41$, $p = .04$), and diversity ($r = .-5$, $p = .014$) of network connections. Transitivity, marker of the interconnectedness of neighboring nodes, was also significantly more impaired in patients with LLD when compared to controls. Depression severity and cognitive reserve did not impact these associations.

Conclusions: WMH increase structural connectome vulnerability uniquely in late-life depression and are associated with alterations in brain network properties demonstrated reduced brain network strength, diversity, degree, and transitivity (a measure of local connectivity).

Keywords: Connectome, Late-life Depression, White Matter

Disclosure: Embodied Labs, Blueprint, Advisory Board, Keywise, Stock / Equity

W117

Re-Examining the Primary Drug Target of Esketamine at its Antidepressant Doses

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Background: Clinical studies demonstrated robust, rapid and sustained antidepressant action of ketamine and esketamine given once or repeatedly by intravenous infusion. Esketamine nasal spray (SpravatoTM) has been approved by the US FDA for treatment resistant depression in March 2019. Ketamine is known as a non-competitive antagonist for the NMDA receptor (NMDAR), and esketamine is about two-fold more potent for the NMDAR than ketamine. Several lines of data emerged recently questioning the NMDAR as the primary antidepressant target of ketamine. We conducted a series of simulations and experiments to re-examine the primary targets of ketamine, esketamine and some of their metabolites.

Methods: The unbound brain Cmax levels of ketamine and esketamine were simulated based on reported parameters (Schaffer et al., 2014) and the plasma Cmax levels at human antidepressant doses (Zarate et al., 2012 and JRD FDA debriefing 2019). Drug target profiles of ketamine enantiomers and selected metabolites at 10 μM were examined using radioligand binding assays at Cerep. The affinity of esketamine for distinct binding sites of NMDAR was evaluated with [3H]CGS 19755 (a NMDAR glutamate site antagonist), [3H]TCP (a NMDAR pore site, also known as PCP site, antagonist) in rat cerebral cortical tissue. The NMDAR subtype selectivity was evaluated with a functional FLIPR assay using CHO cells expressing human recombinant NMDAR subtypes. Both agonist and antagonist effects of esketamine on μ- and κ-opioid receptors (MOR and KOR, respectively) were evaluated in CHO cells expressing rat recombinant receptors.

Results: Simulation showed that the brain unbound Cmax levels of ketamine and esketamine are approximately 0.9 and 0.4 μM. CEREP drug target profile studies of ketamine enantiomers and esketamine major metabolites did not reveal any target on which the action exceeds the cutoff threshold (50% effect at 10 μM), including dopamine, serotonin and norepinephrine receptors and transporters, and opiate receptors. Esketamine did not bind to the glutamate site of the NMDAR. The Ki- and IC50-values of esketamine and arketamine for NMDAR PCP site are 0.26 and 0.45, and 1 and 1.8 μM, respectively. The FLIPR assay showed that esketamine is more potent than arketamine for all NMDAR NR2A-D subtypes. Neither esketamine nor arketamine showed obvious selectivity for NMDAR subtypes. The EC50 values of esketamine for MOR and KOR are above 10 μM (i.e., the highest concentration used in the experiments). Both CEREP binding and functional studies failed to show any effect of ketamine enantiomers or selected metabolites on the nicotinic acetylcholine receptor α7.

Conclusions: The simulated brain unbound Cmax value and the NMDAR PD data together support engagement of NMDAR, but not MOR and KOR, at antidepressant doses of esketamine. Previous human studies showed that ketamine at the antidepressant dose range displays PET tracer binding of the NMDAR pore, and produces effects of NMDAR blockade, such as increased EEG gamma oscillation power and glutamate release. The increase in glutamate release has been shown to correlate with depression improvement in patients with major depressive disorder. Since there is no evidence of respiratory depression or profound euphoria in studies using ketamine or esketamine in the treatment of depression, functionally significant MOR stimulation by ketamine and esketamine is unlikely.

Keywords: Ketamine, Esketamine, NMDAR, MOR, Depression

Disclosure: JNJ, Stock / Equity

W118

Gen-ECT-ic: An International Consortium to Study Extreme Depression and Response to Electroconvulsive Therapy

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Background: Genome-wide association studies have demonstrated that the genetic burden associated with depression correlates with depression severity. Genetic studies of patients at the severe end of the depressive disorder spectrum, such as those with depression who are prescribed electroconvulsive therapy (ECT) are likely to be fruitful.

ECT has controlled risk and logistical demands and it is placed at the end of depression treatment algorithms despite ECT being the most acutely effective form of treatment for depressive

disorders. Understanding the genetics and biology of ECT response could lead to more personalized decisions on treatment.

Methods: ECT providers and researchers from around the world have formed the Genetics of ECT International Consortium (Gen-ECT-ic). Gen-ECT-ic will organize the largest sample collection to study the genomics of depressive disorders and response to ECT. Retrospective and prospective clinical data collection will be facilitated by a uniform data collection approach that is flexible and that will incorporate data from many clinical practices.

Gen-ECT-ic invites all ECT providers and researchers to join its efforts.

Results: 4Gen-ECT-ic has a goal of recruiting 25,000 subjects into the study over the next several years. The genomic and clinical data collected from these subjects will allow the study of those with depressive disorders that receive ECT relative to the general population as well as relative to those with mild-moderate depressive disorders. We estimate 10,000 of these subjects to include clinical response and side effect data and analysis will include associations of genomic risk scores with treatment response/side effect of cognitive impairment. The statistical power for minimal detectable genomic relative risks for various allele frequencies, as well as power calculations to detect associations between genetic risk scores and clinical response to ECT/ cognitive impairment based on different parameters will be presented.

Conclusions: Gen-ECT-ic invites all ECT providers and researchers to join its efforts.

Keywords: Electroconvulsive Therapy, Genome-wide Association Studies, Genetics of depression, Treatment-Response

Disclosure: Nothing to disclose.

W119

Explainable AI Approach Reveals Treatment Responders in a Randomized Controlled Trial of BTRX-246040, a Potent and Selective NOP Receptor Antagonist

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Background: BTRX-246040, a potent and selective antagonist of the human nociceptin receptor (NOPR), has been studied in patients with neurobehavioral disorders including alcohol use disorder and major depressive disorder (MDD). BTRX-246040 is orally bioavailable, brain penetrant and exhibits centrally-mediated activity in vivo in preclinical models and in humans. The purpose of the NEP-MDD-201 study was to extend the clinical characterization of BTRX-246040 to better understand the relationship between self- report and quantitative measures of affect, motivation and cognition. Here, we describe the novel application of machine learning approaches which enabled us to objectively identify subtypes of patients with MDD who were most responsive to NOP receptor antagonism.

Methods: NEP-MDD-201 was a double-blind, placebo-controlled, multicenter Phase 2a study with BTRX-246040 in MDD patients to evaluate the efficacy, safety, and tolerability of BTRX-246040 administered orally once daily at up to 80 mg for 8 weeks (clinicaltrials.gov identifier: NCT03193398). This study carried forward elements from a previous MDD study and added clinical scales and quantitative behavioral assessments that measure anhedonia. Efficacy was assessed using the Montgomery-Asberg Depression Rating Scale (MADRS), the Clinical Global Impression of Severity (CGI-S) and Improvement (CGI-I), and the Facial Expression Recognition Task (FERT). Anhedonia was characterized with both patient-reported outcome measures (Dimensional Anhedonia Rating Scale [DARS] and Snaith Hamilton Pleasure Scale [SHAPS]) and computerized tasks (Probabilistic Reward Task [PRT] and Effort Expenditure for Reward Task [EEfRT]). We used data from qualitative scales and quantitative measures as input variables for an algorithm that produced explainable output (explainable AI or XAI). Our previous work on rule mining and interpretable machine learning in clinical psychiatry was the basis for building predictive models to identify who will respond to drug or placebo. To answer this question, we leveraged three components of our XAI system which had not previously been combined (by us or anyone): (1) Feature selection (2) Prediction of treatment outcome and (3) Explainability.

Results: 104 patients were randomized and the Complete Analysis Set (CAS) comprised 73 patients. There was no separation between patients randomized to BTRX-246040 or placebo on primary or secondary endpoints and there was no clear association with any individual scale or task with treatment response. Predictive models were built using only baseline data to predict the estimated MADRS at week 8 for each individual under treatment and placebo separately. Setting the threshold as a 4-point difference in the predicted MADRS at week 8 between treatment and placebo, we identified individuals who have better predicted outcome under active treatment (treatment-indicated) whereas others who have better predicted outcome under placebo (placebo-indicated). The effect sizes (Cohen's d) were 0.68 and 0.59 for treatment- and placebo-indicated subgroups, respectively, compared to 0.03 for the CAS. The identified subgroups were stable over time (e.g. weeks 1, 2, 4, 6, 8 and follow-up), even though the models were built only to predict week 8. We explored a series of explainable models, based on the number and types of input features, and found the compact nested rule lists were sufficient to support explainable enrichment strategies.

Conclusions: The NEP-MDD-201 study did not apply a specific enrichment strategy. However, the inclusion of multidimensional assessments proved valuable because they provided insights across multiple symptom dimensions and because they enabled more robust treatment prediction models. In addition to practical lessons about deployment of these tools in multisite trials, we learned that no single analytical approach is sufficient to predict placebo vs. treatment responders. In fact, explainable models were only achievable through a combination of analytical approaches. Using only baseline data, the XAI analysis identified a group of patients who respond to BTRX246040. Generating an explainable (and testable) rule list creates an opportunity to conduct additional studies in which one can prospectively enrich for patients who are more likely to respond to NOP receptor antagonism. The overall profile of BTRX-246040, with its novel mechanism of action, encourages further clinical development. To best test the enrichment approach, we propose a study design similar to NEP-MDD-201 that applies a rule-based enrichment strategy.

Acknowledgments: We thank Kerensa Saljooqi, Atul Mahableshwarkar and Humberto Gonzalez for their assistance with data collection and analysis.

Keywords: Major Depressive Disorder (MDD), Anhedonia, Digital Assessment, Machine Learning

Disclosure: BlackThorn Therapeutics, Employee, Stock / Equity.

W120

Psilocybin-Assisted Group Therapy for Demoralization in Older Long-Term AIDS Survivors: A Safety and Feasibility Pilot Study

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Background: Older long-term AIDS Survivors (OLTAS) are people living with HIV >50 years old and who were diagnosed prior to the advent of combined antiretroviral therapy. Compared to HIV seronegative peers, OLTAS suffer higher rates of depression, anxiety, trauma exposure, substance use and risky sexual behaviors. Demoralization is a syndrome common among LTAS and other palliative care patients (point prevalence up to 53%) and is characterized by poor coping and a sense of helplessness, hopelessness and a loss of meaning and purpose in life. Psilocybin is a 5HT2AR agonist and psychedelic that can improve depression and anxiety in patients with cancer when combined with individual psychotherapy, possibly by enhancing the capacity of meaning-making.

Methods: We conducted an open-label Phase I trial of psilocybin-assisted group therapy for gay-identified OLTAS who suffer from moderate-to-severe demoralization. Participants completed 4 preparatory group therapy visits, one psilocybin administration visit (0.3-0.36mg/kg po), and then 4-6 group integration therapy visits. Primary outcomes included adverse events and rate of recruitment and retention. Primary clinical outcome was change in demoralization from baseline to end-of-treatment (3 weeks post drug). Secondary outcomes included self-report measures of complicated grief, depression, and PTSD. This trial was approved by the UCSF IRB.

Results: 18 participants enrolled in the trial with 100% retention at end-of-treatment. In addition to several non-serious expected adverse psilocybin reactions, zero serious adverse reactions and two unexpected non-serious reactions were detected. Changes from baseline to end of treatment were found in Demoralization Scale-II (DS-II; mean difference (SD) = 6.67 (6.51), 95% CI 3.43-9.9, $p = 0.0004$, Hedge's g=0.99); Center for Epidemiologic Studies of Depression Scale-Revised (CESD-R) (mean difference (SD) = 8.94 (14.73), 95% CI 1.62-16.27, $p = 0.02$, Hedge's g=0.76); Inventory of Complicated Grief-Revised (mean difference (SD) = 6.22 (6.74), 95% CI 2.87-9.58, $p = 0.001$, Hedge's g=0.52); PTSD Check List-5 (mean difference (SD) = 9 (11.47), 95% CI 3.29-14.71, $p = 0.004$, Hedge's g=0.74). One-way ANOVA of DS-II demonstrated a main effect of F=9.04, dF=4, $p<0.0001$ with post-hoc Tukey pair-wise testing revealing significant differences between baseline and 1-week post drug ($p < 0.01$), baseline and 3-weeks post drug ($p<0.05$), but no significant difference between 3-weeks post drug and 3-month follow-up.

Conclusions: This was the first modern trial to demonstrate the safety, feasibility, and preliminary efficacy of a psychedelic-assisted group therapy for any disorder. Group therapy merits further study as an efficient means of delivering psilocybin therapy to patients with complex medical and psychiatric needs. Preliminary results resemble prior findings of rapid improvement in mood and anxiety symptoms in patients with cancer. Our results suggest that psilocybin therapy should be studied further in controlled trials of not only mood and anxiety disorders, but also trauma-related disorders (e.g. PTSD and complicated grief) and demoralization. Efficacy, however, will continue to be a challenge to assess without proper blinding.

Keywords: Aging, Psychological Distress, Psychedelics

Disclosure: Nothing to disclose.

W121

Healthy Women who Use Oral Contraceptives Show Distorted Serotonin Brain Architecture Relative to Non-Users

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Background: The lifetime incidence of depression reaches 21% in women, which is about twice the incidence in men. Strong epidemiological evidence supports that women are at higher risk for depression during hormonal transitions, i.e. peripartum and perimenopause. This may extend to exogenous hormone exposure; a large register-based Danish study of more than one million women showed that initiating oral contraceptives (OCs) is associated with subsequent use of antidepressants targeting serotonin (5-HT) neurotransmission [Skovlund CW et al., JAMA Psychiatry, 2016. 73(11): p. 1154-1162]. The mechanisms behind remain elusive, however, OCs may affect 5-HT brain architecture, i.e. reproductive hormones can affect the level of 5-HT synthesis, reuptake, degradation and 5-HT receptor expression. No studies have addressed whether OCs affect serotonergic architecture in terms of 5-HT4 receptor (5-HT4R) binding, which is sensitive to 5-HT manipulation and provides an indirect biomarker for in vivo brain 5-HT levels [Haahr ME et al., Mol Psychiatry, 2014. 19(4): p. 427-32].

We investigate if brain 5-HT4R binding differs between OC users and non-users among healthy women. In a secondary analysis within the OC user group, we explore if 5-HT4R binding differs between 2nd- and 3rd generation OCs. Further we explore if the 5-HT4R binding is associated with plasma estradiol.

Methods: [11C]-SB207145, ligand for 5-HT4R, PET imaging data were available from the Cimbi database for 55 healthy women, of which 17 used OCs (mean age of users = 25.5 vs. non-users = 26.1, $p = 0.71$). Five brain regions, considered important for depression pathophysiology, were co-registered to an MRI image and 5-HT4R non-displaceable binding potential (BPND) was determined using the simplified reference tissue model with cerebellum as a reference region.

The association between use of OC and regional BPND was evaluated using multiple linear regression models adjusting for age, scanner type (GE-Advance vs. HRRT Siemens PET scanner), injected [11C]-SB207145 mass per bodyweight and familial risk for depression. The type of OC was known for 13 of the 17 users, all were combined oral contraceptives (COCs), mainly 2nd- and 3rd generation COCs, which allowed us to explore if the BPND differs between users of OCs containing gestodene (GSD, $n = 4$) vs. levonorgestrel (LNG, $n = 6$).

Results: We found a negative association between BPND and use of OC in all explored regions with the following percentage difference to non-users; pallidostriatum: -7.2% (CI[-13.6:-0.3], $p = 0.04$), caudate nuclei: -8.6% (CI[-15.2:-1.4], $p = 0.02$), hippocampus (Graph 1): -11.2% (CI[-19.3:-2.2], $p = 0.02$), anterior cingulate: -9.6% (CI[-16.9:-1.8], $p = 0.02$), frontal cortex: -9.6% (CI[-16.1:-0.1], $p = 0.05$). Further, 5-HT4R BPND in prefrontal cortex is positively associated to plasma estradiol levels in OC users, but not in nonusers (slope difference, $p = 0.02$). Women using 3rd generation COC showed higher BPND in frontal cortex compared to 2nd generation COC; 16.9%, (CI[6.1:28.7], $p = 0.009$). P-values are uncorrected.

Conclusions: Women using OCs have lower cerebral 5-HT4R binding compared to non-users. We propose that this reflects an effect of OC hormone exposure on 5-HT4R expression, potentially mediated by reduced plasma estradiol, rather than a change in serotonergic tone. This offers a plausible link between OC exposure and subsequent risk of developing depression, particularly in hormone sensitive individuals. Future intervention studies must elucidate if withdrawal from OCs rescues 5-HT4R brain architecture and help identify women who are sensitive to OCs.

Keywords: Serotonin, Estradiol, Ovarian Steroids, Human Brain Imaging, PET Imaging

Disclosure: Nothing to disclose.

W122

Structural Connectivity in Currently Medicated Treatment-Resistant vs. Treatment-Responsive Depression: A Diffusion Tensor Imaging Study

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Background: Findings of changes of structural connectivity in major depression have been variable, and many prior studies excluded patients taking medications. Antidepressant medications may have neurobiological effects independent of antidepressant effects, and treatment-resistant depression (TRD) patients may have a different neurobiological response to treatment compared to treatment-responsive patients. The current study compared structural connectivity in medicated MDD patients with and without current, severe depression using region-of-interest analyses.

Methods: In a cross-sectional design, 30 patients with TRD (current Hamilton Depression Rating Scale-24 ≥20) were compared with 57 patients who had responded to treatment (HDRS <10 for at least 2 months). Both groups of patients had been on stable doses of two antidepressant medications at adequate doses for 8 weeks or longer at the time of imaging. Diffusion tensor imaging was acquired at 3T along 60 directions. Mean fractional anisotropy (FA) was calculated bilaterally for several specific regions of interest from the JHU white matter atlas: cingulum-cingulate gyrus, cingulum-hippocampus, anterior corona radiata, anterior limb of internal capsule, and uncinate fasciculus. Mean FA for each region was compared between groups using a general linear model with age and gender as covariates.

Results: The TRD and treatment-responsive groups did not show significant demographic differences; as intended by study design, the TRD group showed significantly higher scores on multiple depression scales. Mean number of current antidepressant medications was very close to 2 in both groups. Only one region of interest, the left anterior limb of the internal capsule, differed significantly between groups: the TRD group showed greater FA, indicating greater white matter integrity compared to the treatment-responsive group, in this region ($p = 0.035$).

Conclusions: This is the first study, to our knowledge, comparing structural connectivity measures, all in medicated patients, with TRD vs. treatment-responsive depression. Ongoing analyses will include other frontal regions of interest, as well as whole-brain tractography. This study highlights the potential importance of thinking about TRD as depression that persists despite potentially effective treatment, thereby accounting for changes that might be associated with treatment but not efficacy for depression.

Keywords: Treatment-Resistant Depression, Diffusion Tensor Imaging (DTI), Antidepressant agents, Brain Structural Connectivity

Disclosure: Eli Lilly and Company, Employee

W123

Lumateperone (ITI-007) in the Treatment of Bipolar Depression: Results From a Randomized Clinical Trial

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Background: While depressive episodes are far more prevalent than manic episodes in patients with bipolar disorder, approved treatments for bipolar depression are limited. Additionally, approved medications are associated with a range of side effects including weight gain and metabolic risks, hyperprolactinemia, and extrapyramidal symptoms. Treatment options specifically for major depressive episodes associated with bipolar II disorder are even more limited, with quetiapine, which has considerable metabolic burden, as the only currently approved treatment. Lumateperone (lumateperone tosylate, ITI-007) is an investigational drug for the treatment of neuropsychiatric disorders, including schizophrenia and bipolar depression (major depressive episodes associated with bipolar I and bipolar II disorder). Lumateperone has a unique mechanism of action that simultaneously modulates serotonin, dopamine, and glutamate neurotransmission. In the treatment of schizophrenia, lumateperone was shown to be efficacious, safe, and well tolerated, with a safety profile similar to placebo.

This Phase 3 randomized, double-blind, parallel-group, placebo-controlled multinational study (NCT03249376) investigated the efficacy and safety of lumateperone monotherapy in patients with bipolar I or bipolar II disorder experiencing a major depressive episode.

Methods: Patients aged 18-75 years with a clinical diagnosis of bipolar I or bipolar II disorder who were experiencing a current major depressive episode were eligible to participate in the study. Patients were required to have a Montgomery-Åsberg Depression Rating Scale (MADRS) total score ≥ 20 and a Clinical Global Impression Scale-Bipolar Version-Severity (CGI-BP-S) score ≥ 4 at screening and baseline. Patients were randomly assigned 1:1 to lumateperone 42 mg or placebo, administered orally once daily in the evening for up to 6 weeks. The primary and key secondary efficacy endpoints were change from baseline to Day 43 in the MADRS total score and CGI-BP-S total score, respectively, analyzed using a mixed-effects model for repeated measures (MMRM) approach in the intent-to-treat (ITT) population. Secondary efficacy endpoints included percentage of patients achieving response (MADRS improvement ≥ 50%) and remission (MADRS total score ≤ 12) at Day 43. Safety assessments included treatment emergent adverse events (TEAEs), laboratory parameters, vital signs, extrapyramidal symptoms (EPS), and suicidality.

Results: During the 6-week study, 377 patients received treatment (placebo, n = 189; lumateperone 42 mg/day, n = 188) and 333 patients completed treatment (87.4% in each group). Mean MADRS and CGI-BP-S scores at baseline were 30.5 and 10.2, respectively, indicating that patients had moderate-to-severe depression symptoms. Patients in the lumateperone 42-mg group had significantly greater mean improvement on MADRS total score change from baseline to Day 43 compared with placebo (least squares mean difference [LSMD] = −4.59; 95% confidence interval [CI] = −6.34, −2.83; effect size vs placebo = −0.56; P<.0001). Lumateperone treatment was associated with significant MADRS improvement in both patients with bipolar I (LSMD = −3.95; 95% CI = −5.92, −1.99; effect size vs placebo = −0.49; P<.0001) and bipolar II (LSMD = −7.04; 95% CI = −10.92, −3.16; effect size vs placebo = −0.81; P=.0004).

The lumateperone 42-mg group also had significantly greater mean improvement in CGI-BP-S total score compared with placebo (LSMD = −0.94; 95% CI = −1.37, −0.51; effect size vs placebo = −0.46; P<.0001). The MADRS response rate was significantly greater at Day 43 for lumateperone compared with placebo (51.1% vs 36.7%; odds ratio = 2.98; P=.0001); lumateperone treatment was associated with significantly higher remission rates relative to placebo (P=.0176).Only 3 TEAEs occurred in ≥ 5% of patients receiving lumateperone and at rates greater than placebo: headache (17.6% vs 10.1%), somnolence (8.5% vs 1.1%),

and nausea (6.4% vs 2.1%). EPS-related TEAEs were rare, with 1 event of dyskinesia (0.5%) in the lumateperone 42-mg group.

Conclusions: Lumateperone 42 mg significantly improved depression symptoms in both patients with bipolar I and bipolar II depression. Drug-placebo differences in MADRS reduction were of similar magnitude relative to antipsychotics recently approved for bipolar depression. Significantly greater rates of response and remission for lumateperone versus placebo suggest that the effects of lumateperone were clinically meaningful. Consistent with previously reported placebo-controlled and open-label studies in patients with schizophrenia, lumateperone was generally well tolerated in patients with bipolar disorder. These results suggest that lumateperone 42 mg may be a promising new treatment for bipolar depression associated with either bipolar I or bipolar II disorder.

Keywords: Bipolar Depression, Bipolar I & II Disorder, Lumateperone, ITI-007

Disclosure: Intra-Cellular Therapies, Inc, EmployeeStock / Equity

W124

Deep Brain Stimulation (DBS) of the Supero-Lateral Medial Forebrain Bundle (slMFB) in Treatment-Resistant Depression (TRD) – FORESEE III – Study Design and First Results

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Background: Deep brain stimulation (DBS) is currently under research as alternative treatment method for treatment-resistant depression (TRD). Different brain targets are under investigation and antidepressant efficacy has been demonstrated with rapid as well as sustained response. Recently, two study trials have been terminated due to a failed futility analysis. In another RCT, DBS to the supero-lateral medial forebrain bundle (slMFB) did not show significant differences between sham and active stimulation eight weeks after onset of DBS either. Furthermore, effects of discontinuation of stimulation are unknown but important due to safety reasons. These aspects are now intergrated in a new study design: FORESEE III study (registered @ clinicaltrials.gov with Identifier NCT03653858). Here we are going to present details on the ongoing study.

Methods: FORESEE III is a randomized, sham-controlled, double-blind study. Stimulation is activated one week or 17 weeks after implantation of the DBS system. After 17 weeks of sham stimulation, stimulation is activated in Group B and continued for six months. After six months of continued stimulation, a responder assessment is conducted. Response is defined as > 50% mean reduction of MADRS over a period of six months (area under the curve). Responders are then randomized to a group with continued or discontinued stimulation. Criteria for relapse/ event are defined as an augmentation of MADRS > 5 points in two consecutive trials. If an event occurs, re-onset of stimulation will take place. Altogether, 47 patients will be included in the study and duration of study differs between 12 to 19 months per patient.

Results: The study started in September 2018. Until today, 20 patients have been screened for study entry. One patient had to be excluded due to MRI anomalies. 19 patients have been included in the study (9m and 10f) and until today DBS is implanted in 16 patients (with a mean age of 45.7 and a mean duration of current episode of 10.1 years). All patients were treated with > four antidepressants, psychotherapy (> 20h) and electronconvulsive therapy (> 6 sessions). Not eligible for the study were 157 patients. Those patients demanded participation in the study but were excluded due to different reasons (mainly lack of ECT).

Conclusions: FORESEE III has the goal to investigate the antidepressant efficacy of DBS to the slMFB in TRD in a larger patient population. Until today, studies have included 8, 16 and 4 patients. Additionally, eight weeks of sham stimulation were not enough to show a significant difference between sham and active stimulation. Effects such as insertion effects are discussed. A longer sham-stimulation phase is needed to better understand those effects. Furthermore, effects of discontinuation of stimulation are unknown. There is only a case series of slMFB discontinuation showing rapid re-occurrence of depressive symptoms. A systematic investigation of this effect is urgently needed due to safety issues.

Keywords: Deep Brain Stimulation, Treatment-Resistant Depression, Superolateral Medial Forebrain Bundle, Randomized-Controlled Trial

Disclosure: Nothing to disclose.

W125

Eicosapentaenoic Acid (EPA) Supplementation in Major Depressive Disorder Patients With Chronic Inflammation: Dose-Dependent Increases in EPA Metabolites and Specialized Pro-Resolving Lipid Mediators

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Background: Eicosapentaenoic acid (EPA) supplementation is an effective treatment option in major depressive disorder (MDD) associated with chronic low-grade inflammation. EPA serves as a precursor in several enzymatic pathways generating EPA derivatives and specialized pro-resolving lipid mediators (SPMs). These molecules serve important roles in cell signaling, including the activation of molecular pathways that lead to the resolution of inflammatory processes and the restoration of tissue homeostasis. The objective of this study was to assess the effect of different doses of EPA on plasma concentrations of EPA metabolites and SPMs in MDD patients.

Methods: In a 2-site study, 61 MDD patients with screening IDS-C scores >25, body mass index >25 kg/m2 and serum C-reactive protein >3 mg/L were randomized to 4 treatment arms for 12 weeks: 1) Placebo; 2) EPA 1 g/d; 3) EPA 2 g/d; and 4) EPA 4 g/d. The EPA capsules had a 4:1 EPA:DHA ratio. Plasma EPA (as mol%) and SPMs (pg/ml) were measured in study completers at baseline and at the end of treatment ($n = 11$, 12, 12 and 13 in the placebo, EPA 1, 2 and 4 g/d arms, respectively) by liquid chromatography/ mass spectrometry.

NCT02553915

Results: Plasma EPA and SPM concentrations did not change in the placebo group during 12 weeks of treatment. There was a trend for a dose-dependent effect of EPA supplementation on median (final relative to baseline) plasma EPA levels (4, 4 and 7 fold in the 1, 2 and 4 g/d arms, respectively). Similarly, we observed dose-dependent increases in EPA-derived metabolites with anti-inflammatory properties and SPMs: median concentrations of 18-hydroxyeicosapentaenoic acid (18-HEPE), derived through cytochrome P450 enzymatic pathways and precursor of resolvins (Rv) E1-3, were increased 7, 8, and 15 fold, respectively; 15-HEPE, derived from 15-lipoxygenase enzymatic conversion, was increased 4, 3 and 7 fold; 5-HEPE, derived from the 5-lipoxygenase pathway, was increased 4, 5 and 9 fold, respectively. Of the EPA-

derived SPMs, RvE1 was undetected in all treatment groups, while RvE2 was detected in half of the subjects at baseline and after treatment, with dose-dependent increases (1.2, 4.6 and 5.6 fold, respectively). RvE3 was detected only after supplementation, dose-dependently (in 2, 3 and 6 patients, respectively).

Conclusions: Our results show robust increases in plasma EPA, EPA-derived metabolites and SPMs following EPA supplementation with 4 g/d. After 1 and 2 g/d supplementation, similar but more modest increases in these parameters were observed. Future analyses will test whether the dose-dependent increases in SPMs are associated with reduced inflammation and improved clinical benefits.

Keywords: Major Depressive Disorder, Inflammation, Omega-3 Fatty Acid

Disclosure: http://mghcme.org/faculty/faculty-detail/maurizio_favaAdvisory Board, Consultant, Patent

W126

Opioid Use and Schizophrenia

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Background: The opioid crisis in the United States has spread exponentially with opioid use disorder (OUD) related overdoses increasing by 200% since 2000, accounting for 66% of all drug overdoses in the USA (1). With more than 70,000 overdose deaths related to OUD, the opioid crisis has claimed more lives than the AIDS epidemic (2). Patients who suffer from both a psychotic spectrum disease, such as schizophrenia, and OUD are at extremely high risk of accidental or intentional overdose.

Methods: Systematic review of the literature including database search of PubMed, PsycInfo, EBSCO, and Google scholar with the Boolean combination of the following search terms: (opioid OR opiate) AND (schizophrenia OR schizoaffective OR schizophreniform OR schizotypal OR schizo*). Articles not written in English were excluded. The remaining articles were reviewed based on title and abstract and selected based on relevance. Thereafter, remaining articles underwent full-text review to determine relevance.

Results: Initial search yielded 614. Based on title and abstract analysis, 567 articles were excluded. The remaining 47 articles underwent full text review. In total, 28 relevant articles were included in this systematic review. Recent meta-analysis data shows that 41% of patients with schizophrenia have comorbid substance use disorder (3) and patients with OUD have an 8x higher prevalence of schizophrenia compared to non-OUD patients(4). Opioid use is also associated with a significantly increased risk for conversion from schizotypal personality disorder to schizophrenia compared to cannabis [hazard ratio (HR) of 2.74 (1.38-5.45) versus HR 1.30 (1.01-1.68)] (5).

Despite its prevalence, there have been very few investigations into the dual diagnosis of opioid use disorder and schizophrenia and whether medication assisted therapy for opiates can be used in combination with antipsychotics. It is known, however, that treatment with opioid agonist therapy (OAT), such as methadone or buprenorphine is associated with improved antipsychotic adherence (6). Within patients who have this comorbidity, the subset of patients who are also in contact with the justice department or in correctional facilities have been found to have significantly lower rates of OAT treatment compared to both patients without schizophrenia and patients with schizophrenia who have not been incarcerated (7). With regards to antipsychotic treatment, currently olanzapine and clozapine have the best studied data of efficacy for patients with this comorbidity (8). Additionally, a combination of aripiprazole and topiramate has some evidence from industry sponsored investigations (9).

Conclusions: In the above-mentioned studies as well as other investigations on substance use and comorbid schizophrenia, opioid use is often not the focus, rather part of supplemental data. We thus are advocating for more research focus on this very vulnerable population of patients as progress is made to combat the opiate crisis.

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Keywords: Opioid Addiction, Schizophrenia Subtypes, Opiate Epidemic, Opiate Addiction

Disclosure: Nothing to disclose.

W127

Assessment of Ketamine Binding of the Serotonin Transporter: A Human In-Vivo, Bolus-Infusion [11C]DASB Positron Emission Tomography Study

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Background: Modulation of the glutamatergic system and neuroplastic changes are thought to underlie ketamine's rapid and profound antidepressant effects. In addition, recent studies propose a significant role for the serotonergic (5-HT) system, underscored by clinical reports of serotonergic syndrome following ketamine administration. 5-HT depletion was shown to abolish ketamine's antidepressant properties in rodent studies (1). In addition, ketamine was shown to occupy the serotonin transporter (SERT) in primates (2). The SERT is a target for many antidepressant substances. Our previous study did not detect occupancy of

ketamine at the SERT at an antidepressant dose (0.50 mg/kg bodyweight ketamine) utilizing [11C]DASB positron emission tomography (PET) (3). As higher ketamine doses are increasingly explored as a therapeutic option in antidepressant treatment, elucidation of ketamine's molecular binding profile at higher dose-ranges is of growing importance. We thus aimed to assess whether ketamine occupies the SERT at the higher end of the sub-anesthetic dose-range using a bolus-infusion [11C]DASB PET protocol.

Methods: 10 (24.50 +/- 3.69 years of age) healthy males completed the protocol. This study is considered a pilot study for further exploration of the SERT's role in ketamine's antidepressant effects. Following confirmation of physical and psychiatric health, subjects were measured twice with [11C]DASB PET (General Electric Advance full-ring scanner). PET1 served as baseline, while ketamine was administered during PET2. For definitive assessment of ketamine's SERT occupancy, the drug administration and PET measurement protocol was optimized to increase ketamine plasma levels during radioligand equilibrium. [11C]DASB was given in a bolus/infusion scheme based on a previous publication by our group (4). 100 min of dynamic PET (20 frames of 5 min each) commenced 40 min after start of radioligand infusion. During PET2, 0.80 mg/kg bodyweight racemic ketamine were administered intravenously over 50 min starting 60 min after begin of radioligand administration. T1-weighted magnetic resonance images (MRI, 3 Tesla Siemens PRISMA) were utilized for structural colocalization. Nondisplaceable binding potential (BPND) was calculated for 4 SERT-rich regions of interest using average activity measured during tracer equilibrium (last 30 min of PET) with the cerebellum as reference region. Occupancy (%) = (1-BPNDPET2/BPNDPET1)*100. All subjects provided written informed consent. This study was approved by the Ethics Committee of the Medical University of Vienna and performed according to the Declaration of Helsinki.

Results: Mean +/- SD occupancy (%) was -0.95 +/- 19.14, 2.42 +/- 14.34, 1.06 +/- 11.72, and -4.66 +/- 33.50 for the amygdala, thalamus, midbrain, and striatum, respectively. While positive values suggest occupancy, negative values indicate SERT BPND increase from PET1 to PET2.

Conclusions: This study utilized a protocol designed to optimize potential for detection of ketamine binding of the SERT. Nevertheless, we did not elucidate occupancy at this dose (0.80 mg/kg bodyweight) and values were within the published test-retest variability of [11C]DASB PET. The administered dose is comparable to the species-specific equivalent given in animal studies that demonstrated occupancy. Thus, our study is in contrast with these previous reports (2). In addition to potential inter-species differences, the broad range of occupancy values indicated by the high SD suggest inter-individual differences, for which variability in metabolism presents a potential explanation. Consideration of ketamine plasma levels may provide explanation for this discrepancy. In summary, our results suggest that ketamine does not relevantly occupy the SERT in humans at this subanesthetic dose.

Clinicaltrials.gov: NCT02717052

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Keywords: Ketamine, Positron Emission Tomography (PET), Serotonin Transporter

Disclosure: Janssen, Honoraria, Austroplant, Eli Lilly, AOP Orphan Pharmaceuticals, AbbVie, Bristol-Myers Squibb, Gilead, Spouse, W. L. Gore & Associates, Honoraria

W128

Ethnicity as a Major Predictor of Lithium Response in the Treatment of Bipolar Disorder

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Background: Lithium remains as the first-line treatment in the maintenance of Bipolar Disorder (BD). However, individual response to lithium is variable, with about 30% of patients considered full responders. Multiple genetic studies have suggested that lithium response could have a genetic basis but only a small number of studies have explored the effect of ethnic component in the prediction of lithium response in BD. The aim of this study was to test the hypothesis that the ethnic component plays a major role in defining the outcome of lithium treatment in bipolar patients.

Methods: A total of 172 bipolar patients from a population originated from a founder effect in South America, who had received treatment with lithium for at least 3 months consecutively, were comprehensively phenotyped using the "Diagnostic Interview for Genetic Studies" (DIGS), the "Young Mania Rating Scale" (YMRS), the "Hamilton Rating Scale for Depression" (HAMD) and the "Alda Scale" for lithium response. A set of 11 classical markers for ethnic dissection were genotyped and a complex set of variables related to lithium response together with the probability of ethnical endorsement (White, Amerindian, and African) were evaluated as predictors using advance recursive partitioned analysis (ARPA).

Results: A total of 172 patients (59 [34.3%] male, 26 [15.1%]) were included in this study. The global rate of lithium response in this cohort was 15.11% (95%CI =10.28-21.55). When comparing both groups of response, it was found that lithium responders had few depressive episodes (3.9 ± 5.6 vs. 2 ± 2.1, P = 0.002), less African ancestry (0.2 ± 0.2 vs. 0.1±0.1, P = 0.005) and more European ancestry (0.3 ±0.3 vs. 0.5 ±0.3, P=0.024) than non-responders. When using ARPA including demographic and clinical features in addition with genetic ancestry as a predictor, a 13-node classification tree for predicting response to lithium was derived, being Amerindian ancestry the most important predictor, followed by Amerindian and European ancestry variances, and African and European ancestries. Clinical variables such as illness duration, number of depressive episodes, total number of affective episodes and number of manic episodes, were also found to be important predictors. This ARPA-based predictive model yields remarkable performance (Se = 93.8%, Sp = 94.5%, CCR = 89.1% and AUC = 92.4%).

Conclusions: Ethnic endorsement was found to be a major predictor of lithium response in BD patients. These classification trees including both clinical and genetic variables might be suitable to be used by clinicians in the guidance and approaching of the treatment of bipolar disorder.

Keywords: Bipolar Disorder, Lithium Response, Pharmacogenetics, Predictor of Treatment Response

Disclosure: Nothing to disclose.

W129

Antidepressant Treatment Depalmitoylates Gsalpha and Removes it From Lipid Rafts: A Mechanistic Rationale for This Cellular Hallmark of Antidepressant Response

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Background: All antidepressants examined thus far, including "atypical" compounds such as HDAC6 inhibitors and ketamine, increase cAMP by translocating the G protein Gsalpha from cholesterol-rich lipid rafts to non-raft membrane regions where they activate adenylyl cyclase. We have suggested that this is a bio signature of effective antidepressant treatment. Gsalpha is palmitoylated near the amino terminal, and this post-translational modification appears to be relevant for the membrane localization of Gsalpha. We hypothesized that one effect of sustained antidepressant treatment (and brief ketamine treatment) would be to remove the Palmitoylation group from Gsalpha, allowing it to translocate from lipid rafts.

Methods: C6 glioma cells were treated for 3 days with antidepressants of each major class as well as the HDAC inhibitor, tubastatin. They were also treated with ketamine for 15 minutes. Association between tubulin and Gsalpha was determined by co-immunoprecipitation. Direct interactions between tubulin and Gsalpha were determined by surface plasmon resonance. Palmitoylation status of Gsalpha was determined by Mass Spectrometry. Samples were treated with n-ethylmaleimide (NEM) prior to mass spec analysis. The free serine (S3) in depalmitoylated Gsalpha binds NEM, allowing more complete quantitation of depalmitoylated Gsalpha.

Results: Sustained treatment of cells with escitalopram, fluoxetine, phenelzine or desipramine resulted in loss of palmitoylation of Gsalpha. Olanzepine or R-citalopram did not have this effect. Treatment of cells with palmostatin b, a drug preventing depalmitoylation, blocked the antidepressant-induced translocation of Gsalpha from lipid rafts. These same drugs decomplexed Gsalpha and tubulin in lipid raft membranes. The effect of antidepressants on this was indirect, as none of these drugs altered the kinetics of tubulin-Gs association as determined by surface plasmon resonance.

Conclusions: Translocation of Gsalpha from lipid rafts is a consistent biosignature for antidepressant efficacy that may be useful for both diagnostic and prognostic purposes. The data in this study point toward a molecular rationale for this phenomenon.

Keywords: GPCR, G Protein, Biomarker, Cyclic AMP, Adenylyl Cyclase

Disclosure: Otsuka, Consultant, Pax Neuroscience, Stock / Equity, Pax Neuroscience, Stock / Equity(Spouse)

W130

Esketamine Nasal Spray for Rapid Reduction of Depressive Symptoms in Adult Patients With Major Depressive Disorder at Imminent Risk for Suicide: Results From the Phase 3 Program

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Background: Major depressive disorder (MDD) is the psychiatric condition most commonly associated with suicide. Due to delayed onset of action, currently available antidepressants are of limited utility in patients with MDD experiencing acute suicidal ideation (SI) with intent. In a recent proof-of-concept study, esketamine, the S-enantiomer of ketamine, as nasal spray (ESK) plus comprehensive standard-of-care (SOC) demonstrated rapid improvement in depressive symptoms, among patients with MDD at imminent risk for suicide. A global registration program comprised of two phase 3 studies was conducted to confirm the efficacy and safety of ESK + comprehensive SOC vs placebo nasal spray (placebo) + comprehensive SOC in this patient population. This is the first drug development program for this important and understudied patient population for whom there is great unmet medical need.

Methods: ASPIRE-1 (NCT03039192) and ASPIRE-2 (NCT03097133) were double-blind (DB), randomized, placebo-controlled, phase 3 studies conducted in adult patients (aged 18-64 years) with MDD (DSM-5 criteria and confirmed by Mini International Psychiatric Interview [MINI]) who had active SI and intent, required psychiatric hospitalization, and had moderate to severe depression with a Montgomery-Åsberg Depression Rating Scale (MADRS) total score >28. Patients were randomized (1:1) to ESK 84 mg or placebo twice-weekly for 4 weeks (DB phase, days 1, 4, 8, 11, 15, 18, 22, and 25) along with newly initiated or optimized SOC antidepressant(s). During follow-up phase, patients were monitored on days 28, 32, 35, 39, 46, 53, 67 and 90 after treatment. Primary endpoint: Change from baseline in the MADRS total score at 24 h post first dose. Key secondary endpoint: change from baseline in the Clinical Global Impression–Severity of Suicidality–Revised (CGI-SS-R) at 24 h post first dose. Treatment-emergent adverse events (TEAEs) were monitored.

Results: A total of 456 patients (ASPIRE-1: 226; ASPIRE-2: 230) were randomized (229, ESK + comprehensive SOC; 227, placebo + comprehensive SOC); 379 (83%) completed DB treatment. Pooled efficacy analysis set included 226 patients in ESK + comprehensive SOC group and 225 in placebo + comprehensive SOC group. Baseline characteristics were comparable between two studies and both treatment groups. The mean (SD) age of patients was 40.1 (13.00) yr and the majority were women (60.8%). Mean (SD) baseline MADRS total score was 40.4 (5.82). The majority of patients (90%) in both treatment groups were moderately to extremely suicidal, as measured by CGI-SS-R. Patients in the ESK + comprehensive SOC group showed improvement in MADRS total score vs placebo + comprehensive SOC group (difference of least squares mean [LSM] [95% CI]: -3.8 [-5.75; -1.89]) at 24 h post first dose. Treatment differences (LSM [95% CI]) based on MMRM for change in baseline MADRS total score at 4 h post dose (-3.4 [-5.05; -1.71]) and day 25, 4 h post dose (-3.4 [-5.36; -1.36]) numerically favored the ESK + comprehensive SOC group. Change in baseline MADRS score at 24 h post first dose also favored ESK + comprehensive SOC group for all prespecified subgroups. Difference (95% CI) between treatment groups in percentage of patients achieving remission (MADRS score ≤12) at day 25 (4 h post dose) was 13.1% (4.03; 22.19), favoring the ESK+ comprehensive SOC group. Although improvement in CGI-SS-R was observed in both treatment groups, estimated treatment differ-

ences were not statistically significant. All indices of suicidality numerically favored ESK+ comprehensive SOC at 4 h and 24 h post dose and day 25. Most common TEAEs (≥20%) observed in ESK + comprehensive SOC vs placebo + comprehensive SOC group during the DB treatment phase were dizziness (38% vs 14%), dissociation (34% vs 6%), nausea (27% vs 14%), somnolence (21% vs 10%), and headache (20% vs 20%), respectively.

Conclusions: In this first global registration program, treatment with ESK + comprehensive SOC demonstrated significant benefits by rapidly reducing depressive symptoms in this vulnerable and heretofore understudied population. The severity of suicidality improved in both treatment groups, though the difference in improvement between groups was not statistically significant. Safety findings were consistent with the established safety profile of ESK.

Keywords: Esketamine Nasal Spray, Major Depressive Disorder, Suicidal Ideation

Disclosure: Janssen R&D, Employee, Johnson & Johnson, Stock / Equity

W131

A Deficit of Doublecortin Immature Neurons in Dentate Gyrus in Untreated Major Depressive Disorder: Possible Reversal by SSRIs Correlates With Clinical State

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Background: Adult hippocampal neurogenesis (AHN) is the process by which new neurons are generated from multipotent neural progenitor cells (NPCs) in the dentate gyrus (DG). In rodents, AHN plays a critical role in memory preservation and encoding (Akers et al., 2013), recovery from stress (Anacker et al., 2018), pattern separation (Clelland et al., 2009) and emotional regulation (Sahay et al., 2007). We reported that individuals with major depressive disorder (MDD) treated with selective-serotonin reuptake inhibitors (SSRIs) have more NPCs (Boldrini et al., 2009) and granule neurons (Boldrini et al., 2014) selectively in the anterior DG compared with untreated MDD, suggesting antidepressants may increase NPC proliferation and differentiation or cell survival. However, no postmortem human study has investigated the effects of MDD and SSRIs on neuronal differentiation. Many studies use doublecortin (DCX) as a marker of immature neurons (Knoth et al., 2010) while several others find it expressed in glial cells (Liu et al., 2018, Sorrells et al., 2018). Therefore, we characterized the glial or neuronal phenotype of DCX+ cells in human DG. Using RNAscope (ACDbio, Inc.) fluorescent in situ hybridization (FISH) and immunofluorescence (IF), we analyzed co-localization of DCX with neuronal markers, including Neuronal Nuclear marker (NeuN) and Neuron-specific class III β-tubulin (TUJ1). We then used RNAscope FISH and IF to test co-localization of DCX with microglia marker Iba1, oligodendrocyte marker Olig2 and astrocytic marker glial fibrillary associated protein (GFAP). We then compared numbers of neuronal and glial DCX+ cells in anterior, mid and posterior DG from non-psychiatric controls (Control), untreated MDD (uMDD) and MDD treated with SSRIs (MDDT).

Methods: Frozen hippocampi were fixed and processed for immunohistochemistry (IHC), IF and RNAscope FISH, confocal laser scanning microscopy and stereology. Clinical and neuropathological data were obtained by psychological autopsy and brain examination. Medication status was confirmed by toxicology screenings. We analyzed the anterior, mid and posterior DG from Control (n = 32), uMDD (n = 19) and MDDT (n = 13) age 25-67 years. The anterior DG was defined as the portion from the most rostral appearance of the DG to the beginning of the lateral geniculate (visible in coronal brain sections); the mid DG spanned the lateral geniculate; and the posterior DG went from the end of the lateral geniculate to the caudal end of the DG. We used Global Assessment Scale (GAS) score as a measure of functioning and symptom severity. High GAS score reflects superior functioning while low GAS score indicates a greater possibility of self-harm. We classified MDDT as "responders" if their GAS score was 50 or above. Mean GAS scores for non-psychiatric controls, uMDD and MDDT were 85.3 ± 8.9, 55.4 ± 20.9; and 41.0 ± 22.9 respectively.

To verify the phenotype of DCX+ cells, we employed IF and RNAscope FISH following our published protocols (Boldrini et al., 2018, Tartt et al., 2018). For RNAscope FISH, three fresh frozen sections from the anterior-mid hippocampus were used. For IF and IHC, we used free floating sections fixed in 4% paraformaldehyde, at 2-mm interval throughout the anterior, mid and posterior DG. Experiments were run with a negative control to distinguish signal from noise. Non-neurogenic regions were assessed to ensure antibody specificity. Fluorescent images were acquired on a Leica 2-Photon confocal microscope using 40x and 63x oil objectives to ascertain markers co-localization and cell morphology. Unbiased stereology was performed to quantify cell numbers (Stereoinvestigator, MBF, Inc.).

Results: We found that DCX-immunorective (IR) cells were fewer in uMDD vs. controls ($p = 0.000163$) in anterior DG. MDDT with high GAS had more DCX-IR cells than uMDD ($p = 0.000214$), while MDDT with low GAS had similar numbers of DCX-IR cells compared to uMDD in anterior DG.

Similarly, SSRI responders (high GAS) had more DCX mRNA+ cells than non-responders (low GAS) in anterior-mid DG ($p = 0.043946$). Controls had more DCX mRNA+ cells vs. both uMDD ($p = 0.000965$) and SSRI non-responders ($p = 0.000872$).

Using FISH, we found that, in all groups, 80-100% of DCX+ cells also expressed neuronal marker TUJ1. Furthermore, we found mRNAs and proteins for DCX and NeuN co-localized in several cells of the DG. Our experiments show close proximity between DCX and glial markers, but these mRNAs and proteins do not co-localize in the same cells.

Conclusions: No study assessed DCX+ cells in treated and untreated MDD compared with controls. Fewer cells expressing DCX protein and mRNA in uMDD suggests less AHN which could be due to downregulated DCX gene expression through unknown molecular mechanisms. Fewer DCX+ cells in uMDD selectively in anterior DG, could be linked to impaired emotional responses in MDD, a function regulated by the anterior hippocampus through the frontal lobe-amygdala circuit. More DCX+ cells in SSRI responders (high GAS) vs. non-responders (low GAS), suggests more AHN could have a role in the antidepressant response. However, in postmortem studies cause and effect cannot be discerned, and we cannot conclude that increased neuronal maturation is a result of antidepressant treatment or that newborn neurons contribute to treatment response. Our findings using IF and FISH demonstrate that DCX+ cells are neuronal and suggests that DCX expression in glial cells (Paredes et al., 2018) may reflect pathological conditions, e.g.epilepsy (Liu et al., 2018) and dementias (Verwer et al 2007).

Keywords: Adult Neurogenesis, Major Depressive Disorder (MDD), Antidepressants, Doublecortin, Glia

Disclosure: Nothing to disclose.

W132

Anterior Insula Sensitivity to Negative Stimuli is Associated With Adaptive Changes in Problem-Solving Style During Integrated Collaborative Care for Comorbid Depression and Obesity

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Background: Depression is associated with maladaptive problem-solving styles. To address this, problem-solving therapies treat depression by reinforcing adaptive problem-solving. However, it remains largely unknown how problem-solving ability relates to neural circuit function in depression. Using an a-priori defined circuit-based biotype model of neural dysfunction, we examined this relationship in an integrated collaborative care intervention for comorbid depression and obesity which incorporates a problem-solving therapy component.

Methods: Participants with comorbid depression and obesity were randomly assigned to either 12-month integrated collaborative care intervention that included problem-solving therapy with as-needed antidepressant medication for depression and a video-based behavioral weight loss treatment or to usual care. At baseline and follow-up visits at 2, 6, and 12 months, a sub-sample of participants ($n = 108$) underwent BOLD functional neuroimaging during a viewing of facial affect paradigm. Composite and constituent region scores were derived for a neural circuit associated with sensitivity to negative affective stimuli using an SPM 8.0-based pipeline. Constituent regions of interest included amygdala, anterior insula, and anterior cingulate cortex. Problem-solving ability (orientation and style) was measured with the Social Problem-Solving Inventory-Revised: Short Form (SPSI-R:S) at baseline and 6- and 12- month follow-up. We examined first whether changes in the negative affect circuit between baseline and 2-month visit were associated with changes in problem-solving ability and second whether this association differed between treatment groups.

Results: The negative affect circuit composite score showed an inverse relationship with changes in the Impulsivity/Carelessness Style (ICS) subscale at 6 months and the Avoidance Style (AS) subscale at 12 months (all $p<.05$), suggesting that increased sensitivity to negative stimuli was associated with reduced maladaptive problem-solving styles. Examining regions within the circuit revealed that this relationship was attributable to activation in anterior insula, which similarly showed a bilateral inverse relationship with the AS and ICS subscales, as well as a positive relationship with overall SPSI-R:S score (all $p<.05$). For the composite circuit score and the anterior insula region, associations consistently interacted with treatment group such that in the integrated intervention group they were more positive for AS and ICS, and more negative for overall SPSI-R:S score, relative to usual care. Main effects and interactions for anterior insula remained consistent for problem-solving style measured at both the 6- and 12-month follow-up visits and across right and left hemispheres (all $p<.05$). No associations were observed with the Rational Problem Solving, Positive Problem Orientation, or Negative Problem Orientation subscales (all $p>.05$).

Conclusions: Altered neural activity in the negative affect circuit was differentially associated with changes in problem-solving style for integrated treatment relative to usual care. Within this circuit, anterior insula sensitivity appeared to play a key role. Reduction in maladaptive problem-solving style was associated with increased neural sensitivity to negative stimuli for treatment as usual, whereas this relationship was reversed for patients receiving integrated intervention with problem-solving therapy. These findings shed light on the neural mechanism of action in integrative care and problem-solving interventions. They furthermore identify a potential neural marker of adaptive change in problem-solving style for individuals with depression and obesity.

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

Disclosure: Nothing to disclose.

W133

The Effect of Ketamine on Reinforcement Learning in Treatment-Resistant Depression

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Background: Unlike any existing antidepressant treatments, ketamine has been shown to have anti-anhedonic properties in treatment-resistant depressed (TRD) patients, yet very little is known about the underlying cognitive mechanisms that drive this. An important cognitive facet of anhedonia may be altered reward and punishment learning. This type of learning relies in part on interactions between the striatum, which encodes prediction errors (PEs: the difference between expected and obtained outcomes), and orbitofrontal cortex, which encodes the expected values of outcomes. We hypothesized that a core mechanism driving symptoms in TRD is an imbalance between these systems, and that alterations in this balance may constitute an important mechanism of ketamine's beneficial effects. We therefore predicted that TRD patients would be characterized by a lower reliance on representing the expected values of outcomes as compared to healthy subjects, which has previously been observed with negative symptoms in schizophrenia, and that ketamine would ameliorate this process.

Methods: Twenty-four TRD patients (recruited 32: 16 women) and thirty-five healthy controls (HC; recruited 37: 16 women) completed a probabilistic reinforcement learning paradigm assessing reward and punishment learning, followed by a transfer test phase. A subgroup ($n = 15$) of the TRD patients performed the same task 24 hours following ketamine (0.5mg/kg) and placebo infusions in a randomized, double-blind, crossover trial. To offer mechanistic insight into behavior, a computational framework was used to examine task performance. Three reinforcement learning models were used to compare different hypotheses of the cognitive strategy participants may use to solve the task: 1) an actor-critic model that learns through PEs; 2) a Q-learning model that learns by representing the expected values of options; and 3) a hybrid model where the actor-critic model is modulated by the Q-model, as represented by a mixing parameter. Parameters for all models were estimated using maximum likelihood, and the best fitting model was assessed with the Akaike information criterion (AIC). To examine differences in reinforcement learning, the parameter values from the winning model were compared between the groups using two-sample t-tests. Paired t-tests were used to examine the effects of ketamine. Bonferroni correction was used to control for multiple comparisons.

Results: Trial-by-trial performance on the task was best modelled using the hybrid model for patients and healthy controls, as well as for patients post ketamine and placebo infusion. HCs had a numerically higher mixing parameter (M: 0.53, SEM: 0.05), compared with TRD patients (M: 0.42, SEM: 0.05), where higher values indicate greater reliance on Q-values. However, this

difference did not achieve significance (t(57)=1.52, p = 0.13, Hedge's g=0.41). Similarly, ketamine did not significantly affect the mixing parameter in TRD patients relative to placebo (t(14) =0.81, p = 0.43, d=0.20). None of the other parameters of the winning model were significantly different between groups (all t<1.94, all p>0.061), nor did ketamine affect these parameters (all t<1.67, all p>0.12).

Conclusions: Consistent with previous research, we found that healthy individuals used a mixture of stimulus-response (actor-critic model) and action-value (Q-learning model) associations in a simple reinforcement learning task. This was also true for TRD patients. However, patients were not characterized by significantly different parameters relative to healthy individuals, suggesting intact reinforcement learning processes. Crucially, and contrary to our predictions, there were no significant differences between groups in the mixing parameter. As such, the data provide no evidence that TRD patients significantly differed from healthy individuals in their use of reinforcement learning strategies (PEs vs incorporation of expected values) to guide their decisions. Importantly, ketamine treatment also did not influence performance on this task, indicating that ketamine may exert its beneficial effects through modulation of other computational mechanisms than those that rely on basic reinforcement learning signals. Future efforts in identifying a mechanistic explanation of ketamine's beneficial effects will likely provide insight into who may benefit from this treatment.

Keywords: Ketamine, Treatment-Resistant Depression, Reinforcement Learning, Computational Psychiatry

Disclosure: Nothing to disclose.

W134

Rhythms and You (RAY): Pilot Data From a Randomized Trial of an Online Intervention for Managing Bipolar Disorder in Primary Care

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Background: Over a third of individuals with bipolar spectrum disorders (BD) are treated exclusively in primary care settings, and yet, primary care providers (PCPs) are ill-equipped to manage this complex and disabling illness. BD-specific psychotherapy is a key element of effective care, and yet, evidence-based BD-specific psychotherapies are typically unavailable in primary care settings. Over 4 billion people worldwide have access to the internet, making this ubiquitous technology an appealing option for extending the reach of psychotherapy. Rhythms and You (RAY) is a fully automated, internet-based, intervention for BD designed for use in primary cares settings. RAY is based on principles of Interpersonal and Social Rhythm Therapy, an evidence-based psychotherapy for BD. The primary goal of RAY is to help individuals regularize their social rhythms to entrain underlying disturbances in circadian and sleep/wake regulation. These factors are increasingly recognized as playing important roles in the pathogenesis of BD. This pilot study examined the feasibility and acceptability of delivering RAY to individuals with BD who receive care from PCPs.

Methods: Individuals were recruited from primary care offices using Best Practice Alerts (embedded alerts in the medical record system that remind doctors to refer potentially eligible patients) and by advertisement. After giving informed consent, participants (n = 47) with BD I, II or NOS were randomly assigned to receive RAY, RAY + Clinical Helper (CH) or Adjunctive Reading Material (ARM), in addition to usual care. Those assigned to RAY received access to 12 interactive, online, modules released weekly over 12 weeks. Lessons are designed to be completed in 15-20 minutes. CH calls occurred weekly, lasted approximately 5 minutes, and focused on promoting participant engagement with the RAY application. ARM participants received 12 weekly, brief readings via email about BD and health. Participants were assessed by telephone and via computer at baseline, 4, 8 and 12 weeks.

Results: Participants were 41.8 (±13.5) years old and mostly female (n = 35; 75%). Over half (n = 25; 53%) met criteria for BD I. Mean depression scores on the Quick Inventory of Depressive Symptoms (QIDS) at baseline were moderately elevated (14.1 ±5.7). Baseline mean Internal State Scale-Activation (ISS-AC) scores, a proxy for hypomania, were mildly elevated but with large variability (232.6 ±123.6). QIDS scores declined significantly over time (F=4.437, df=1, p = 0.049) but there was no significant time *group interaction (F=0.682, df=2, p>0.05). ISS-AC scores did not change over time (F=1.65, df=1, p >.05), nor was there a significant time*group interaction. Brief Social Rhythm Scores improved significantly over time (F=11.5, df=1, p = 0.003) but there was no time*group interaction. Overall rates of satisfaction (Client Satisfaction Questionnaire, CSQ) were high 25.1 ±5.5. Although there were no statistically significant differences among groups (F=1.2, df=2, p >0.05), mean CSQ scores were numerically highest in RAY-CH (27.6) versus RAY (23.7) and ARM (24.1).

Conclusions: Conclusions that can be drawn from this randomized trial are limited by the small sample size. However, these preliminary data demonstrate the feasibility of remotely delivering a new online intervention focused on regularizing social rhythms for individuals with BD. Participants assigned to RAY-CH had somewhat greater satisfaction with their treatment than RAY alone, suggesting that acceptability is enhanced by adding a small amount of human support to the automated intervention, a finding that is consistent with other studies of online interventions for psychiatric disorders. Future research should include conducting an adequately powered RCT to evaluate the effectiveness of RAY as an adjunctive treatment for BD in primary care.

Keywords: Psychotherapy, Bipolar Disorder, Internet

Disclosure: American Psychiatric Association Publishing, Royalties, Myriad Genetics, Grant, Wolters Kluwer, Royalties

W135

Neural Correlates of Long-Term Exposure to Lithium in Adults With a Childhood Diagnosis of Bipolar Disorder who Have Been Prospectively Characterized for up to 18 Years of Illness

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Background: Bipolar Disorder (BD) is a chronic mental illness characterized by mood fluctuations (recurrences) and often requiring lifelong treatments. Mood stabilizers represent the first treatment for acute episodes and prophylaxis of recurrences in patients with BD. Among mood stabilizers, Lithium has shown to be an effective treatment of acute mania, prophylaxis of recurrences, and prevention of suicide attempts. Most neuroimaging studies have looked at the effect of Lithium cross-sectionally. These studies have shown that taking lithium at the time of scan was associated with increased volumes of the amygdala, hippocampus, frontal, temporal, and parietal lobes, and widespread increased cortical thickness. Yet, the effect of long-term exposure to lithium on the brain and how this affects mood

recurrence in BD remain unclear. By following youth with BD for up to 18 years, the Course and Outcome of Bipolar Youth (COBY) study has identified demographic and clinical risk factors predicting trajectories of different BD courses. The present study seeks to elucidate the influence of long-term exposure to Lithium on different structural brain measurements and how alterations in these measures correlate with severity of mood symptoms at scan.

Methods: Clinical data including weekly assessment of depressive/manic symptoms (Psychiatric Status Ratings range 0-6) and pharmacological treatments (medications classes and doses) were collected at each follow-up visit (average between visits=12.42 months) for up to 18 years, using the Adolescent Longitudinal Interval Follow-Up Evaluation. Medications included mood stabilizers, antidepressants, antipsychotics, and stimulants. Structural neuroimaging data was also collected in 66 BD participants (mean age [SD] = 26.3 [3.9] years, M/F=34/32; R/L=57/9). Structural brain measurements included cortical thickness, local cortical gyrification, and cortical and subcortical volumes in 47 brain regions. Gyrification is an index of deepness of sulci. Severity of mood symptoms were assessed at the time of the scan using the Hamilton Depression Rating Scale (HDRS), Young Mania Rating Scale (YMRS), and Mood and Anxiety Questionnaire (MASQ). Data from 34 healthy controls (HC; mean age [SD] = 26.4 [4.7] years, M/F=18/16; R/L=27/7) were used as reference.

A three-level analytic approach was used.

Level 1: Medication data on the use of lithium, non-lithium mood stabilizers, antidepressants, antipsychotics, and stimulants were binary coded (yes/no). The annual sum of weeks using Lithium for each year of follow-up was imported to SAS. Latent class analysis using TRAJ was used to derive different lithium-trajectories.

Level 2: To identify structural brain measurements that better explain our outcome of interest (i.e., lithium-trajectories identified in Level 1 analysis), a regularized linear model was performed using glmnet in R. Glmnet fits a generalized linear model via penalized maximum likelihood using lasso, ridge, or elastic-net techniques to rank predictors importance (nonzero coefficients). These nonzero coefficients allow selection of the most import predictors and removal of variables with negligible effect on outcome. Demographic, clinical, and neuroimaging data were potential predictors in Level 2 analysis.

Level 3: Stronger predictors identified in Level 2 analysis were imported into SPSS as best predictors for our model. Multivariate Analysis of CoVariance, MANCOVA and post-hoc analyses were used to assess the effect of. Age and gender were covariates in these analyses. Data from heathy controls (HC) was used as reference. False Discovery Rate was used to account for multiple comparisons.

The effect of other medication classes (non-lithium MS, AD, AP, SM) on main findings was explored.

Results: Level 1: BD were classified into BD with long-term lithium exposure (N = 52) and BD with no/inconsistent use of lithium (N = 24).

Level 2: Local gyrification measures in the following regions were the stronger predictors of long-term lithium exposure: left/right medial orbitofrontal cortex (mOFC), right lateral orbitofrontal (lOFC), and superior parietal cortex.

Level 3: MANCOVAs confirmed that there was a main effect of lithium exposure in the left (F=7.4, P=0.008) and right (F=8.7, P=0.005) mOFC, and in the right lOFC (F=9.0, P=0.004). Specifically, BD participants with long-term exposure to lithium showed deeper gyrification of all three OFC regions than BD participants with no/inconsistent exposure. There was no between-group difference in the superior parietal cortex.

When compared to HC, BD participants with long-term exposure to lithium showed deeper gyrification of the right lOFC (F=4.8, p = 0.033) but there were no differences in the left and right mOFC (P>0.005). Conversely, BD participants with no/inconsistent exposure to Lithium showed lower gyrification in the left (F=5.6, p = 0.020) and right (F=5.8, p = 0.019) mOFC, but there were no differences in the lOFC (P>0.005).

Further analyses revealed that the deeper gyrification in the right lOFC was associated with lower depressive symptoms at scan (HDRS: r = −0.252, P=0.041; MASQ: r = −0.250, P=0.043).

There was no effect of other medication classes in these regions.

Conclusions: Our findings suggest that long-term lithium exposure in BD patients can promote significant normalizing effects in the gyrification of the mOFC. In addition, the deeper gyrification of the lOFC and its association with lower depressive symptoms might reflect a compensatory mechanism in BD.

Keywords: Bipolar Disorder, Lithium, Neuroimaging, Medial Orbitofrontal Cortex, Lateral Orbitofrontal Cortex

Disclosure: Nothing to disclose.

W136

A Meta-Analysis of Combinatorial Pharmacogenetic Guided Antidepressant Treatment for Major Depressive Disorder

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Background: Advancements in the clinical implementation of combinatorial multi-gene pharmacogenetically guided treatments in psychiatry have been driven primarily by industry. These efforts have been criticized by the FDA and others as putting the proverbial cart before the horse. Questions remain about the clinical utility of pharmacogenetics in psychiatry. The aim of this work is to use meta-analysis to address those questions, in particular for Major Depressive Disorder (MDD).

Methods: A binary random effects model and the DerSimonian-Laird method for meta-analysis were used. We identified four studies that met the criteria for inclusion; prospective controlled trials of combinatorial pharmacogenetically guided treatments. The total N = 2098. Analysis was performed with the Open MA software package.

Results: The pharmacogenetically guided interventions were significantly superior to treatment as usual (TAU) in terms of response: OR = 1.538, (95% CI, 1.227-1.927) p < 0.001). Statistical heterogeneity was non-significant and low for response: Q = 2.278, p = 0.320, I squared = 12.187%. The pharmacogenetically guided intervention was superior to TAU in terms of remission; OR = 2.3, (95%CI, 1.124-4.688) p = 0.023. Statistical heterogeneity for remission was significant and high, however, (Q = 19.203, p< 0.001, I squared = 84.378%). The corresponding NNT for remission is 11.5. There was not clear evidence of publication bias, however the number of total studies was small and only the largest study had rigorous methodology.

Conclusions: A growing body of evidence supports the use of combinatorial pharmacogenetic methods in depression treatment which appear to have clinically significant effects and probable clinical utility. The effect on response to treatment was smaller than remission but more stable as it had less heterogeneity. Due to the small number of studies and few with rigorous methodology, these results should be viewed cautiously but suggest that further optimization and study of these methods is warranted, and that they may be clinically useful.

Keywords: Depression, Pharmacogenetic Response, Treatment-Response, Antidepressant Response, Remission

Disclosure: Nothing to disclose.

W137

Presence of Cardiometabolic Risk Factors in Bipolar Disorder

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Background: Recent work from the Rochester Epidemiology Project identified a significant association between bipolar disorder (BD) and MACE, a composite measure of myocardial infarction, ischemic and hemorrhagic stroke, percutaneous coronary intervention, cardiac bypass graft surgery, and total mortality (Morgan et al., 2019). This study was conducted to further evaluate cardiometabolic risk factors in BD patients compared to general population controls.

Methods: Cardiometabolic risk factors were quantified in participants diagnosed with BD (confirmed by the Structured Clinical Interview for DSM-IV) from the Mayo Clinic Individualized Medicine Biobank for Bipolar Disorder (n = 661), and age-sex-race-matched controls from the Mayo Clinic Biobank (n = 706). Cardiometabolic risk factors were analyzed as continuous variables and binary high/low risk variables (i.e. waist circumference (WC) ≥102 cm in men or ≥88 cm in women, BMI ≥25 kg/m2, triglycerides ≥150 mg/dl, total cholesterol (TC) ≥240mg/dL, HDL ≤40 mg/dl, LDL ≥190 mg/dL, systolic and diastolic blood pressure (BP) ≥130/80 mm Hg, and fasting plasma glucose ≥110 mg/dl. Associations between risk factors, cardiometabolic medications, and BD illness were conducted using linear and logistic regression.

Results: BD patients had higher BMI (BD=30.1± 6.6, C = 29.2± 6.2, p = 0.007), WC (BD=104.9± 18.0, C=97.9± 16.7, p < 0.001), triglycerides (BD=149.4±97.5, C = 121.2±61.9, p < 0.001), systolic BP (BD=124.3±17.7, C=118.1±13.9, p < 0.001) and diastolic BP (BD=75.1± 11.1, C = 72.9± 9.2, p = 0.006), when compared to controls. Binary analyses showed similar significant differences: elevated triglycerides (36.9% vs. 26.2%, p < 0.001), lower HDL levels (23.4% vs. 14.9%, p < 0.001), elevated systolic (35.2% vs. 18.8%, p < 0.001), and diastolic BP (33.0% vs. 23.4%, p = 0.001). Proportions of antihypertensive and antidiabetic medication use were not significantly different; however, in comparison to controls, a significantly lower proportion of BD patients used lipid-lowering medications (39% vs. 53%, p < 0.001), and a higher proportion used thyroid supplementation (29% vs. 20%, p < 0.001). After adjusting for all cardiometabolic medication use, BD association with cardiometabolic variables remained significant for WC, triglycerides, systolic and diastolic BP, but not for BMI. When analyses were stratified by sex, we found that female BD patients had higher rates of abnormal fasting glucose (17.3% vs. 9.3%, p = 0.006) compared to controls.

Conclusions: In this matched set of BD cases and controls, we observed differences in cardiometabolic risk factors even after adjusting for use of cardiometabolic medications. These controlled data further delineate cardiometabolic risk factors that contribute the increased risk of cardiovascular disease in patients with bipolar disorder. The physiological targets of central adiposity / elevated triglycerides and increased vascular tone (i.e. elevated systolic and diastolic pressures) warrant further investigation into the temporal relationship between these objective measurement abnormalities and bipolar symptom onset and whether there are markers (i.e. cardiometabolic candidate genes, pathway analysis, family history) associated with either mood or cardiometabolic disease onset.

Keywords: Bipolar Disorder, Cardiometabolic Risk, Epidemiology

Disclosure: Nothing to disclose.

W138

A Preliminary Two-Site, Open-Label, Non-Randomized Trial, Comparing Focal Electrically Administered Seizure Therapy (FEAST) and, Right Unilateral Ultra-Brief Pulse Electroconvulsive Therapy (UBP-RUL ECT)

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Background: Focal Electrically Administered Seizure Therapy (FEAST) is a form of electroconvulsive therapy (ECT) which maximally focuses the electrical stimulus to the frontal lobe. Recent open-label studies suggest that FEAST's enhanced focality may lead to reduced cognitive side effects (1, 2), however there are no studies directly comparing FEAST to other forms of ECT (such as Right Unilateral Ultra-Brief Pulse ECT (UBP-RUL-ECT). We subsequently compared the efficacy, and cognitive side effect burden of FEAST and UBP-RUL-ECT.

Methods: In a non-randomized, open-label fashion, 39 depressed adults were recruited after being referred for ECT. Twenty received FEAST (14 women; age 45.2+12.7), and 19 received UBP-RUL-ECT (16 women; age 43.2+16.4). Cognitive effects were measured using the time to reorientation (assessed at each treatment), and the Columbia Autobiographical Memory Interview Short-Form (CAMI-SF; assessed prior to, and following the treatment course). Efficacy was measured using the Hamilton Rating Scale for Depression (HRSD24; assessed prior to, and following the treatment course).

Results: Those receiving FEAST, had a numeric reduction in mean time to reorientation (FEAST = 6.6+5.0 minutes; UBP-RUL-ECT = 8.8+5.8 minutes; p = ns; Cohen's d=0.41). Similarly, participants receiving FEAST had a numerically improved consistency score on the CUMI-SF (FEAST = 69.2+14.2%; UBP-RUL-ECT = 63.9+9.9%; p = ns; Cohen's d=0.43) Average reduction in HRSD24 was nearly equivalent between the two groups (average reduction: FEAST = 60.7+18.5%; UBP-RUL-ECT = 59.2+21.4%; p = ns), while response rate numerically favored FEAST (50% reduction in HRSD24: FEAST = 65%; UBP-RUL-ECT = 57.9%), and remission rate numerically favored UBP-RUL-ECT (HRSD24 ≤ 10: FEAST = 35%; UBP-RUL-ECT = 47.4%).

Conclusions: These data further support the hypothesis that treatment with FEAST may have a milder cognitive side effect profile as compared to conventional ECT, while retaining similar efficacy. A blinded, randomized trial in a larger sample will be needed to definitively determine if this is the case.

Keywords: Electroconvulsive Therapy, Depression, Brain Stimulation

Disclosure: Nothing to disclose.

W139

Interleukin-1β: A Promising Inflammation Biomarker in Treatment Resistant Bipolar Depression

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460

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Background: Adjunctive inflammatory modulation improves remission rates in treatment-resistant bipolar depression (TRBDD), but reliable biomarkers are needed to characterize the biosignature of TRBDD and the mechanisms underlying treatment response. Additionally, there is a need for novel therapeutic strategies and interventions at the level of inflammatory cytokine signaling. To that end, this molecular profiling study aims to describe TRBDD and treatment response from the standpoint of interleukin-1 Beta (IL-1β) and the Tryptophan-Kynurenine Pathway (TRP/KYN).

A number of clinical trials have already demonstrated the efficacy of adjunctive inflammatory modulation with the cyclooxygenase-2 (COX-2) inhibitor, celecoxib (CBX), in major depressive disorder (MDD). This study, to our knowledge, is the first to replicate the efficacy of CBX add-on therapy in TRBDD. In addition, this study is the first to address the need for molecular work characterizing the cytokine signature of TRBDD and the putative role of IL-1β in the mechanism(s) of treatment response.

Based on the premise that IL-1β induces and maintains a pro-inflammatory state through chronic activation of COX-2, we hypothesized that COX-2 inhibition via CBX add-on should improve response rates, at least in part, by ameliorating the total pro-inflammatory cytokine burden promoted by IL-1β.

Methods: This was a randomized, double-blind, two-arm, placebo-controlled study consisting of a screening visit, a 2-week washout, and a 1-week placebo run-in phase. Subjects who met study criteria and were not placebo-responders underwent a physical exam, medical history, routine laboratory tests, and completed a number of rating instruments. At their baseline visit, they were rated in a blinded manner, as if they had been actively treated. If they continued to score ≥18 on the 17-item Hamilton Depression scale (HAM-D), they were randomized to receive ESC + CBX, or ESC + placebo.

47 TRBDD patients with moderately severe HAMD-17 scores were randomized to receive either escitalopram (ESC) (10mg twice/day) + celecoxib (CBX) (200mg twice daily), or ESC (10mg twice daily) + placebo (PBO) (twice daily). Plasma cytokine levels were measured in both treatment arms at baseline and week 8, and in a healthy control (HC) group of subjects (N = 35) once. A binary logistic regression equation was modeled from this series to predict diagnosis of TRBDD using age, BMI, gender, and IL-1β at baseline.

Results: Patients receiving ESC + CBX had 4.278 greater odds of responding ($p = 0.021$) with NNT=3, and 15.300 greater odds of remitting ($p < 0.001$) with NNT=2, compared with ESC + PBO patients. Patient BMI ($p = 0.003$), baseline IL-1β ($p = 0.004$), and baseline KYN/TRP ($p = 0.001$) were most predictive of TRBDD diagnosis. By Week 8, responders showed a more significant decrease in IL-1β compared to non-responders ($p = 0.049$), effectively normalizing IL-1β compared to HC levels ($p = 0.067$).

Conclusions: The immediate clinical impact of this molecular profiling study involves the role of IL-1β as a candidate prognostic and therapeutic biomarker for CBX augmentation in TRBDD. Elevated IL-1β may prove to be a prognostic marker for treatment resistance and ultimately an important cornerstone of the TRBDD cytokine signature. The decrease in IL-1β over the course of treatment, furthermore, may be a useful therapeutic biomarker for tracking clinical response and remission. More work is needed to elucidate the IL-1β and the TRBDD cytokine signature generally, to further optimize treatment outcomes and personalize psychiatric care.

Keywords: Bipolar I Depression, Depression Inflammation Cytokine, Interleukins, Cyclooxygenase, Kynurenine Metabolism

Disclosure: Nothing to disclose.

W140

Intestinal Microbiota Predict Response to Antidepressant Treatment in a Pilot Randomized Placebo-Controlled Trial of Levomilnacipran in Late-Life Depression

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Background: Late-life depression (LLD) is often accompanied by significant physical and cognitive comorbidity, less responsive to antidepressant treatment and more prone to later relapse than depression in younger adults. However, there are no validated biomarkers proven to be clinically useful in guiding LLD treatment algorithms. Gut microbiome changes occur with aging as well as depression in humans but their association with antidepressant treatment response has not yet been investigated. We assessed changes in gut microbiota with antidepressant treatment and evaluated whether baseline microbiota can predict treatment response in a pilot randomized placebo-controlled trial of Levomilnacipran (LVM) treatment in LLD.

Methods: Seventeen adults (>60yrs) with major depression (41.2% female; mean age=70.2±5.8 years; mean education = 15.9 ±1.5 years) were randomized to either 20-120mg of LVM or placebo for 12 weeks. This trial was registered on clinicaltrials.gov (NCT02466958). Stool samples were acquired at baseline and 12 weeks for 16S-rRNA based fecal microbiome composition analysis. Thirteen subjects (4 LVM and 9 placebo) completed the study. Due to high dropout rate in the LVM group (43%), the LVM and placebo groups were combined for the subsequent analyses. Group differences in microbial community composition were evaluated based on treatment response (HAMD $<= 6$) and timepoint regardless of the treatment received. Statistical significance of differences in microbial beta diversity was assessed using Adonis algorithm, a nonparametric method of analysis of variance. Random forest analysis was used to generate baseline microbiome-based classifier for prediction of treatment response.

Results: Response (HAMD<=6 at the end of treatment) was achieved by 2/4 LVM participants (50%) and 3/9 (33%) of participants in the placebo group. There was a significant association between baseline microbiome composition with treatment response after adjusting for sex and treatment ($p = 0.024$). There were no significant baseline alpha diversity differences measured by species richness (Chao1), phylogenetic diversity (Faith's PD) and species evenness (Shannon index) between responder and non-responder groups. Baseline microbiome-based random forest classifier included 10 genera with high predictive accuracy for treatment response (AUC=0.95). Of these, Ruminococcaceae UBA1819 was decreased in responders compared to non-responders and contributed the most to the classifier accuracy. Also, a trend towards microbial composition change after treatment was found only in responders but not among the nonresponders.

Conclusions: This is the first pilot study using fecal microbiota to predict treatment response in geriatric depression. We also observed longitudinal change in microbial composition associated with treatment response. Our findings need to be confirmed in larger prospective studies.

Keywords: Gut Microbiota, Microbiota-Gut-Brain Axis, Antidepressant Trials, Clinical Outcome Prediction

Disclosure: Nothing to disclose.

W141

ChRERα: Noninvasive, Longitudinal, and Quantitative Opsin Localization for Research and Clinical Optogenetics Applications

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Background: Optogenetics comprises a revolutionary technology for precise manipulation of cellular processes with strong potential as a therapeutic technology for nervous system disorders. However, a critical limitation of existing optogenetic technologies is the inability to monitor opsin expression and localization in the body in a nondestructive and longitudinal manner. This drawback minimizes the applicability of optogenetics to nonhuman primate (NHP) applications as one cannot predict the location of virally-mediated opsin expression downstream of the virus injection site, limiting the ability to target discrete neuronal terminal projection sites for in vivo optogenetic terminal stimulation. Additionally, this limitation presents a barrier to clinical translation of CNS optogenetics applications where clinical decisions and patient outcomes would undoubtedly benefit from longitudinal tracking of opsin expression in targeted areas. We have shown that positron emission tomography (PET) can be used to localize, noninvasively and quantitatively with picomolar sensitivity, virally-mediated chemogenetic construct expression at both the local site of adeno-associated (AAV) injection in the brain as well as at neuron terminal projection sites. This offers the unprecedented ability to visualize molecularly- and anatomically-defined neuronal projections in the living brain, a critical advance for translational neuropsychiatric research. We reasoned that a similar PET-based reporter system for optogenetics that provides noninvasive and longitudinal quantitative assessment of opsin expression would transcend the current barriers facing this technology and would facilitate both advanced optogenetic NHP brain circuit investigations and clinical optogenetics applications.

Methods: We fused the ligand binding domain (LBD) of the human estrogen receptor alpha (ERα) in several positions of the classic Channelrhodopsin-2 (ChR2) opsin to create novel "ChRERα" (pronounced "carrera") fusion opsins. We screened ChRERα variants for in vitro and in vivo efficacy, and for in vitro high-affinity binding to [3H]estradiol and to its fluorinated analog fluoroestradiol (FES), whose radiolabeled analog, [18F]FES, is clinically-approved and currently used for cancer detection. We also performed in vivo studies using PET and [18F]FES to assess expression and localization of ChRERα in vivo.

Results: We identified one ChRERα variant where the in vitro and in vivo functional properties of ChR2 and the binding properties of ERα-LBD to FES were conserved. [18F]FES showed minimal binding to endogenous ERα in brain as their expression level in this organ is very low. In contrast, [18F]FES enabled successful detection of ChRERα at both local AAV-ChRERα injection sites (unilateral prefrontal or motor cortex) and at downstream projection sites (e.g. corticostriatal and corticothalamic terminal projection sites).

Conclusions: With the advance of optogenetics strategies to control cellular activity, the development of noninvasive viral vectors and delivery systems, and the continually increasing knowledge of brain circuit contributions to behavior, a critical hurdle to the translational and clinical implementation of such technologies is an effective, reproducible, and noninvasive reporter system that allows for longitudinal monitoring of the expression of the transduced therapeutic opsin in a sensitive and quantitative manner. PET is a clinical diagnostic tool that offers unique possibilities to remotely track ligand binding to a given target and the ERα-LBD offers a small, inactive, and non-immunogenic protein moiety that is in fact not limited to opsins but can be potentially attached to any effector protein and used as a dock for the clinically-approved PET ligand [18F]FES. With this scalable technology, we now have a way to monitor the expression of various opsins and other proteins that lack a known LBD, and to analyze their neuroanatomical distribution in a noninvasive, quantitative, and longitudinal manner.

Keywords: Optogenetics, PET, Circuit, Neuroimaging, Neuromodulation

Disclosure: Metis Laboratories, Stock / Equity

W142

A Phase 1 Healthy Volunteer Study of the Safety, Tolerability and Pharmacokinetics of TRV250, a G Protein-Selective Delta Receptor Agonist

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Background: The delta opioid receptor (DOR) has been implicated in a diverse array of neuronal processes of potential relevance to major central nervous system disorders. For example, DOR agonists have demonstrated activity in nonclinical models of migraine, nociceptive, inflammatory and neuropathic pain, depression and anxiety. In addition, unlike conventional mu-opioid receptor agonists that are used to treat other painful conditions, studies in rats and monkeys evaluating abuse potential for DOR ligands suggest that these agonists may not demonstrate high abuse liability.

DOR agonists significantly reduce nitroglycerin-evoked hyperalgesia and conditioned place aversion in preclinical migraine models. In addition, DOR agonists reduce KCl-stimulated cortical spreading depression, a phenomenon associated with cortical excitability during migraine. Functional activity at the DOR has also been linked to the calcitonin gene related peptide (CGRP), which is thought to play a key role in migraine. Diffuse dural innervation peptidergic CGRP-expressing C fibers co-express the DOR, suggesting that agonists of the DOR could exert anti-migraine effects in part by inhibition of CGRP release, providing a novel therapy for the treatment of migraine.

Attempts at clinical development of a DOR agonist has been hampered by on-target convulsant effects. Data suggest that β-arrestin2 recruitment plays a critical role in DOR-mediated convulsions. Hence, a potent DOR agonist with reduced β-arrestin2 recruitment could offer an improved therapeutic index over previous candidates in this class.

TRV250 is a novel small molecule agonist of the DOR that acts in a manner preferentially selective for G-protein signaling, with relatively little activation of the β-arrestin2 post-receptor signaling pathway. TRV250 significantly reduces nitroglycerin-evoked hyperalgesia in rodents, a model used to screen candidates for potential utility in acute migraine and is being developed for the acute treatment of migraine in humans. Nonclinical studies indicate that TRV250 shows a substantial reduction in pro-convulsant activity compared to other, non G protein-selective DOR agonists, with an established 50- to 80-fold margin between therapeutic effect and seizures in rodents and monkeys.

Methods: This was a two-part single ascending dose study. Part A included four cohorts of healthy adults (N = 38). Each cohort was dosed on three occasions (placebo and two different dose levels of TRV250, allocated in randomized order and administered by subcutaneous route). In part B, a single cohort (N = 9) of

subjects received an oral dose administration of either TRV250 or placebo in a fed or fasted state. Serial blood samples were obtained for pharmacokinetic determination across a 24-hour post-dose period. Safety assessments included clinical laboratory measures, vital signs, 12-lead ECGs, and electroencephalograms pre- and post-dosing.

Results: TRV250 was well tolerated in both study parts. There were no serious adverse events, and all treatment-related adverse events were mild in severity. There were no clinically significant changes in any safety parameters. Specifically, no subject experienced abnormalities in EEGs, and no subject experienced a change from baseline in rate-corrected QT interval (QTcF) greater than 60 msec, or an absolute QTcF interval greater than 480 msec at any post-dosing observation. Peak and total plasma exposure to TRV250 increased in a dose-proportional manner following 0.1 to 30mg SC doses, with the mean half-life ranging from 2.39 to 3.76 hours. Oral bioavailability of TRV250 ranged from 14% (fasting) to 20% (fed) relative to SC dosing, while administration with food reduced the rate of absorption as reflected by a modest delay in median Tmax and a slight reduction in Cmax.

Conclusions: In this first-in-human study, TRV250, a G protein-selective DOR agonist, showed safety, tolerability and a pharmacokinetic profile supporting its potential use in the treatment of acute migraine.

Keywords: Delta Opioid Receptor, Phase 1 Study, Migraine

Disclosure: Trevena, Inc., Employee

W143

Cariprazine Alleviates Core Behavioral Deficits in the Prenatal Valproic Acid Exposure Model of Autism Spectrum Disorder

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Background: Autism spectrum disorder (ASD) is a frequent neurodevelopmental condition with pediatric prevalence values as high as 1:59 in the United States. It is characterized by core symptoms including socio-communicational deficits and restricted/repetitive behaviors. The core symptoms may be associated with a wide variety of additional symptoms such as intellectual disability, attention deficit, hyperactivity, seizures, sleep problems, etc. There is a large unmet medical need in ASD: symptoms may be severe and there is no approved pharmacotherapy for the treatment of the core symptoms. Risperidone and aripiprazole are marketed for the treatment of irritability associated with autistic disorder in pediatric patients. Cariprazine (Vraylar® in US; Reagila® in Europe), a dopamine D3-preferring D3/D2 and serotonin 5-HT1A receptor partial agonist drug, has already been approved for the treatment of schizophrenia and bipolar I disorder in adults, and its investigation in patients with ASD is warranted. The aim of this non-clinical study was to investigate the effects of cariprazine, in comparison with risperidone and aripiprazole, in a widely accepted and translationally sound animal model of ASD on behavioral endpoints representing the core symptoms of the disorder.

Methods: To induce an autistic-like condition in the offspring, time-mated Wistar Han Rcc rats were treated with 600 mg/kg VPA i.p. on gestational day 12.5. Male offspring were tested for behavioral deficits at a juvenile age (postnatal days [PND] 30-32) and at young adult age (PND 59-60). The behavioral test battery employed included social play (PND 30), open field (PND 31-32), social approach-avoidance (PND 59) and social novelty recognition (PND 60). Animals were dosed orally, once a day for 8 days, with test compounds (cariprazine, risperidone, aripiprazole) or vehicle (5% Tween 80 in distilled water) before behavioral assessment. Cariprazine was administered at the doses of 0.003, 0.01, 0.03 and 0.1 mg/kg. Risperidone was administered at 0.1 mg/kg, while aripiprazole was given at the dose of 1 mg/kg. The histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA, 5 mg/kg, i.p.) was included as an internal control. Animals were randomly assigned to each treatment group, with blocked analysis of behavior conducted between 8am and 11am to reduce potential bias between groups ($n = 8$). All procedures conformed to the guidelines of the National Institutes of Health for the care and use of laboratory animals, were approved by the Animal Research Ethics Committee of University College Dublin (Ireland), and were carried out in strict compliance with the European Directive 2010/63/EU regarding the care and use of laboratory animals for experimental procedures.

Results: In the social play paradigm, cariprazine showed a dose-dependent and complete reversal of defective play and general social behavior, reaching significance at the 0.1 mg/kg dose. Risperidone and aripiprazole failed to improve play or general social behavior in this assay. In the social approach-avoidance test, cariprazine dose-dependently and fully reversed the social deficit, reaching significance at the highest dose tested. In this assay, risperidone and aripiprazole were similarly effective, also causing full reversal. Dose-dependent, significant rescue of defective social memory was observed in the social recognition memory test following treatment with cariprazine from the dose as low as 0.03 mg/kg. Risperidone and aripiprazole had similar effects in this assay. Stereotypical/repetitive behaviors (circling and excessive grooming), present in the open field, were reduced by all applied doses of cariprazine as well as by risperidone and aripiprazole. Hyperactivity (distance travelled and center crossings) in the open field was also significantly decreased by cariprazine, i.e. the distance covered was significantly reduced at the dose of 0.1 mg/kg, while center crossings were diminished at the lower dose of 0.01 mg/kg. Likewise, risperidone and aripiprazole displayed efficacy in this trial.

Conclusions: In the present study, cariprazine effectively reversed core behavioral deficits and hyperactivity present in juvenile and young adult autistic-like rats. Risperidone and aripiprazole were inactive in the social play paradigm, though they behaved similarly to cariprazine on other endpoints. This differentiation of cariprazine from the other two compounds is of particular importance, considering that social play is also a principal indicator of healthy development in humans, and social play is the earliest form of social behavior directed towards peers and not the mother. The results presented here indicate that cariprazine may be a useful pharmacotherapy against the symptoms of ASD in humans.

Keywords: Cariprazine, Autism Spectrum Disorder, Neurodevelopmental and Behavioral Deficits, Pharmacotherapy

Disclosure: Allergan, Employee

W144

Investigating how the Prefrontal -Lateral Hypothalamic Circuit Modulates Social Dominance

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Background: Both humans and mice live in groups organized by social hierarchies. By adjusting behavior based on their social rank, animals decrease unnecessary aggression and save energy.

Although hierarchies are central to successful group dynamics, the neural basis of dominance behaviors remains poorly understood. Cross-species evidence suggests that the medial prefrontal cortex (mPFC) is crucial for social dominance behaviors (Wang et al., 2011, Zhou et al., 2017, Zink et al., 2008). Given the role of the lateral hypothalamus (LH) in homeostatic functions, and its connectivity with the mPFC, it is well-positioned to help modulate social behaviors in a rank-dependent manner.

Methods: Considering that dominant animals typically exercise priority access to resources, we designed a novel behavioral task, the "reward competition assay". This assay utilizes a trial structure to facilitate statistical comparisons wherein mice compete for a reward that is signaled by a tone. Male mice were trained to associate a tone with an Ensure reward and once they learned the task, they were paired with a cage mate to compete for the reward. To validate this task, we ranked mice using the tube test (Wang et al., 2011) and tested them on the reward competition assay. Across the session, dominant mice (as defined by tube rank) won more rewards than subordinates, as the percent of rewards obtained for the dominant mice was higher (paired t-test; % rewards subordinate vs dominant $p < 0.01$; $n = 12$). Using this novel reward competition assay we investigated the role of mPFC-LH projectors in social dominance.

Results: Our preliminary data show that optogenetic stimulation of the mPFC->LH projectors increased winning in subordinate mice (paired t-test; % rewards obtained OFF vs ON sessions $p = 0.0047$; $n = 5$). However, we saw no effects of stimulation when mice were performing the reward task alone, indicating that mPFC->LH projectors modulate social competition for a reward, but not reward-seeking behavior. Furthermore, we used wireless electrophysiology to investigate how mPFC activity relates to social dominance during the reward competition assay. We recorded mPFC single units in both dominant and subordinate mice ($n = 4$) while they competed for Ensure rewards. Preliminary data suggest that mPFC single units represent competition as they fired differently in trials when there were high vs low levels of competition for the reward ($n = 74$ single units recorded).

Conclusions: Considering our data, we hypothesize that the mPFC-LH pathway carries social rank information that is integrated with other homeostatic systems such as those regulating energy balance.

Keywords: Social Behaviors, Medial Prefrontal Cortex, Circuit Optogenetics, Lateral Hypothalamus, Social Dominance

Disclosure: Nothing to disclose.

W145

Cortical Network Activity Predicts IGF-1 Treatment Response in Rett Syndrome

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Background: Rett Syndrome is a neurodevelopment disorder associated with mutations in the gene MECP2, which is involved in the development and function of cortical networks. This dysfunction results in specific electrophysiological abnormalities which are associated with clinical features. Insulin-like growth factor 1 (IGF-1) has been shown to ameliorate the symptoms of Rett in animal models and in early clinical trials, but it remains unclear whether IGF-1 treatment impacts the underlying network architecture in patients with Rett.

Methods: In this study, we performed clinical assessment and resting-state EEG recordings in eighteen patients with Rett, nine of which were treated with IGF-1. We repeated the assessment at baseline and twelve months following treatment. Network measures were derived using statistical modelling techniques based on inter-electrode coherence measures.

Results: We demonstrate that IGF-1 treatment is associated with alterations in network measures, and that there are differences in network architecture associated with clinical response. Further, we show that network measures capture heterogeneity within the Rett Syndrome population that is not evident clinically, and that there are electrophysiological differences between treatment responders and non-responders prior to IGF-1 treatment.

Further, we assessed the ability of these computational biomarkers to predict treatment response. Using derived network measures, we trained a support vector machine model and we demonstrate that pre-treatment network measures can predict treatment response in unseen patient data with 100% accuracy (100% sensitivity & 100% specificity) in this small group.

Conclusions: These results further underline the importance of network pathology in this disorder and highlights the potential for approaches using these techniques to better characterise disease and allow more targeted treatment.

Keywords: Rett Syndrome, Insulin-Like Growth Factor 1, Quantitative EEG

Disclosure: Nothing to disclose.

W146

Disruption of Adult Hippocampal Neurogenesis Following Androgen Deprivation

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Background: At some point almost half of all prostate cancer patients receive androgen deprivation therapy. This involves surgical castration, the administration of an androgen receptor antagonist or a gonadotropin-releasing hormone analog that reduces plasma testosterone levels. However, an important side effect experienced in many patients is impairment of cognitive function. This is manifested by deficits in executive functioning, spatial abilities and working memory. In the dentate gyrus of the hippocampus, throughout adulthood neural stem cells and progenitors proliferate and differentiate into new neurons. This phenomenon is known as adult neurogenesis and is involved in some aspects of memory and learning. The goal of this study was to test the hypothesis that androgen deprivation disrupts adult hippocampal neurogenesis and to compare and contrast three approaches to androgen deprivation that are analogous to how human prostate cancer patients are treated.

Methods: Androgen deprivation was produced in male mice by surgical castration, the administration of the androgen receptor antagonist flutamide, or leuprolide, a gonadotropin-releasing hormone analog ($n = 8$/group). Sham surgery and saline-treated controls were included in the experimental design. The mice were sacrificed 35 days after castration or the beginning of drug treatment. Using immunofluorescent double-staining and confocal microscopy, the number of BrdU-positive cells and percentage of BrdU-positive cells that co-express NeuN were used to determine neuronal survival, whereas proliferation was assessed by counting the number of Ki-67-positive cells and percentage of Ki-67-positive cells that co-express nestin and doublecortin. The data will be analyzed by ANOVA, followed where appropriate by Newman-Keuls tests to detect differences among treatment groups. The criterion for rejection of the null hypothesis will be set at $p<0.05$.

Results: All three treatments disrupted hippocampal neurogenesis as both neuronal proliferation and survival were decreased in the dentate gyrus of the hippocampus. In contrast, neurogenesis was not affected in the subventricular zone, another region where adult neurogenesis occurs.

Conclusions: As adult hippocampal neurogenesis is involved in memory and learning; these results support the conjecture that disruption of adult hippocampal neurogenesis might underlie some of the cognitive impairment found in prostate cancer patients undergoing androgen deprivation therapy. Supported by DOD grant W81XWH-16-1-0429.

Keywords: Hippcampus, Adult Hippocampal Neurogenesis, Androgen, Cognition, Animal Models

Disclosure: Nothing to disclose.

W147

Kinase Network Activity Changes in the Frontal Cortex in Depressed-Suicide Subjects

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Background: Suicide is one of the top 20 causes of death worldwide, but few interventions consistently reduce suicidal thoughts and behaviors. Our limited understanding of the neurobiology of suicide hinders development of efficacious and safe interventions. Protein kinases are essential molecules that fine-tune signaling in complex biological networks. Postmortem studies have identified significantly altered gene and protein expression levels of kinases in depressed subjects who die by suicide. However, measuring individual kinase expression alone is not sufficient to understand the "kinome", the complex network of kinase interactions which represents the intrinsic state of kinases. Studying altered kinase activity, not just changes in expression levels, better reflects the altered state of kinases in disease. The "kinome" has not yet been studied in depressed subjects who died by suicide.

Methods: We investigated changes in serine/threonine kinase activity in the dorsolateral prefrontal cortex of depressed subjects who died by suicide and comparison subjects using a chip-based peptide array system (PamGene12). Male ($n = 10$ per group, pooled) and female ($n = 10$ per group, pooled) depressed-suicide subjects and corresponding healthy control subjects ($n = 10$ per group, pooled) were compared. All samples were run in triplicate. Random sampling analysis (z-score cutoff +/-2) was applied to identify over- and under-represented kinases. In silico confirmation analysis was conducted using Kaleidoscope, an R shiny program that collates publicly available postmortem transcriptome datasets. The kinase data undergo connectivity mapping using the Library of Integrated Network-based Cellular Signatures (LINCS) database. Connectivity mapping links changes in patterns of gene expression induced by altered kinase network activity in depressed-suicide subjects, with chemical perturbagens that induce similar and opposing patterns of change in gene expression.

Results: Our study identified sex-specific, functional changes in kinases in the frontal cortex of depressed subjects who died by suicide. We demonstrate large scale abnormalities in activity (peptide intensity log2 fold change cutoff >0.20 or < -0.20) of kinases in depressed subjects who die by suicide. In female subjects, mitogen activated protein kinase (MAPK) family kinases, ERK, P38 and JNK were significantly altered (z-score +/- 4). In male subjects, novel kinases including Aurora kinase (AUR) and WNK were identified (z-score +/- 3). Kinases that were common to males and females, including AMP-activated protein kinase (AMPK), were also identified.

In silico confirmation analysis identified a significant decrease ($p<0.05$) in AUR gene expression in male depressed-suicide subjects, a kinase not previously implicated in this disorder. AMPK gene expression was significantly reduced in depressed-suicide female subjects ($p<0.05$) but significantly increased in male subjects ($p<0.05$) in the DLPFC.

AMPK overexpression (female analysis) and knockdown (male analysis) gene expression networks were generated in the LINCS database. Chemical perturbagens (disconcordance score < -0.70 female analysis; < -0.44 male analysis) were identified.

Conclusions: Taking a network-level approach, we identified novel and predicted kinase activity changes in male and female depressed-suicide subjects. There is a central role for dysregulated signal transduction in severe mental illness, and our data support kinase network activity as a high-yield pathophysiological substrate for investigation of the molecular deficits contributing to depression and suicide. Kinases have the potential to modulate complex behaviors like suicide but are yet to be exploited therapeutically. Studying the kinome will increase our understanding of the neurobiology of suicide and may lead to the development of novel interventions for suicide.

Keywords: Major Depressive Disorder (MDD), Suicide, Postmortem Brain Tissue, Kinases

Disclosure: Nothing to disclose.

W148

Lithium Sarcosine Delays Disease Onset, Prolongs Survival and Reduces Neurological Deficits in a Transgenic Mouse Model of Amyotrophic Lateral Sclerosis by Its Synergistic Dual Therapeutic Mechanisms

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of motor neurons, leading to muscular weakness, atrophy, and eventual paralysis and death. The ubiquitously expressed enzyme Cu2+/Zn2 + superoxide dismutase (SOD1) is the first identified gene associated with ALS. Spinal cord slice from embryonic SOD1-G93A mice show a disequilibrium between excitatory and inhibitory neurotransmission. One of the mechanisms implicated in ALS is the excitotoxicity whereas dysregulation of NMDA function such as D-serine can contribute significantly to the development of ALS. Drugs such as riluzole and memantine which delay the disease progression and prolong the life span of SOD1-G93A mice while attenuate NMDA activity. Thus, regulating NMDA activity to reach an equilibrium is critical to develop novel therapeutics for ALS.

Glycine regulates excitatory glutamatergic neurotransmission by acting as an obligatory co-agonist of NMDA receptor. Enhancing NMDA neurotransmission via the glycine site is safer than the glutamate site while excessive D-serine in the extrasynaptic NMDA receptor is toxic. Synaptic concentrations of glycine are regulated by glycine transporter-1 (GlyT-1) and GlyT-2. At inhibitory glycinergic synapse, GlyT-1 is widely expressed in glial cells and functions in lowering extracellular glycine concentration to modulate inhibitory neurotransmission. At excitatory glutamatergic synapses, GlyT-1 is colocalized with NMDA receptor on both glial and neuronal cells where it tightly maintains glycine concentration at subsaturation levels. Inhibition of GlyT-1 in these

areas may lead to increased synaptic glycine level and resulting in a potentiation of NMDA function. Therefore, inhibition of GlyT-1 could be a therapeutic approach for various CNS disorders that modulates either inhibitory glycinergic or excitatory glutamatergic neurotransmission or both.

Methods: Sarcosine, also known as N-methylglycine, exhibits NMDAR co-agonist and glycine receptor agonist properties. In addition, sarcosine is a competitive inhibitor of GlyT-1. Sarcosine has been studied in proof-of-concept clinical trials for treating several CNS disorders including schizophrenia, depression, Parkinson's disease with dementia, attention deficit hyperactivity disorder and obsessive-compulsive disorder. The clinical benefits of sarcosine are considered to arise from its inhibition of GlyT-1, but other possible mechanisms such as modulating inhibitory glycinergic or excitatory glutamatergic neurotransmission may also contribute to its therapeutic activity. Lithium has neurotropic and neuroprotective effects in neurologic diseases such as stroke, traumatic brain injury, Huntington's disease, Alzheimer's disease and fragile X syndrome. Lithium also gains attention as a potential therapeutic agent for ALS. However, findings from both rodent and human clinical trials are equivocal. This raises the possibility that the single agent therapeutic is not good enough for a degenerative disorder like ALS. In the present study, we produce lithium salt of sarcosine to investigate whether this novel compound has synergy of the dual mechanisms of sarcosine and lithium, and determine whether it can elicit a greater effect than either lithium or sarcosine alone; in delaying the onset of motor dysfunction, disease progression and prolonging the lifespan in SOD1-G93A mice model of ALS.

Results: Our findings reveal ithium sarcosine has better physicochemical properties, including water solubility, than lithium carbonate or sarcosine alone. In comparison to the MK-801-treated group, both lithium chloride (212 mg/kg; 5 mmol/kg) and lithium sarcosine (480 mg/kg; 5 mmol/kg) displayed a significant lower locomotor activity whereas sarcosine (445 mg/kg; 5 mmol/kg) did not. Moreover, lithium sarcosine displayed better rescue/protective effects on MK801-induced hyperlocomotion than lithium chloride. The plots of cumulative disease onset against the age revealed that a right shift of the curve in mice treated with lithium sarcosine. The onset of impaired motor function was 95.1±6.1, 102.1±4.1, 109.7±2.2 days for the saline control, lithium carbonate, and lithium sarcosine group respectively ($p < 0.05$). Both lithium carbonate and lithium sarcosine treatments also improve survival. The mean survival time for mice treated with lithium carbonate was 131.5±4.6 days, for lithium sarcosine was 139.3±2.8 days, which were about 2 and 10 days longer than those treated with saline (129.3±2.7 days).

SOD1-G93A mice in saline control group exhibited a significantly reduced rod-staying latency from 11 weeks of age and declined afterwards. However, both lithium carbonate and lithium sarcosine treatments improved the latency in SOD1-G93A mice and the first significant decrease on rotarod performance was observed at 15 and 16 weeks of age, respectively. The result of wire-hanging test showed an improved trend for lithium sarcosine treated SOD1-G93A mice between 16 to 19 weeks of age. In addition, lithium sarcosine group exhibited delayed neurological deficit and the first changes in stride length occurred at 18 weeks of age, instead of 15 weeks in controls, in the footprint analysis of step length.

Conclusions: Overall, lithium sarcosine has superior physicochemical properties and delays disease onset, prolongs survival and reduces neurological deficits presumably due to its synergistic dual therapeutic mechanisms

Keywords: ALS, NMDA, Novel Therapeutics

Disclosure: SyneuRx, Patent

W149

Frontoparietal Network Alteration as a Possible Biomarker to Predict Alzheimer's Disease

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Background: Alzheimer's disease (AD) is an irreversible age-related neurodegenerative disorder that leads to progressive loss of memory and other cognitive abilities that interfere with activities of daily living. Currently, there are no effective treatments that could prevent the disease, halt its progression or delay its onset. The existence of an effective biomarker for early detection of AD would facilitate improved diagnosis and the design of an early mechanism-based therapeutic outcome. Coordination of neural activity in the prefrontal cortex (PFC) and parietal cortex (PC) is critical for attentional control and mnemonic operations and these cognitive processes are affected in AD. Here we hypothesized that desynchronized cortical oscillations precede the onset of AD.

Methods: We utilized the triple transgenic (3xTg-AD) mice, which harbors mutations in three genes (human presenilin-1, human amyloid precursor protein and human tau) to examine cortical electroencephalographic (EEG) activity in the PFC-PC networks for spectral power, spectral coherence, and permutation entropy. The neurophysiological changes were assessed in a longitudinal study design in young-adult animals at time points when the pathogenic features (Aβ accumulation and tau pathology) are not observed. EEG head mounts with 6-pin connectors were implanted to record electrophysiological activity in awake 3 months old wild-type (WT) and 3xTg mice, animals. Because nicotinic cholinergic transmission is impacted in AD, we also assessed the effects of systemic nicotine administration (0.18 and 0.36mg/kg) on cortical activity. EEG data was recorded in 5s epochs every 10 minutes for a total of 80 minutes. EEG recordings were conducted again with the same manipulations when the animals attained 6 months of age.

Results: We observed genotypic differences in the delta frequency band form in the PFC under baseline (saline) conditions regardless of age ($F(1, 8)=109.17$, $p<.0001$). Moreover, nicotine administration regardless of dose reduced the delta and theta power in the PFC of 3xTg-AD mice, while an opposite pattern was observed for the beta and gamma power in these mutants. Frontoparietal coherence in the low gamma (30-58 Hz) and delta bands was significantly higher in 3xTg mice in both the saline and low nicotine dose condition ($p<.05$ vs WT), but not in the high dose nicotine condition. PFC permutation entropy was reduced in 3xTg mice in all treatment conditions at both 3 months and 6 months, respectively (all $p<0.05$ vs WT).

Conclusions: Increased EEG coherence in 3xTg-AD mice is reflective of compensatory activation of PFC-PC network as an adaptive response to neural changes that may precede the onset of AD pathology. Moreover, genotypic differences in the band power following nicotine administration indicate that these alterations in cortical oscillations could be linked to nicotinic cholinergic modulation that presumably might have been impacted before the progressive development of Aβ plaques and tau tangles. Furthermore, lower permutation entropy in 3xTg-AD mice suggest that this measure is sensitive to cortical changes that may underlie consistent oscillatory abnormalities observed in the delta and gamma bands. Collectively, our findings indicate that neurophysiological changes in the frontoparietal network are evident well before the pathological features of AD emerge in a mouse model. Further research is warranted to assess the translational utility of these biomarkers to predict AD in clinics.

Keywords: Alzheimer's Disease, Frontoparietal, EEG, Transgenic Mice, Oscillations

Disclosure: Nothing to disclose.

W150

Increased Dopamine System Function in the Fab Rodent Model of Alzheimer's Disease

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Background: Individuals affected by Alzheimer's disease (AD) often experience comorbid psychosis, which severely diminishes the quality of life for the patient and their family. Because of the potential risk antipsychotic medications present to the elderly, there is an immediate need to establish novel alternative therapies. Psychosis (including hallucinations and delusions) has been demonstrated to be associated with a dysregulation of the dopamine system. We have previously demonstrated that psychosis observed in schizophrenia, may be attributed to aberrant regulation of dopamine neuron activity by the hippocampus. Because the hippocampus has been identified as a site of pathology in AD, we posit that it may also be a key region contributing to comorbid psychosis in AD.

Methods: We used the ferrous amyloid buthionine (FAB) rodent model of AD to model a sporadic form of the disease. We utilized in vivo electrophysiology to examine alterations in hippocampal activity and dopamine system function.

Results: FAB rats display both structural and functional alterations in the hippocampus, which is accompanied by a decrease in spontaneous low frequency oscillatory activity. Additionally, FAB rats exhibit robust increases in dopamine neuron population activity, consistent with what is observed in other models of psychosis.

Conclusions: These data suggest that aberrant hippocampal activity in AD may contribute to dopamine dependent psychosis. We believe that understanding the pathophysiology leading to comorbid psychosis in AD, will lead to novel targets for the treatment of this disease.

Keywords: Alzheimer's Disease, Dopamine, Hippocampus

Disclosure: Nothing to disclose.

W151

Assessment of Phosphodiesterase-4D as a Novel Target in Treatment of Memory Loss Using a Translational Mouse-Model Approach

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Background: Cyclic nucleotide phosphodiesterase-4 (PDE4) consists of four subtypes, three of which are expressed in mammalian brain (PDE4A, B, D). Clinical research indicates therapeutic potential for inhibitors of PDE4 for neuropsychiatric illnesses such as depression and dementia. However, dose-limiting side effects of existing inhibitors, which inhibit all PDE4 subtypes with equal potency, has stalled drug development in this area. While it has been suggested that development of subtype-selective PDE4 inhibitors (e.g., exhibiting much greater potency for inhibiting PDE4D compared to PDE4A, B, and C) is difficult. This is due to the high conservation of the catalytic site of PDE4, across subtypes, to which classical inhibitors bind. Recent collaboration with Tetra Discovery has involved assessment of newly synthesized allosteric inhibitors of PDE4 that exhibit at least 100-fold PDE4B or PDE4D selectivity. Despite the promise of new, subtype-selective inhibitors of PDE4, there remains the challenge of identifying animal models that are predictive and translate well to the human condition.

Methods: Our laboratory has established a humanized mouse line that contains the primate-specific sequence in the N-terminal region of PDE4D enzyme that confers inhibitor selectivity. These mice were separated into 8 groups (10 male mice/group), which were treated with different doses of PDE4D inhibitor BPN14770 in the presence of scopolamine. Morris Water Maze, step-down passive avoidance, and Y-maze spontaneous alteration behavioral tests were performed to elicit the effects of BPN14770 on various types of learning and memory after scopolamine induced neurological deficits. A global, quantitative proteomics/systems-biology analysis were conducted to identify differentially regulated neuroplasticity pathways occurring in memory-associated brain regions, such as the hippocampus and cortex, between humanized PDE4D mice and wild-type littermates with scopolamine-induced memory deficits following PDE4D inhibition. A one-way ANOVA following a Dunnett's test to determine whether the means differed significantly from the vehicle-treated and BPN14770-treated group in the presence of scopolamine.

Results: The results suggested that BPN14770 was 100-fold more potent in enhancing spatial memory and aversive memory in the Morris water maze, Y-maze and step-down passive avoidance tests, in humanized PDE4D mice than those of wild-type littermates. The further well-characterized neurosystems-biology analysis suggested that BPN14770 acted on genes related to biological markers of AD such as, cAMP, phospho-CREB, BDNF, synaptophysin and PSD95, which result in memory enhancement.

Conclusions: These findings provide support for the utility of allosteric PDE4D inhibitors in mediation of memory and identify biomarkers related to treatment of memory deficits associated with AD.

Keywords: Phosphodiesterase-4 (PDE4), Memory, Alzheimer's Disease

Disclosure: Nothing to disclose.

W152

AMPA Receptor Modulators Selective for TARP-Gamma8: Improving the Regional Specificity of CNS Drugs by Targeting Accessory Proteins

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Background: The alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA) subtype of ionotropic glutamate receptors mediates the majority of fast synaptic transmission within the mammalian brain. The ubiquitous expression of the primary subunits of AMPA receptors (AMPARs), and the lack of pharmacological selectivity amongst them, preclude regional or neuronal subtype specificity. In vivo, AMPARs comprise a variety of accessory proteins. Of particular interest, TARP-gamma8 is highly expressed in the hippocampus, amygdala, and cortex, regions of the brain implicated in several psychiatric and neurological disorders.

Methods: We used high-throughput screening to discover compounds that selectively modulate AMPARs containing TARP-gamma8. Subsequent medicinal chemistry efforts were used to

improve potency and pharmacokinetics of the hits. Assays were developed to measure target occupancy and functional effects of the compounds in vivo.

Results: These compounds possess a novel mechanism-of-action, characterized by TARP-dependent negative modulation of AMPAR function. Lead molecules with oral bioavailability and high brain penetration allowed demonstration of a strong relationship between pharmacokinetics and pharmacodynamics. The compounds show anticonvulsant, anxiolytic, and analgesic profiles in rodent. Molecules in this class provide large safety margins in preclinical species relative to non-specific AMPAR antagonists due to their improved regional specificity.

Conclusions: AMPAR modulators selective for TARP-gamma8 have the potential to be novel treatments for anxiety/depression, bipolar disorder, temporal lobe epilepsy, and/or chronic pain.

Keywords: AMPA, AMPA TARP Gamma8, Epilepsy, Bipolar Disorder

Disclosure: Janssen R&D, Employee

W153

Selective Melatonin MT2 Receptor Ligands Induce an Opioid-Mediated Antiallodynic Effect by Activating the Opioid System in the Descending Brainstem Antinociceptive Pathway

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Background: Neuropathic pain (NP) is a health problem leading to suffering, reduced productivity, and health care costs, for which few treatments are available. Opioids are not fully effective in NP and cause addiction, tolerance and risk of death. Furthermore, misguided prescription of opioid medications led to the Opioid Crisis. Analgesic supraspinal acting drugs such as opioids modulate pain via the brainstem descending pathway which includes the periaqueductal gray (vlPAG) and its projections to the rostral ventromedial medulla (RVM). Two types of neurons, both modulated by opioids, have been characterized in the RVM: ON cells which are pronociceptive and OFF cells which are antinociceptive. Opioid receptors (Or), μ- and δ-opioid receptors (μ-Or and δ-Or, respectively), are also expressed in the PAG neurons. Recently, we showed that melatonin (MLT), an endogenous neurohormone binding to two G-protein-coupled receptors (MT1 and MT2), exerts antiallodynic effects in both animals and humans, likely with involvement of Or. Using an animal model of NP, this study examined whether the selective MT2 receptor partial agonist UCM924 has antiallodynic properties in rodents following a spared nerve injury (SNI). We tested the efficacy of UCM924 to modulate ON and OFF neurons in the RVM. To investigate the potential mechanism linking MT2 receptors and Or, we determined whether the MT2 analgesic effect is reversed by Or antagonists or prevented in μ-Or and δ-Or knock out mice (μ-Or-/- and δ-Or-/-, respectively). We also investigated the localization of MT2 and Or in the PAG. Finally, we explored the efficacy of UCM924 to induce the gene expression of the endogenous Or ligands proenkephalin (PENK) and pro-opiomelanocortin (POMC) after treatment with UCM924 in the PAG.

Methods: SNI was performed according to Decosterd & Woolf in 5 week old male rats and 6 weeks old wild type wt (C57bl/6), μ-Or-/- and δ-Or-/- male mice. 14 days after SNI, rats and mice developed mechanical allodynia was measured using von Frey filaments. The rats then received a subcutaneous (s.c.) or intra-PAG injection of the vehicle (VEH), UCM924 (20 mg/kg or 10 μg), in addition to one of the following antagonists: the selective MT2 antagonist 4P-PDOT 10 mg/kg or 100 μg; the non-selective Or antagonist naloxone 1 mg/kg or 1 μg; the selective μ-Or antagonist CTOP 1 μg; or the selective δ-Or naltrindole 1mg/kg or 1 μg and 10 min later received UCM924. The mice received s.c. injection of the VEH, or UCM924 (20 mg/kg). For in-vivo extracellular recordings in rats, 14 days after SNI, a cannula was stereotaxically lowered above the left vlPAG using coordinates from Paxinos & Watson. VEH or drug solutions were administered into the vlPAG in a volume of 1 μL. After implantation of the cannula, an electrode was similarly lowered into the RVM. ON cells were identified by a burst of activity while OFF cells were identified by their pause in the firing when a nociceptive (hind paw pinch) stimulus was applied. For immunohistochemistry (IHC), knock-in μ-OR-mcherry mice ($n = 4$) were perfused and slices containing PAG were incubated with primary MT2 antibodies, then visualized under confocal microscope. For gene expression, 14 days after SNI, wt mice received s.c. injection of the VEH, or UCM924 (20 mg/kg). After 3 hours, PAG was collected, and following total RNA extraction, the samples were processed for quantitative polymerase chain reaction (real time qRT-PCR) to assess POMC and PENK mRNA levels.

Results: IHC revealed that MT2 are located in the rostral third of the PAG where μ-Or are also abundant. Neuropathic rats treated with UCM924 ($n = 10$), s.c. or through intra-PAG injections, displayed an antinociceptive effect compared to veh ($n = 7$, $p < 0.001$ s.c. and $n = 8$, $p < 0.001$ intra-PAG), that was MT2 mediated since it was blocked by the selective MT2 antagonist 4P-PDOT ($n = 8$; $p < 0.001$, s.c.). It was also opioid-mediated since the analgesic effect was blocked by naloxone ($n = 7$, $p < 0.001$ s.c.; $n = 6$, $p < 0.001$ intra-PAG), and particularly μ-Or mediated since it was blocked by the selective μ-Or antagonist CTOP ($n = 6$, $p < 0.001$ intra-PAG). However, the δ-Or antagonist naltrindole only partially blocked this effect ($n = 6$, $p < 0.001$ s.c.; $n = 11$, $p < 0.001$ vs UCM924 and $p < 0.001$ vs veh intraPAG). We observed that UCM924 into the vlPAG decreased the firing of ON cells ($n = 4$, $p < 0.001$), and increased the firing of OFF cells ($n = 4$, $p < 0.001$), which is characteristic of most analgesic drugs. Further experiments showed that the selective MT2 4P-PDOT ($n = 6$, $p < 0.001$ vs UCM924), the non-selective Or (naloxone) ($n = 4$, $p < 0.001$ vs UCM924) and the selective μ-Or antagonists (CTOP) ($n = 4$, $p < 0.001$ vs UCM924), but not the selective δ-Or antagonist (naltrindole) ($n = 4$, $p>0.05$ vs UCM924) blocked the decrease of ON cell and the increase of OFF cell activity produced by UCM924. Neuropathic wt and δ-Or-/- mice treated with UCM924 ($n = 10$) s.c., displayed an antinociceptive effect compared to veh ($n = 9$, $p < 0.001$ s.c.). No antiallodynic effect was displayed after UCM924 treatment in neuropathic μ-Or-/- mice ($n = 10$, $p < 0.001$ s.c.). These results confirm μ-Or, but not δ-Or, involvement in MT2-induced antiallodynia. Finally, in the PAG of SNI wt mice, PENK, but not POMC, mRNA levels were upregulated following UCM924 treatment ($n = 5$, $p < 0.01$).

Conclusions: Together, these data show that selective MT2 agonists have analgesic properties through the descending modulatory pathway and this effect is mediated by μ-OPr via enkephalin involvement. Thus, MT2 receptors may represent a novel pharmacological target in the treatment of NP.

Keywords: Opioid, Melatonin, Neuropathic Pain, MT2 Receptor

Disclosure: Patent holder, Patent

W154

Duration of Illness and Cortical Thickness in Trichotillomania: Preliminary Evidence for Illness Change Over Time

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Background: Trichotillomania is a psychiatric condition characterized by repetitive pulling out of one's hair, leading to marked functional impairment. The aim of this study was to examine the association between duration of trichotillomania and structural brain abnormalities by pooling all available global data.

Methods: Authors of published neuroimaging studies of trichotillomania were contacted and invited to contribute de-identified MRI scans for a pooled analysis. Freesurfer pipelines were used to examine whether cortical thickness and sub-cortical volumes were associated with duration of illness in adults with trichotillomania.

Results: The sample comprised 50 adults with trichotillomania (100% un-medicated; mean [SD] age 34.3 [12.3] years; 92% female). Longer duration of illness was significantly associated with lower cortical thickness in bilateral superior frontal cortex and left rostral middle frontal cortex. Volumes of the a priori sub-cortical structures of interest were not significantly correlated with duration of illness (all $p>0.05$ uncorrected).

Conclusions: This study is the first to suggest that trichotillomania is associated with biological changes over time. If this finding is supported by prospective studies, it could have important implications for treatment (i.e. treatment might need to be tailored for where a person is in the course of illness as opposed to a treatment for the disorder itself). Viewed alongside prior work, the data suggest the existence of brain changes in trichotillomania associated with vulnerability (excess thickness in right inferior frontal cortex) may differ from those associated with chronicity (reduced thickness in medial and superior frontal cortex). Longitudinal research is now indicated.

Keywords: Trichotillomania, Human Neuroimaging, Staging

Disclosure: Takeda Pharmaceuticals, Grant, Roche, Promentis

W155

Rapid Improvement of OCD Symptoms and Antidepressant Response to Deep Brain Stimulation of the Superolateral Medial Forebrain Bundle in OCD – a Case Series

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Background: Deep brain stimulation (DBS) to nucleus accumbens, the ventral capsule, the ventral striatum, the subthalamic nucleus and the inferior thalamic peduncle have been proposed as treatment option for severe, treatment resistant obsessive-compulsive disorder (OCD). A meta-analysis showed that 60% of patients treated with DBS to one of these targets responded and that overall symptom reduction was 45.1% measured by Yale-Brown Obsessive Compulsive Scale (Y-BOCS) (Alonso et al., 2015). Notable treatment effects (35 and 50% YBOCS reduction after 1 year) in two patients with treatment resistant OCD who received DBS to another target region, the superolateral medial forebrain bundle (slMFB), have been reported by our research group (Coenen et al., 2017). Up to now, rapid and lasting (up to 4 years) antidepressant effects of slMFB DBS have been observed for treatment-resistant depression. Under the assumption of a dysfunctional reward system in depression, these effects can be explained by the slMFB's crucial role in the reward system. As the slMFB is also a structure connecting relevant target regions in OCD treatment, it seems to be a suitable DBS target for this disorder itself.

Methods: Eight patients (4 f, 4 m) suffering from severe, treatment-resistant OCD have been treated with DBS to the slMFB (electrodes: model DB2202-Directional, BostonScientific and pulse generator: vercise Gevia (rechargeable), BostonScientific (7 patients) and vercise (not rechargeable), BostonScientific (1 patient)) following the protocol described in Coenen et al. (2017). Patients were between 25 and 52 years old, mean duration of illness was 20 years (range 12 to 27 years). All patients received various adequate pharmacological treatments, psychotherapy with exposure, inpatient and most of them outpatient treatments before DBS. None of these treatments had substantial lasting effects on OCD symptoms. One patient (#4) had been treated with DBS to the VC/VS for the last 9 years, and one patient (#7) with DBS to the NAcc for the last 4 years, but OCD symptomology did not decrease substantially.

Implantations and stimulation onset took place between December 2017 and June 2019. OCD symptoms (via Y-BOCS) and depressive symptoms (via Montgomery Asberg Depression Rating Scale, MADRS) were measured at least at two baseline visits and then about every 3rd month after stimulation onset as part of our clinical routine. Bilateral stimulation has been performed continuously and stimulation parameters have been adjusted individually in various titration visits. Frequency and pulse width have been kept unchanged at 130 Hz and 60µs. Minimal current applied to the slMFB was 1.5 mA and maximal current was 3.9 mA.

As this is a one to one case series, treatment was neither blinded nor controlled.

Results: Mean Y-BOCS score of at least two preoperative visits was 31.04 (SD = 6.21). At stimulation onset Y-BOCS mean score was 19.57 (SD = 4.89) (data available for seven patients) and after one month of stimulation mean score had dropped to 11.80 (SD = 7.33) (data available for 6 patients) which results in a mean reduction rate of 55.33% compared to baseline. 6 out of 8 patients experienced rapid and remarkable reduction of OCD symptoms post-surgery, 4 of them reached response (35% reduction in Y-BOCS score) directly at stimulation onset. Two to 17 month data are available for 6 patients and show stable response in 5 of them. Patient #4 shows noticeable improvement in several months, but response is unstable so far.

In all but one (#4) patients a reduction of depressive symptoms was observed. Mean Reduction rate of MADRS-score was 48.95% (SD = 28.23) after one month of stimulation (data available for seven patients) compared to baseline. Over time depressive symptoms seem to decline even more. After 5 months of stimulation MADRS-reduction rate was 59.42% (SD = 24.3) (data available for 5 patients).

Related adverse events were reported during parameter adjustment in form of accommodation problems or diplopia. On several occasions, patients reported increased restlessness. After parameter adjustment adverse events disappeared immediately.

Conclusions: These results support the proposal of the slMFB as target region for DBS in the treatment of OCD as the majority of our patients showed fast and stable decrease of OCD symptoms. As we would expect in the light of the results of slMFB stimulation in depression, depressive symptoms declined as well. As OCD and depressive symptoms seem to decline simultaneously the suggestion of a shared relevant disease network is encouraged.

Two of our patients had been treated with DBS to other brain targets before without notable success. Both of them did not show immediate response. It might be speculated that these patients are especially treatment resistant. On the other hand, one of these patients reports improvement occasionally but relapses quite quickly. For the other patient we do not have follow-up data yet, therefore improvement is not excluded. To generate more insight into the mechanism of action of this treatment on a metabolic level, PET-studies might be useful.

The data we are presenting here are obtained from an uncontrolled, unblinded case series. As results are extremely promising they call for a controlled randomized long-term study that assesses treatment response and treatment safety systematically.

Keywords: Obsessive-Compulsive Disorder (OCD), DBS, Superolateral Medial Forebrain Bundle

Disclosure: Nothing to disclose.

W156

Preliminary Data Examining the Neural Substrates of Obsessive-Compulsive Disorder in Patients With Neurodegenerative Disorders

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Background: Obsessive-compulsive disorder (OCD) is characterized by recurrent, persistent, intrusive thoughts and repetitive behaviors or mental acts. While OCD is thought to have a prevalence of around 2% in the general population, it is underdiagnosed, undertreated, and the neural substrates of the disorder are incompletely understood. Numerous studies have suggested that cortico-striatal circuits are important in OCD but how these different areas contribute to symptomology remains unclear. Studies have also suggested that OCD may be associated with an imbalance of goal-directed and habitual behavior, possibly through involvement of the same circuits implicated in the generation of OCD symptoms. While often not considered OCD per se, ruminative thoughts similar to obsessions and repetitive behaviors similar to compulsions are prominent in certain neurodegenerative illnesses. However, associations between these symptoms and regional atrophy in patients with neurodegenerative processes has been understudied. Two neurodegenerative disorders which may be especially relevant to OCD are frontotemporal dementia (FTD), which is primarily a disorder of cortical degeneration; and Huntington's disease (HD), primarily a disorder of striatal degeneration. Importantly, patients with both of these syndromes have prominent repetitive thoughts and behaviors similar to OCD that occur early in the course of the disorder. We hypothesize that regional degeneration in these disorders will correspond to subsets of OCD symptoms and deficits in goal directed/habitual behavior.

Methods: In this pilot study, we examined the characteristics, severity, and frequency of OCD-like behavior in 14 patients with either HD or FTD (7 of each) using the Yale-Brown Obsessive Compulsive Scale, Neuropsychiatric Inventory, Frontal System Behavior Scale, and DSM-5 level 1 cross-cutting measures. MR images were acquired on a 3.0T Philips Achieva Quasar Duel Magnet Scanner at Columbia. Each subject's T1 weighted MPRAGE image was used to generate measures of global and regional brain volume using FreeSurfer software. Brain volume calculations followed Walhovd et al., 2018 and volumetric ROI for vPFC, DLS, and other regions of interest in the cortico-striatal circuit were defined a priori. As a first pass, we looked for correlations between ROI volumes in regions of interest and severity of compulsive behavior as assayed using the Y-BOCS in FTD and HD patients.

Results: In preliminary data, we show that the types of OCD-like symptoms in FTD and HD are remarkably different. Specifically, FTD patients have more prominent compulsive behaviors whereas HD patients exhibit more pronounced obsessive thinking. Preliminary structural MRI data supports that degeneration patterns are generally different in HD and FTD and that degeneration in some cortical regions may be associated with OCD severity across disorders.

Conclusions: Our findings in this pilot data set provide preliminary evidence that OCD symptoms may be related to degeneration of specific brain regions in FTD and HD. As we continue this project, we plan to also examine performance of goal directed and habitual behaviors in this same population. Through continuation of this work we hope to better understand the mechanistic and neuroanatomical bases of obsessive thinking and compulsive behavior with the goal of ultimately using this knowledge to generate novel treatments and targeted interventions.

Keywords: Neurodegeneration, Obsessive-Compulsive Spectrum Disorders (OCDS), Huntington's Disease, Frontotemporal Dementia

Disclosure: Nothing to disclose.

W157

Large-Scale Tractography of the Anterior Limb of the Internal Capsule in Obsessive Compulsive Disorder: Disease Correlates and Predictors of Capsulotomy Outcome

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Background: Treatment refractory obsessive compulsive disorder (OCD), a highly debilitating condition, can be effectively treated with capsulotomy, a neurosurgical procedure targeting the anterior limb of the internal capsule (aLIC). Multiple fronto-subcortical tracts course through the aLIC with some degree of topographical organisation. Identifying tracts within the aLIC predictive of optimal clinical outcome can enhance the precision of capsulotomy or neurosurgical procedures to enhance efficacy and decrease side effects. Recent deep brain stimulation studies of the ventral internal capsule (VIC) and subthalamic nucleus (STN) in OCD have identified tracts targeting the dorsolateral prefrontal cortex (dlPFC) and dorsal cingulate.

Methods: 100 OCD and 88 matched healthy controls were scanned using a diffusion spectrum imaging sequence with 41 undergoing capsulotomy with followup between 6 to 91 months post-surgery. Yale Brown Obsessive Compulsive Scale, and depression and anxiety scales were assessed pre- and post-surgery. Quantitative measures of fractional anisotropy (FA) and normalised counts of the streamlines passing through the aLIC connecting functionally defined prefrontal regions of interest (dorsolateral, ventrolateral and ventromedial prefrontal, orbitofrontal, dorsal cingulate and pre-supplementary motor area) with subcortical regions (thalamus, striatum and STN) were assessed between OCD and healthy controls. To assess specificity of the findings, whole brain tract-based spatial statistics was also assessed. dlPFC, orbitofrontal and dorsal cingulate tracts were then correlated with obsessive-compulsive symptom improvement post-capsulotomy covarying for other clinical measures.

Results: A topographical dorsal-ventral and anterior-posterior organisation of prefrontal tracts was statistically confirmed with hierarchical clustering based on the shortest linkage identified for lateral prefrontal cortices, then OFC, dACC and pre-SMA. Relative to healthy controls, OCD patients had lower FA of dorsolateral, ventrolateral prefrontal, orbitofrontal, dorsal cingulate and pre-SMA (FDR $p<0.05$ corrected) streamlines to thalamus, striatum and subthalamic nucleus. These findings were specific to the aLIC streamlines as whole-brain TBSS identified lower FA of various prefrontal regions and superior longitudinal fasciculus but not aLIC. Greater pre-operative FA and normalised counts in OCD patients of dlPFC and thalamic and subthalamic tracts were associated with better obsessive-compulsive symptom improvement post-capsulotomy controlled for depression, anxiety and duration of followup. With controlling for other clinical variables, dorsal cingulate and orbitofrontal tracts were no longer significantly correlated with capsulotomy outcomes.

Conclusions: We highlight a specific decrease in FA of prefrontal streamlines to thalamic and subthalamic structures through the aLIC in a relatively large population of OCD subjects. The topographical clustering in the aLIC emphasizes that specific anatomical targeting of lesion or stimulation surgery are likely to influence overlapping clustered prefrontal regions (e.g. tracts from dorso- and ventrolateral prefrontal cortices overlap and may be difficult to dissociate) but require additional dorsal, ventral or posterior targeting or contact stimulation to simultaneously influence other prefrontal regions. Critically, we show that the dlPFC tracts through the aLIC predict optimal obsessive-compulsive capsulotomy outcomes, similar to dlPFC prediction of VIC and STN DBS outcomes. These findings have implications for enhancing the precision of neurosurgical targeting in OCD.

Keywords: Neurosurgery for Psychiatric Disorders, Diffusion Tractography, Obsessive Compulsive Disorder

Disclosure: Nothing to disclose.

W158

Comparison of Dynamic Brain States in Body Dysmorphic Disorder and Healthy Controls Using Leading Eigenvector Dynamics Analysis

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Background: Body dysmorphic disorder (BDD) is marked by preoccupation with misperceived appearance flaws, which they believe render them ugly and disfigured. The consequences are profound, with a high prevalence of suicide attempts (25%) and hospitalization (50%). In contrast to its impact on quality of life, it is understudied, and the neurobiological models to explain vulnerability to BDD are scarce. Further, the study of brain functional connectivity (FC) in BDD has been even less explored. Given the fact that FC can evolve over time, it is important to assess dynamic changes in FC and how they may differ in people with BDD compared to the healthy controls. Adopting a recently developed method to examine dynamic FC called leading eigenvector dynamics analysis (LEiDA), re-emerging FC states during rest can be well-characterized. LEiDA calculates dynamic FC at an instantaneous level and reveals patterns of blood oxygen level-dependent (BOLD) phase coherence, or FC states, that reoccur over time. In this study, differences in occurrence and duration of FC states were compared between the adults with BDD and the controls. This approach may provide novel neurobiological profiles associated with BDD pathophysiology.

Methods: 12 adults with BDD and 12 healthy controls ages 18-40 were included in this study. The participants were scanned with a 3T Siemens MR scanner to acquire T1-weighted (T1w) anatomical images (TR/TE=2.3s/0.00227s, flip angle=8°), and resting-state BOLD fMRI images (TR/TE=0.72s/0.037s, flip angle=52°, time points=570). The participants were instructed to rest with their eyes open during the 7-min fMRI acquisition. The data were preprocessed using fMRIPrep 1.4.0. The T1w image was corrected for intensity non-uniformity. Brain tissue segmentation of cerebrospinal fluid, white matter, and gray-matter was performed on the brain-extracted T1w. Volume-based spatial normalization to standard MNI space was performed through nonlinear registration. For the fMRI data, a deformation field to correct for susceptibility distortions was estimated. Based on the estimated susceptibility distortion, an unwarped BOLD reference was calculated for a more accurate co-registration with the anatomical reference. Head-motion parameters with respect to the BOLD reference were estimated before spatiotemporal filtering. The BOLD time-series were slice-time corrected and were resampled onto their native space by applying a single, composite transform to correct for head-motion and susceptibility distortions. The BOLD time-series were then resampled into standard MNI space. Automatic removal of motion artifacts using independent component analysis (ICA-AROMA) was also performed on the BOLD on MNI space time-series after removal of non-steady state volumes and spatial smoothing with an isotropic, Gaussian kernel of 6mm FWHM. Anatomical Automatic Labeling (AAL) atlas was used to parcellate the brain into N = 116 brain areas and the preprocessed BOLD signals were averaged over all voxels belonging to each brain area. LEiDA was then performed to compute the BOLD phases, the instantaneous BOLD synchronization matrix, and the Leading Eigenvector at each frame from the fMRI data. The Leading Eigenvectors were then clustered into recurrent Functional Networks, and the probability and lifetime of each Functional Network were computed. The number of clusters (K) was varied over a range between 5 and 15. Each functional connectivity (FC) state change was examined for each K and statistically compared between the two groups using a permutation-based independent sample t-test (10000 permutations).

Results: Several brain states demonstrated different properties between the BDD and control group in terms of both BOLD phase coherence probabilities and lifetimes. The most prominent results were associated with the fronto-parietal network for K=5 brain states, both in terms of probabilities (uncorrected $p = 0.029$, corrected $p = 0.15$) and lifetimes (uncorrected $p = 0.004$, corrected $p = 0.02$), both of which were lower in the BDD group compared with controls.

Conclusions: This represents the first examination of whole-brain dynamic functional connectivity in the resting state in individuals with BDD. Using relatively high temporal resolution fMRI, results suggest that the fronto-parietal network's phase coherence lasts significantly shorter, and may appear less often, for individuals with BDD compared with controls. Given previous findings of executive dysfunction, including difficulties with cognitive flexibility and set-shifting, these results provide insight into potential underlying abnormal temporal patterns of intrinsic connectivity states in BDD.

Keywords: Dynamic Functional Connectivity, Leading Eigenvector Dynamics Analysis, Body Dysmorphic Disorder, Resting State Networks

Disclosure: NOCD, Inc., Consultant, Pfizer

W159

Development of Selective M5 Muscarinic Acetylcholine Receptor Negative Allosteric Modulators for the Prevention of Opioid Misuse and Relapse

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Background: Due to the considerable risk for development of opioid use disorder (OUD) following prescription opioid use and the limitations with currently available OUD medications, it is critical to identify novel, non-opioid based treatments for prevention of opioid misuse and/or relapse. Accumulating evidence suggests that selective negative allosteric modulators (NAMs) of the M5 muscarinic acetylcholine receptor (mAChR) may provide an alternative therapeutic approach for OUD. Previous studies have shown that the M5 mAChR subtype is localized within the mesocorticolimbic reward circuitry. In addition, the M5 NAM ML375 attenuated opioid, cocaine and ethanol drug seeking behaviors in rodents without affecting opioid-induced antinociception. Using more optimized shorter-

acting M5 NAMs, including VU6008667, we report that selective inhibition of M5 can attenuate the acquisition and maintenance of opioid drug-seeking behaviors in rodents after both acute and chronic administration.

Methods: In the present study, we used male Sprague Dawley rats to evaluate the effects of repeated daily dosing of VU6008667 on the acquisition of μ-opioid agonist oxycodone self-administration (SA) under an FR1 schedule (between subject design; n = 6-8 rats/treatment group). In addition, the dose-dependent effects of VU6008667 on oxycodone SA under a fixed ratio 3 (FR3) schedule of reinforcement (within subject design; n = 14 rats) and on cue-induced reinstatement of oxycodone SA after saline substitution (between subject design; n = 9 rats/treatment group) were investigated. We also examined the effects of the M5 NAM ML375 on the reinforcing strength of increasing doses of oxycodone under a progressive ratio (PR) schedule of SA to determine if increasing doses of oxycodone could surmount the blocking effects of the M5 NAM mechanism (within subject design; n = 14 rats). Finally, we performed in situ hybridization experiments, both alone and in combination with retrograde tracing, allowing us to understand the specific neuronal populations where M5 was expressed within the mesolimbic and mesocortical circuitry (n = 5/treatment group). Statistical comparisons were made using one- or two-way ANOVAs followed by Dunnett's post hoc tests.

Results: VU6008667 is a highly selective M5 NAM with optimized physicochemical properties, including shorter half-life and increased central penetration, for acute and sustained dosing in animal models of OUD. Acute daily administration of VU6008667 blocked the acquisition of oxycodone self-administration in rats ($p < 0.01$). VU6008667 also produced a robust dose-dependent reversal of oxycodone SA under an FR3 schedule of reinforcement ($p < 0.01$), but did not have an effect on sucrose pellet-maintained responding within the same dose range. Additionally, VU6008667 dose-dependently attenuated cue-induced reinstatement of oxycodone SA following saline substitution. Moreover, the ability of ML375 to attenuate the reinforcing strength of oxycodone was observed across a broad range of oxycodone doses as assessed under a PR schedule of reinforcement. Anatomically, we demonstrated a high degree of co-localization of M5 mRNA with tyrosine hydroxylase mRNA positive neurons within the ventral tegmental area, the majority of which project to the nucleus accumbens.

Conclusions: Taken together, these findings suggest that selective functional antagonism of the M5 mAChR may represent a novel, non-opioid-based treatment for the prevention of prescription opioid misuse and treatment of OUD.

This work is funded by NIDA Grant R01DA37207, NIH T32 Training Grant in CNS Drug Discovery, and Ancora Innovations.

Keywords: M5 Muscarinic Receptor, Negative Allosteric Modulator, Opioid Use Disorder, Oxycodone

Disclosure: Lundbeck, Grant, Ono Pharmaceuticals, Ancora Innovations, Psychogenics, Consultant

W160

Fatty Acid Amide Hydrolase Relates to Impulsivity in Antisocial Personality Disorder: A [11C]CURB Positron Emission Tomography Study

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Background: Antisocial personality disorder (ASPD) is a common yet understudied psychiatric condition that presents with high rates of violence. Although several structural imaging studies have reported brain abnormalities in ASPD, much less emphasis has been placed on attempting to understand the neurochemistry underlying the disorder and its symptom clusters. Here, we used [11C]CURB positron emission tomography (PET) to measure fatty acid amide hydrolase (FAAH) density in the prefrontal cortex (PFC) and ventral striatum (VS). FAAH is an enzyme the metabolizes anandamide, an endocannabinoid that binds to CB1 receptors in the central nervous system. Higher levels of FAAH are linked to reduced CB1 neurotransmission, which shows a relation with aggression in animal models. The PFC and VS were chosen as the primary regions of interest (ROI), given their involvement in the aggression of ASPD.

Methods: We recruited 10 males with ASPD who had convictions for violent offenses and three males with ASPD and comorbid schizophrenia (SCZ) with convictions for violent offenses. 13 healthy controls were also sampled. None of the ASPD males without SCZ had comorbid mood, psychotic, or substance use disorders. All individuals provided negative urine drug screens on the screening and scanning days. Each participant underwent one [11C]CURB PET scan. The ROI, including PFC and VS, were automatically delineated using ROMI, an in-house program. An irreversible two-tissue compartment model with arterial input function was used to fit the time activity curves and provide an identifiable net influx constant [$Ki = K1 \times k3/(k2 + k3)$], where K1 (mL × cm-3 × min-1) is the plasma-to-tissue transfer constant, k2 (min-1) is the tissue-to-plasma transfer constant, and k3 is the rate constant of irreversible trapping of the tracer in tissue due to the reaction of the radioligand with the enzyme and is proportional to the concentration of catalytically active FAAH. The composite parameter λk3 is a reliable index of FAAH activity. Subjects also underwent a standard T1-weighted MRI scan to aid in ROI delineation of the PET images. We also collected blood samples to test for a functional polymorphism of the FAAH gene (rs324420, C385A) that is rare (homozygotes represent 2-6% of the population).

Results: There were no differences in terms of FAAH density in the PFC and VS between ASPD subjects and healthy controls. Similarly, FAAH density did not differ between ASPD and ASPD +SCZ. However, in the combined ASPD group, total impulsivity levels (measured using the Barratt Impulsiveness Scale 11) were positively correlated with PFC FAAH level ($r = 0.57$, $p = 0.040$) and VS FAAH level ($r = 0.56$, $p = 0.048$).

Conclusions: These results suggest that components of the endocannabinoid system, namely FAAH, have relevance for understanding symptom clusters and common antecedents of violence in ASPD. We are continuing to recruit ASPD subjects to increase our sample size.

Keywords: Antisocial Personality Disorder, Positron Emission Tomography Imaging, Endocannabinoid System, Impulsivity

Disclosure: Nothing to disclose.

W161

Galantamine-Memantine Combination Effective in Dementia: Translate to Schizophrenia and Beyond?

Abstract not included.

W162

Glutamatergic Neurometabolite Levels in Patients With Severe Treatment-Resistant Schizophrenia: A Cross-Sectional 3T Proton Magnetic Resonance Spectroscopy Study

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Background: To date, only four studies have examined glutamatergic neurometabolite levels, using proton magnetic resonance spectroscopy (1H-MRS) in patients with treatment-resistant schizophrenia (TRS) and their findings remain inconsistent in the anterior cingulate cortex (ACC) and basal ganglia. Moreover, TRS patients in these studies had mild to moderate illness severity, which is not entirely reflective of what is seen in clinical practice. Overall, the inclusion of patients with limited severity may be contributing to the inconsistencies seen amongst the previous findings.

Methods: In this study, we compared glutamate plus glutamine (Glx) levels in the dorsal ACC (dACC) and caudate among TRS patients, non-TRS patients, and healthy controls (HCs), using 3T 1H-MRS (PRESS, TE=35ms). TRS criteria were defined by severe positive symptoms (i.e. ≥5 on 2 Positive and Negative Syndrome Scale (PANSS) positive symptom items or ≥4 on 3 positive symptom items) despite optimal antipsychotic treatment.

Results: A total of 95 participants were included (29 TRS patients [PANSS=111.2±20.4], 33 non-TRS patients [PANSS=49.8 ±13.7], and 33 HCs). dACC Glx levels were higher in TRS group versus HCs (group: $F[2,75]=4.74$, $p = 0.011$; TRS vs. HCs: $p = 0.012$). No group differences were identified in the caudate. There were no associations between Glx levels in the dACC or caudate and clinical severity in both patient groups.

Conclusions: Our results suggest that higher Glx levels in the dACC might be related to the pathophysiology underlying TRS. Moreover, higher dACC Glx levels in TRS group may not be attributable to higher symptom severity but may reflect the difference in disease subgroup between TRS and non-TRS.

Keywords: Treatment Resistant Schizophrenia, Glutamate, MRS

Disclosure: Nothing to disclose.

W163

Identifying Multimodal MRI Characteristics of Individual Schizophrenia Patients: Influences of Illness Duration and Antipsychotic Therapy

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Background: Multivariate pattern recognition (MVPR) and machine learning generally has made possible the neuroimaging-based classification of individual psychiatric patients. Here we apply multiple kernel leaning to utilize multimodal MRI in the individual identification of antipsychotic-treated and never-treated schizophrenia patients at both early and later stage of illness.

Methods: High resolution 3D T1 imaging and resting-state fMRI were acquired from 373 schizophrenia patients including never-treated first episode ($n = 179$) and chronic ($n = 30$), and antipsychotic-treated first-episode ($n = 71$) and chronic ($n = 93$) schizophrenia patients, and 373 age, gender and sample size matched healthy controls for each of the four groups. Functional measures including regional homogeneity (ReHo), amplitude of low-frequency fluctuation (ALFF) and fractional amplitude of low-frequency fluctuation (fALFF) and grey matter volume (GMV) were extracted using the Automated Anatomical Labeling template and cortical thickness (CT) and surface area (SA) were extracted using the Desikan Atlas. These ROI-based brain measurements were selected as input features in multiple kernel learning classifier in each of the four matched patient-control groups. We used a five-fold cross validation strategy to test the accuracy and sensitivity of the classifiers. The process was repeated for 20 times independently to avoid potential bias caused by the random partition of subsets. Statistical significance of accuracy and sensitivity was determined by permutation tests repeated 1000 times (thresholded at $p < 0.001$). In addition, we carried out general linear model analyses in the most discriminating measurement in classification to identify illness-related alterations between each matched patient and control group using age and gender as covariates. Results were corrected for multiple comparisons using the false discovery rate (FDR, $p<0.05$).

Results: By applying fused functional and structural neuroimaging data in large samples ($n = 746$), we revealed that classification accuracies were similarly moderate in never-treated first-episode (69%) and chronic schizophrenia patients (71%) vs. their matched controls, but were significantly higher ($P<0.001$) in treated first-episode patients (88%) and chronic schizophrenia patients (93%). GMV was the most discriminating measure in schizophrenia patients except for antipsychotic-naïve first-episode patients in which ReHo was the most important feature. Treated patients showed consistently higher GMV in right pallidum and reduced GMV in left medial and middle frontal regions, right superior frontal regions and left anterior cingulate gyrus compared with untreated patients, which enhanced case control discrimination in treated individuals. The four patient groups shared common altered GMV in bilateral hippocampus, left medial orbital frontal and bilateral calcarine cortex compared with healthy controls.

Conclusions: Combining structural and functional MR features using multiple kernel learning led to moderate case-control separation in untreated patients and significantly more robust case identification in antipsychotic treated patients. These findings suggest that well-characterized brain changes associated with antipsychotic treatment may robustly enhance patient-control discrimination above and beyond intrinsic effects of disease processes. Classification accuracy did not differ between early and later course patients. Anatomic changes in bilateral hippocampus, left medial orbital frontal and bilateral calcarine cortex consistently contributed to case identification across the four patient-control group pairs.

Keywords: Schizophrenia, Antipsychotics, Machine Learning Classification, Multimodal Neuroimaging, Antipsychotic-Naïve First-Episode Schizophrenia

Disclosure: Nothing to disclose.

W164

Anatomic Abnormalities of Hippocampal Subfields in Never-Treated and Antipsychotic-Treated Long-Term Ill Schizophrenia Patients

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Background: The hippocampus is considered a key structure in the pathophysiology of schizophrenia (SCZ). Hippocampal volume reductions have been more pronounced in chronic SCZ compared to first-episode or prodromal SCZ. It remains unclear whether certain structural changes in the hippocampus are related to antipsychotic treatment and whether they progress over the course of illness. Volumetric alterations have been reported in specific hippocampal subfields and related to clinical manifestations. We compared volumes and hemispheric asymmetry of

hippocampal subfields between never-treated and antipsychotic-treated patients with long-term schizophrenia relative to matched healthy controls.

Methods: High-resolution T1-weighted images were acquired from never-treated (N-SCZ, n = 29) and matched antipsychotic-treated (T-SCZ, n = 40) schizophrenia patients and healthy controls (HC, n = 40). Hippocampal segmentation was conducted using FreeSurfer software (version 6.0). Thirteen subfields were examined: the granule cell layer of dentate gyrus (GCL) -head (H), GCL -body (B), Cornu Ammonis (CA) 4-H, CA4-B, CA2/3-H, CA2/3-B, CA1-H, CA1-B, molecular layer (ML) -H, ML-B, hippocampal tail (Hip-T), subiculum (Sub) -H, and Sub-B. Analyses of subfield volumes and laterality effects were conducted using multivariate analysis of covariance. Post-hoc t-tests were used to compare subfield data across groups. Correlations between subfield volumes and clinical variables were investigated. Linear regression analyses were conducted to identify subfields with significant age effects on anatomic measures, and age-related trajectories of these measures were compared across groups.

Results: Both N-SCZ and T-SCZ displayed significantly smaller volumes in bilateral hippocampus than HCs (p = 0.0032−0.048). Significant group differences were found in GCL-H, GCL-B, CA4-H, CA4-B, CA2/3-H, ML-B, Hip-T and Sub-B bilaterally, and CA2/3-B and CA1-B in the right hemisphere (F=4.00−13.07, p = 8.7×10-6 −0.021, η2p = 0.072−0.201). These volume differences survived FDR correction for multiple comparisons. Post-hoc pairwise tests showed that N-SCZ had significantly smaller subfields than T-SCZ in the right GCL-H, GCL-B, CA4-H, CA4-B, CA2/3-H, CA2/3-B, CA1-B and ML-B (p = 3.7×10-4−0.043). No patient group differences were significant in the left hemisphere.

Subfield volumes in N-SCZ were negatively correlated with PANSS total scores in the left GCL- H, ML-H and Sub-B, and right GCL-H, CA4-B and CA3-H (r = −0.442−-0.382, p = 0.021−0.049). Subfield volumes were inversely associated with illness duration in the left CA1-B, right CA4-H and CA2/3-H, and bilateral GCL-H for N-SCZ (r = −0.443−-0.384, p = 0.021−0.048), while no significant correlations were found for T-SCZ. Positive associations were detected between subfield volumes and chlorpromazine (CPZ) equivalents for T-SCZ in the right GCL-B (r = 0.358, p = 0.027) and CA4-B (r = 0.367, p = 0.023). Linear regression analyses showed significant age-related volume reductions for N-SCZ in the left GCL-H, CA2/3-B, CA1-H, CA1-B, ML-H, ML-B and Sub-H (r = 0.381 −0.442, p = 0.016−0.041), but not in T-SCZ or HCs. Direct comparisons of regression slopes across groups demonstrated accelerated age-related decline in N-SCZ in left ML-B (F=3.32, p = 0.040).

Conclusions: Widely distributed subfield alterations were present in schizophrenia patients. The greater subfield deficits in never-treated patients in the right hemisphere suggest that long-term antipsychotic medication via direct or indirect mechanisms may benefit hippocampal subfields over the longer-term course of illness.

Keywords: Schizophrenia, Antipsychotics, Hippocampal Subfields, Structural Neuroimaging

Disclosure: Nothing to disclose.

W165

Motor Abnormalities as Predictors of Psychopathology and Clinical Outcome in Schizophrenia

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Background: There's still a striking paucity of objective measures that can predict clinical outcome and psychopathology in schizophrenia after an acute psychotic episode. This study introduces a comprehensive assessment of motor symptoms to predict mid-term psychopathology and clinical outcome following an acute psychotic episode.

Methods: We assessed motor symptoms (i.e. Neurological Soft Signs (NSS), parkinsonism, akathisia, catatonia and acute dyskinesia), psychopathology, cognition and psychosocial functioning in a cohort of 96 schizophrenia patients using well-established instruments. We examined the relationship between motor symptoms, psychopathology, cognition and psychosocial functioning. This study also tested the clinical feasibility of this relationship when predicting clinical outcome in schizophrenia patients.

Results: 43 individuals of the patient cohort were examined after a follow-up period of >6 months. At follow-up, patients showed significantly decreased general symptom load, as well as decreased levels of NSS, parkinsonism and catatonia. NSS hard signs (p = 0.001, Bonferroni corrected) and akathisia (p = 0.005, Bonferroni corrected) predicted a decrease of positive and general symptoms as assessed by PANSS over time, as well as an improvement of cognitive function assessed by B-CATS.

Conclusions: In conclusion, the data suggest that higher NSS and akathisia scores are significant predictors of poor clinical outcome and cognitive dysfunction in schizophrenia after an acute psychotic episode.

Keywords: Schizophrenia, Motor abnormalities, Clinical outcome, Psychopathology

Disclosure: Nothing to disclose.

W166

Insula Functional Connectivity in Schizophrenia

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Background: The insula is structurally abnormal in schizophrenia, demonstrating robust reductions in gray matter volume, cortical thickness, and altered gyrification during prodromal, early and chronic stages of the illness. Despite compelling structural alterations, less is known about its functional connectivity, limited by studies considering the insula as a whole or only within the context of resting-state networks. There is evidence, however, from healthy subjects that the insula is comprised of sub-regions with distinct functional profiles, with dorsal anterior insula (dAI) involved in cognitive processing, ventral anterior insula (vAI) involved in affective processing, and posterior insula (PI) involved in somatosensory processing.

Methods: The current study builds on this prior work and characterizes insula sub-region resting-state functional connectivity in a large cohort of male and female individuals with schizophrenia (N = 191) and healthy participants free from psychopathology (N = 196). Group differences in sub-region connectivity were analyzed using independent samples t-test thresholded at cluster-level pFWE<.05 for voxel-wise cluster-defining threshold p = .001 (uncorrected). Similarity between sub-region connectivity patterns was estimated using eta-squared. Hypotheses regarding specific associations between insula sub-region connectivity abnormalities and clinical characteristics related to their functional profiles were tested using both region-of-interest and whole brain approaches.

Results: Functional dysconnectivity of the insula in schizophrenia is broadly characterized by reduced connectivity within insula sub-networks and hyper-connectivity with regions not normally connected with that sub-region. This pattern is reflected in significantly greater similarity of dAI and PI connectivity profiles and significantly lower similarity of dAI and vAI connectivity profiles ($p<.05$). In schizophrenia, hypo-connectivity of dAI correlates with worse cognitive function ($r = .18$, $p = .014$), whereas hyper-connectivity between vAI and posterior superior temporal sulcus correlates with severity of negative symptoms ($r = .27$, $p<.001$).

Conclusions: Overall, these findings reveal altered insula connectivity in all three sub-regions and converges with recent evidence of reduced differentiation of insula connectivity in schizophrenia, implicating functional dysconnectivity of the insula in cognitive and clinical symptoms.

Keywords: Schizophrenia, Resting State Functional Connectivity, Insula, Cognition, Negative Symptoms

Disclosure: Nothing to disclose.

W167

Elucidating Relationship Between Brain Structure and Function in Psychosis: A Multicenter Harmonized Diffusion Tensor Imaging Study

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Background: Because schizophrenia (SCZ) is a devastating brain disorder, a great body of research has been conducted to understand its neurobiology. While white matter deficits have been identified as key features, the development of structural aberrations with disease progression remains unclear. More importantly, the association between aberrant structure and sex, medication or symptomology has yet to be elucidated. A greater understanding of how these factors impact the brain is essential for the course and treatment of individuals.

Our initial study comprised of a large sample of 597 patients with SCZ and 490 healthy controls (HC) demonstrated widespread white matter deficits in SCZ when comparing to HC. Here, we investigate further clinical correlates of those structural abnormalities. First, we try to disentangle influence of age and duration of illness on the observed white matter pathologies. We then evaluate whether medication influences white matter. Thirdly, we assess association of structure with cognition and symptom severity. Finally, we investigate sex specificity in both structure and function.

Methods: Participants were recruited from 13 independent sites as part of separate studies. Diffusion tensor images and clinical measurements were collected and harmonized by our group. Images underwent thorough quality control followed by a harmonization procedure which took place at the level of the raw signal using a novel, well validated method. Images were then registered to IIT Human Brain Atlas and fractional anisotropy (FA) was estimated and averaged for seven tracts: forceps major, forceps minor, cingulum, inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), superior longitudinal fasciculus (SLF) and uncinate fasciculus (UF).

FA values were predicted for patients based on non-linear age trajectory of FA of HC using regression models. Next, deviations from predicted FA values were calculated and used in subsequent analyses.

To study the influence of age and duration of illness on white matter, mediator regression models were utilized with, age and/or duration of illness as independent variable, FA as dependent variable and sex as a covariate. Next, analyses were computed for males and females, separately. Patients were grouped based on current Chlorpromazine equivalent dosage (CPZ) (CPZ=0, CPZ<300mg/day, CPZ 300-1000mg/day, CPZ >1000mg/day) and CPZ group was added to regression model.

Lastly, a structural equation model (SEM) was constructed for the subset of people with complete clinical data ($n = 351$). In this model we aimed to determine direct influence of overall structural impairment on clinical symptoms. Additionally, we assumed that premorbid IQ would mediate the influence of structure on function. Again, analyses were performed for both sexes separately.

Results: Regression analyses revealed a significant influence of duration of illness on FA of the forceps major ($T=3.24$, $p<.001$), forceps minor ($T=3.40$, $p<.001$) and SLF ($T=3.83$, $p<.0001$). While the age effect alone was also significant for those tracts, when including both age and duration of illness in the model, only the latter remained significant. When separating males and females, the association of duration of illness and white matter was significant for males only (forceps major $T=3.55$, $p<.0001$; forceps minor $T=2.75$, $p<.006$; IFOF $T=2.76$, $p<.006$; SLF $T=3.61$, $p<.0001$). The addition of CPZ information to our regression model showed significant effect of CPZ on forceps major FA only ($T=3.93$, $p<.0001$).

For SEM data paths were calculated with asymptotically distribution-free model. Model fit revealed an acceptable overall fit (Root Mean Square Error of Approximation [RMSEA] =.09) which improved when separating sexes (RMSE =0.7). While influence of structural impairment on functional impairment was present for both sexes (standardized estimate=.44, males = .29, females =.52), only in females IQ had a significant impact on structural and functional impairment (females: IQ/structure -.44; IQ/function -.50).

Conclusions: The effect of duration of illness on brain structure supports the notion of progressive white matter degeneration in SCZ and provides evidence that brain structure might contribute to explaining devastating effects of being chronically ill. However, effects are regionally specific and can be easily confounded with age effects.

Our study does not clearly demonstrate the influence of antipsychotic medication on brain structure. In our analysis, medication seems to have at most a small effect on white matter. Alternatively, CPZ at the time of the scan might not be the best measure for evaluating influence of medication on brain structure. Future longitudinal studies controlling for lifetime dosage are needed.

The fact that structural impairments lead to clinical symptoms highlights the importance of white matter in pathophysiology of SCZ. As this effect is partially mediated by influence of IQ, it would suggest the important role of cognitive reserve in modulating clinical symptomatology. Further studies should include measurements of overall functioning and well-being.

Lastly, the association between structure and function seems to be sex specific in SCZ. Men show a stronger impact of chronicity, while for females IQ has a bigger impact on both white matter and symptoms. Our results therefore emphasize the importance of studying sex when trying to develop individualized treatment.

Keywords: Psychosis, Diffusion Tensor Imaging (DTI), White Matter, Symptoms, Neurocognitive Functioning

Disclosure: Nothing to disclose.

W168

Factors Associated With Successful Antipsychotic Dose Reduction in Schizophrenia: A Systematic Review of Prospective Clinical Trials and Meta-Analysis of Randomized Controlled Trials

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Background: It is critical to minimize exposure to antipsychotics in the maintenance treatment of schizophrenia because of troublesome dose-dependent side effects, such as extrapyramidal symptoms, cognitive impairment, and cardiac sudden death. However, because of insufficient knowledge about successful antipsychotic dose reduction, such a clinical effort has been mostly made on a try-and-error basis. The authors conducted systematic review and meta-analysis to identify predictors associated with successful antipsychotic dose reduction in schizophrenia.

Methods: We conducted a systematic literature search for studies examining antipsychotic dose reduction in schizophrenia in March 31, 2019, according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement using the MEDLINE and Embase with the following search terms: ((antipsychotic or neuroleptic or tranquiliz*) AND (dose or dosage) AND (reduce or reduction or low*-dose or minim* or decrease) AND schizophreni* AND adult). The search was filtered with Humans and English. Prospective clinical trials including randomized controlled trials (RCTs) and RCTs examining antipsychotic dose reduction in schizophrenia for systematic reviews and meta-analyses were selected, respectively. Two investigators independently conducted literature search and data extraction for all trials, and meta-analyses for RCTs. We conducted a qualitative analysis of the identified prospective clinical trials in the systematic review. A successful dose reduction was arbitrarily defined herein as any significant superiority or no significant difference in relapse rate in the dose reduction group versus maintenance group, or any significant improvement or no significant change in symptom severity between pre- and post-reduction. In case all included studies identified a certain factor for successful dose reduction while a majority of unsuccessful studies did not show that specific factor for unsuccessful dose reduction, it was considered to be a predictor of successful dose reduction. In meta-analyses, primary outcome was relapse defined per studies. Secondary outcomes were hospitalization, withdrawal from the study, and changes in psychotic symptoms, extrapyramidal symptoms, body weight, and neurocognition. We combined and compared the outcome data between the dose reduction and maintenance groups regarding each extracted outcome. Moreover, we conducted subgroup analyses with the following exploratory factors: publication year, study duration, illness stability, age, treatment setting, duration of illness and treatment, baseline symptom severity, antipsychotic type and formulation, pre- and post-reduction doses of antipsychotics, reduction rates, and duration of reduction. When some factors proved to be significantly associated with the increased risk of relapse, we conducted a further subgroup analysis of only studies that had each of those predictive factors relevant to antipsychotic dose to find other variables independent of antipsychotic dose.

Results: A total of 37 trials involving 2,080 patients were identified. Only 8 studies focused on second-generation antipsychotics (SGAs); no studies investigated long-acting injectable SGAs. Among 24 studies that evaluated relapse or symptom changes, 20 studies (83.3%) met the criteria for successful dose reduction. Study duration of <1 year, >40 years of age, duration of illness of >10 years, and post-reduction chlorpromazine equivalent (CPZE) dose of >200 mg/day were associated with successful dose reduction. Clinical deterioration was mostly re-stabilized by increasing the dose back to the baseline (N = 7/8, 87.5%). Among 18 RCTs involving 1,385 patients, relapse rate was significantly higher in the reduction than the maintenance group (N = 902, risk ratio [RR] = 1.96; 95% CI, 1.23-3.12, P = .005) whereas neurocognition significantly improved (N = 136, standardized mean difference [SMD] = 0.69; 95% CI, 0.25-1.12, P = .002). Subgroup analysis indicated that only post-reduction CPZE dose of ≤200 mg/day was associated with an increased risk of relapse (N = 504, RR = 2.79; 95% CI, 1.29-6.03, P = .009).

Conclusions: Clinicians should consider the risk of relapse in the long run among younger patients with a relatively short illness duration when reducing the doses of antipsychotics, and the final doses may be kept higher than CPZE 200 mg/day. Further studies, especially with SGAs, are warranted to elucidate optimal strategies for successful antipsychotic dose reduction in schizophrenia. Considering substantial heterogeneity in study designs and insufficient quality of the data, optimal antipsychotic dose reduction strategies should currently be guided by individual patient characteristics.

Keywords: Antipsychotics, Dose Reduction, Maintenance Treatment, Schizophrenia

Disclosure: Nothing to disclose.

W169

The Differential Impact of Childhood Maltreatment on Verbal Learning in Psychosis Patients Vs. Healthy Controls

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Background: A robust body of evidence has demonstrated a dose-response relationship between exposure to childhood maltreatment (CM) and psychotic symptoms. However, because CM is neither necessary nor sufficient for the emergence of psychosis, it has been suggested that CM confers risk for these symptoms via its impact on intermediate phenotypes, such as cognitive function. Several studies comparing psychotic disorder patients with and without a history of CM have provided some support for this hypothesis. However, a critical implication of this position has not been adequately explored. Specifically, if CM increases risk for psychosis via its effects on cognition, the impact of CM on cognitive function should be much more pronounced in patients with psychotic disorders relative to healthy individuals. To date, however, few studies have adequately examined this issue. In the present study we sought to examine the impact of CM on verbal learning, a cognitive domain impaired in children exposed to maltreatment and strongly associated with psychosis, using a case-control design well controlled for potential confounds.

Methods: Stable adult outpatients with a psychotic disorder (N = 325) and healthy controls (N = 261) were administered the WRAT-3 Reading Test as an estimated measure of IQ, symptom scales assessing the severity of positive, negative and depressive symptoms, the Childhood Trauma Questionnaire (CTQ) and the Hopkins Verbal Learning Test (HVLT). A linear regression model, covarying for estimated IQ as well as symptom severity, was used to examine the impact of total CTQ score, case-control status and the interaction between case-control status and CTQ score on HVLT performance.

Results: The full model accounted for 38% of the variance in HVLT scores ($F(9,463)=32.32$; $p<.001$) and indicated that estimated IQ ($B = .18$, $p<.001$) and severity of negative symptoms ($B = -.83$, $p<.04$) as well as the interaction between case-control status and CTQ total score ($p<.001$) significantly predicted performance on the HVLT. Closer examination of the interaction effect revealed that while the severity of prior exposure to CM was associated with worse verbal learning in patients ($B = -.10$), it was associated with better verbal learning in controls ($B = .28$). These results suggest that in patients, CM may contribute to psychotic outcomes indirectly through its relationship to impairments in verbal learning. The relationship between CM and verbal learning in controls, however, suggests that verbal learning may index resilience to the adverse effects of CM. Follow-up analyses in a subset ($N = 74$) of the control sample demonstrating a significant relationship between verbal learning scores and a measure of resilience (The Connor-Davidson Resilience Scale: CD-RISC), provided support this conclusion ($r = .32$; $p = .005$; $d = .68$).

Conclusions: These results suggest that verbal learning may index both risk and resilience to the adverse effects of exposure to childhood maltreatment. Given the retrospective nature of this study, however, it is unclear whether CM directly impacted verbal learning or if pre-existing verbal learning ability moderated the impact of CM. Additional prospective studies examining this issue are warranted as the precise identification of the impact of CM on cognitive function may provide insight into the biological processes involved in risk and resilience to psychotic disorders.

Keywords: Childhood Maltreatment, Verbal Learning, Psychosis, Healthy Controls, Resilience

Disclosure: Nothing to disclose.

W170

The Evolution of Cellular and Extracellular White Matter Pathologies in Schizophrenia: A Multi-Site, Harmonized, Free Water Imaging Study

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Background: Free Water Imaging is a diffusion magnetic resonance imaging (dMRI) method which can distinguish between changes affecting the extracellular space from those that originate around neuronal tissue (Pasternak et al., 2009). The ability to differentiate between pathologies that may affect the extracellular space, such as edema or atrophy, from pathologies that may affect tissue-related processes, such as axonal degeneration or altered myelination, improves the biological specificity of commonly used dMRI measures, such as fractional anisotropy (FA). Free Water Imaging has been particularly valuable in dMRI studies of schizophrenia, as there is substantial evidence for both immune activation and white matter deterioration in the pathophysiology of the illness, both of which would be reflected as reductions in FA.

At present, studies employing Free Water Imaging in schizophrenia have only been conducted in populations at discrete stages along the illness spectrum (Pasternak et al., 2012; 2014; Oestreich et al., 2017, Lyall et al., 2018, Tang et al., 2019). These studies suggest that FW elevations may be present at the onset of psychosis (but not before), while reductions in FAt present in more chronic stages of illness. As no longitudinal or large scale FWI analyses have been performed thus far, the lifespan time course of Free Water Imaging metrics (i.e., free water fractional volume (FW) and the fractional anisotropy of the tissue (FAt)) in schizophrenia is presently unknown. To accomplish this goal, we leveraged a recently developed dMRI data harmonization technique to remove scanner and sequence differences from raw dMRI data (Cetin-Karayumak et al., 2019). We collected dMRI data from 13 international sites and created a large sample of schizophrenia patients and healthy controls that ranges from 14 years to 65 years of age. In this work, we characterize lifespan time courses of FW and FAt to understand the potential differential impact of cellular and extracellular white matter pathologies to schizophrenia pathophysiology along an age continuum.

Methods: The dMRI sample in this study consists of a total of 1092 participants, with 600 individuals diagnosed with SZ at different illness stages (383 males, 217 females, age:) and 492 healthy controls-HC (275 males, 217 females, age:). After pre-processing steps including motion correction and skull stripping were completed, all dMRI data was harmonized using rotation invariant spherical harmonics (as described in Cetin-Karayumak et al. 2019), thereby removing nonlinear scanner and sequence differences found across the 13 sites. Average Fractional Anisotropy of the tissue (FAt) and average Free Water (FW) was computed for the whole brain and 14 major white matter tracts (as defined by the Illinois Institute of Technology Atlas, Varentsova et al., 2014)) using the harmonized dMRI data as previously described (Pasternak et al., 2009). Later, FAt and FW were registered to the common template. For the whole brain and each tract, percent change over time was modeled with quadratic curves (the best fitted model: highest adjusted r2) and fitted separately to the SZ and HC populations. Peak age, as well as upper and lower bounds of the model, were estimated after 5000 bootstraps. Whole brain percent differences between SZ and HC for both FAt and FW were modeled at each age, covarying for sex. Effect sizes (Cohen's d) were also computed at each age.

Results: The statistical analyses in this study are presently ongoing. The DMRI data pre-processing and harmonization steps for all 1092 subjects have already been completed. We plan to present lifespan time courses of average FAt and FW for the whole brain as well as the 14 major white matter tracts. These data will also be compared to life span time course of the traditional dMRI metric, fractional anisotropy (FA), which were recently accepted for publication (Cetin-Karayumak et al., Molecular Psychiatry, In Press).

Conclusions: This is the largest Free Water Imaging study in schizophrenia, to date. The combination of a whole-brain brain approach with a more refined region-of-interest analyses on lifespan FAt and FW in 14 major white matter tracts will help to determine which white matter structures exhibit specific cellular and/or extracellular pathologies, as well as the timeline of these pathologies.

Keywords: Free Water, Schizophrenia; Technology, Scanner Harmonization, Diffusion Weighted Imaging, White Matter

Disclosure: Nothing to disclose.

W171

Transcriptional Changes in the Stress Pathway are Related to Symptoms in Schizophrenia and to Mood in Schizoaffective Disorder

Abstract not included

W172

miRNA30a-Related Changes in the Prenatal Stress Model and Modulation by Lurasidone Treatment: Implications for Psychiatric Disorders

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Background: Exposure to early life stress (ELS) produces widespread changes in brain function that may predispose individuals to develop a wide range of psychiatric disorders later in life. This may occur through the epigenetic regulation of gene expression involving changes in DNA methylation as well as miRNA expression. Animal models are particularly useful to investigate the molecular and functional mechanisms that are persistently affected after exposure to ELS and that may represent important targets for pharmacological interventions. On these bases, we performed genome-wide methylation analyses in the hippocampus and prefrontal cortex of adult male and female rats exposed to stress during gestation (PNS), a model that is associated with persistent behavioral and molecular alterations relevant for psychiatric disorders.

Methods: Using MeDIP-chip, we investigated changes in the prefrontal cortex and hippocampus of adult male and female rats exposed to restraint stress during the last week of gestation. After having identified the most promising candidates from this analysis, we investigated their expression profile during postnatal maturation, in order to establish potential changes in their developmental trajectories. We also investigated whether subchronic treatment with the antipsychotic drug lurasidone given during the peripubertal period could prevent or modulate some of the changes brought about by PNS exposure.

Results: We found that a large number of gene promoters were differentially methylated in the prefrontal cortex and hippocampus of adult male and female rats exposed to stress during gestation. An overlap of 138 differentially methylated genes around the transcription start site (-2000 to +500) was observed among the two brain regions and genders. By restricting the overlap to genes that were modulated in the same direction, we identified two genes, which were also interacting together: miR-30a and NEUROD1 that were both less methylated in PNS exposed rats. Accordingly, the expression of miR-30a and of NEUROD1 was significantly increased in adult rats exposed to PNS. Using miRWalk database, we found that miRNA30a is involved in the modulation of pathways related to axon guidance and neurotrophin signaling. Among the genes involved in these two pathways, we decided to validate CAMK2A, c-JUN, LIMK, MAP2K1, MAP2K2, PIK3CA and PLCG1 as miRNA30 targeted genes. We found that the cumulative score of their mRNA level modulation following PNS supported the downregulation of the two identified pathways related to miRNA30a. Importantly, sub-chronic treatment with lurasidone was able to prevent - to a large extent - the up-regulation of miR30a as well as the changes observed on its target genes.

Conclusions: In summary, our genome-wide approach allowed us to identify miR-30a as being persistently affected by PNS through epigenetic changes. This miRNA may represent a master regulator for the increased susceptibility to psychiatric disorders as a long-lasting consequence of early life stress exposure, by affecting pathways related to axon guidance and neurotrophin signaling. Moreover, chronic treatment with the antipsychotic drug lurasidone was able to prevent some of the changes produced by PNS with a major effect on miRNA30a levels as well as on its target genes LIMK, MAP2K2, and PIK3CA.

These effects may be particularly relevant in preventing some molecular alterations induced by ELS exposure, known to be involved in the development of stress-related psychiatric disorders, and thus be useful in minimizing the individual risk of vulnerability.

Keywords: Lurasidone, Epigenetic Modification, miR-30a, Early Life Stress, Neurotrophin Signalling

Disclosure: Sumitomo Dainippon Pharma, Honoraria, Sumitomo Dainippon Pharma, Grant

W173

A Novel Behavioral Paradigm for Measuring Hallucination-Like Perceptions in Mice

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Background: Excessive activity of midbrain dopamine projections is thought to be causal for psychotic experiences such as hallucinations. However, it remains open which neural circuits mediate the observed link between dopamine and hallucinations. Recent methodological developments in neurosciences have yielded powerful tools for the study of midbrain dopamine projections in rodents. To take advantage of these developments, it is crucial to find a reliable behavioural readout of hallucinations in rodents. Here, we built on recent work in human psychosis research relating hallucinations to quantifiable perceptual alterations that are eminently testable in rodents. We aimed at establishing a behavioural paradigm to measure perceptual alterations in rodents as a behavioural readout of hallucinations that would enable the circuit-level interrogation of the observed link between dopamine and hallucinations.

Methods: Following the rationale that hallucinations can be operationalized as false percepts that are experienced with high confidence, we developed a psychometric auditory detection task with time-investment-based confidence reports. In this task, mice are presented with tones of varying strength embedded in noise or with noise only and report whether or not they perceived a tone by poking into one of two choice ports. Correct choices are rewarded with a water reward that is delivered after a variable, unpredictable interval which enables to measure the time mice are willing to invest for a reward as a measure of confidence. To manipulate prior expectations, we varied the ratio between tone-in-noise and noise-only trials. Behavioral data were analyzed using a computational model of statistical confidence. Moreover, we started to measure striatal dopamine activity during the task using fiber photometry.

Results: After training, mice were consistently able to perform the task: their choices were closely fitted by a psychometric curve (explained variance mean 40.0%, range 24.0% to 55.1%) and time investment behavior significantly correlated with the predictions of the statistical confidence model ($r = 0.27$, $p < 0.001$; data from 8 mice, 126 sessions, 67575 trials). We found that prior expectations of hearing a tone induced more hallucination-like percepts, as indicated by an effect of trial ratio on false alarms ($F=30.0$ $p < 0.001$) and confidence in false alarms ($F=7.7$, $p = 0.003$; data from 8 mice, 83 sessions, 48399 trials). We further found preliminary evidence that higher striatal dopamine activity favored hallucination-like percepts, as indicated by higher photometry signals at stimulus delivery preceding false alarms as compared to correct rejections (2 mice, 6 sessions, 3190 trials).

Conclusions: Our new behavioral paradigm generates reliable measures of hallucination-like perceptions in mice and enables

the investigation of the neural circuits mediating the suggested link between dopamine and hallucinations. We suggest that hallucination-like perceptions can serve as a behavioral marker of hallucinations in rodents.

Keywords: Hallucinations, Rodent Models, Dopamine, Auditory Perception, Confidence

Disclosure: Nothing to disclose.

W174

Cognitive Impairment in Never-Treated Schizophrenia Spectrum Individuals

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Background: Cognitive impairment is a prominent feature of schizophrenia along disease progression. However, antipsychotic treatment may influence neuropsychological performance, hence reducing the chance of recognizing the distinctive cognitive features of the disease. We investigated the cognitive function in a group of antipsychotic-naive schizophrenia spectrum individuals and compared their results with a group of healthy controls.

Methods: Four groups of individuals were assessed: 87 individuals at Clinical High-Risk for psychosis (CHR), 91 First-Episode Psychosis patients (FEP), 16 Chronic Schizophrenia patients (CSz) and 77 Healthy Controls (HC). All participants underwent cognitive testing with the MATRICS Consensus Cognitive Battery (MCCB).

Results: Repeated-measures analysis of variance revealed a significant effect of group ($F(1,251)=97.1$, $p<.001$). Paired comparisons revealed a progressive effect, with highest MCCB composite scores in the HC group, compared with all the clinical groups (CHR mean difference (MD)=17.9; FEP MD=38.9; CSz = MD=33; $p<.001$ in all cases). While the CHR group showed higher scores compared with the groups with full-blown psychosis (FEP MD=20.9; CSz MD=15.45; $p\leq.001$ in both cases), no significant differences were found between FEP and CSz groups (MD=5.5, $p = 1.0$).

We also found a significant interaction of group by domain ($F(15,1291)=6.7$, $p<.001$). In all cases, no significant differences were found between the FEP and CSz groups. Interestingly, the CHR group was not significantly different from the other clinical groups in Attention/vigilance, Working memory, Verbal learning, and Social cognition domains ($p\geq.2$ in all cases). On the other hand, the CHR group showed similar scores to the HC group in Visual learning (MD=11, $p = .07$).

Conclusions: We found significant cognitive failures since at-risk stages of schizophrenia spectrum. Interestingly, some cognitive domains related to executive and memory function were similar across all clinical groups, including CSz. Moreover, patients with first-episode psychosis were as impaired as those with chronic disease.

Since considerable cognitive impairment is present from early stages of the schizophrenia spectrum, and the performance of first-episode patients is similar to those at chronic stages, efforts for early detection and cognitive remediation are needed.

Keywords: Cognitive impairments, MATRICS Consensus Cognitive Battery, Schizophrenia, Clinical High-Risk, First-Episode Psychosis

Disclosure: Janssen (Johnson & Johnson), Consultant

W175

Imaging the Endocannabinoid-Metabolizing Enzyme Fatty Acid Amide Hydrolase in Psychosis

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Background: Anandamide and the cannabinoid CB1 receptor are thought to be altered in psychosis, however, it remains unknown whether fatty acid amide hydrolase (FAAH) – the enzyme responsible for maintaining anandamide levels – is altered in the brain in patients with psychosis.

Methods: Sixty four participants, including twenty-four patients with psychotic disorders and 38 healthy control participants completed [11C]CURB positron emission tomography (PET) and MRI scans. We investigated the relation of FAAH with psychotic symptom severity, duration of illness and duration of untreated psychosis.

Results: Psychosis patients did not differ in FAAH compared to healthy controls in any brain region studied ($F1,61.91 = 2.19$, $p = .14$). Patients with greater positive psychotic symptoms had lower FAAH across all regions ($F1,23.73 = 6.43$, $p = .018$), and those with high symptom severity ($n = 9$) had lower FAAH compared to patients with low symptom severity ($F1,23.73 = 8.32$, $p = .008$). Higher FAAH was associated with longer duration of illness ($F1,23.72 = 6.99$, $p = .014$). Across all participants, FAAH was higher in females than in males ($F1,61.88 = 11.78$, $p = .001$) and exhibited marked differences across brain regions ($F5,103.19=27.19$, $p<1x10-16$).

Conclusions: FAAH demonstrated robust regional variations and, in the combined sample, FAAH was higher in females compared to males. Our findings suggest that overall, FAAH is not different in a cross-sectional sample of patients with psychotic disorders of varying severity compared to healthy controls. However, patients with greater positive psychotic symptoms and shorter duration of illness had lower FAAH across all regions. FAAH may be a useful biomarker of disease stage and potential stratification tool for clinical studies targeting psychotic symptoms.

Keywords: Endocannabinoid System, Psychosis, Fatty Acid Amide Hydrolase, PET Imaging

Disclosure: Nothing to disclose.

W176

Application of Rasch Modelling and Principal Component Analysis to Identify Negative Symptom Trajectories in First-Episode Schizophrenia

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Background: The Positive and Negative Syndrome Scale (PANSS) is widely used for assessment of treatment response including change in negative symptom severity in schizophrenia trials. Calculation of a composite negative subscale score provides an apparent clinical measure of negative symptom severity. However, analyzing ordinal data as continuous data using composite score-based methods does not comply with

the fundamental nature of PANSS data. The appropriateness of using the negative symptom subscale score to compare negative symptom severity between subjects and across time can be psychometrically evaluated using Rasch analysis. This method belongs to the family of Item Response Theory (IRT), but unlike IRT models, which seek to fit the response model to the data, the Rasch model requires the data to fit the model. In that sense, Rasch is a statistical model linking unambiguously sufficiency of the total or subscale scores with a statistical fit of the Rasch model. IRT has previously only been applied to data from samples of patients with chronic schizophrenia. Here, in a unique sample of first-episode patients, we aimed to test fit of the PANSS negative subscale by the Rasch model, to investigate latent negative symptom trajectories and to replicate and extend our findings in another and larger data set.

Methods: We analyzed the OPTiMiSE data set comprising 446 first-episode schizophrenia patients treated with open-label amisulpride for 4 weeks. We evaluated the scalability of the PANSS negative subscale using Rasch analysis. Subsequently, we performed a Principal Component Analysis (PCA) using polychoric correlations of the PANSS negative subscale and applied cluster analysis of successive differences in the main component score to identify symptom trajectories. In addition, we are, in ongoing analyses, applying both Rasch analysis and PCA in a combined larger data set comprised of data from the following 3 European first-episode schizophrenia trials: OPTiMiSE ($N = 446$); EUFEST ($N = 498$) and PECANS ($N = 215$). The methods and respective results will be compared to better understand the differences including pros and cons of these alternate statistical methods. All the trials were designed as prospective cohort studies and included both sexes. Since these results concern a re-analysis of data from finalized clinical trials, estimation of effect size and power calculation are not applicable.

Results: The PANSS negative subscale data from the current study did not fit the Rasch model showing low internal consistency ($p \leq 0.005$) across all items. This means that combining information from the individual negative symptom items into a composite score did not make full use of the information contained in each item score. Consequently, this would imply loss of statistical power when using composite score-based methods. PCA revealed one significant component clustering into two significant symptom trajectories: One dominant trajectory with subtle but constant decrease in negative symptom severity across visits, and one minor trajectory with symptom instability across visits. The results of the extended Rasch analyses and PCA from the larger data set of first-episode patients will be presented at the meeting.

Conclusions: The PANSS negative subscale in a first-episode psychosis multinational sample did not possess the necessary psychometric properties to support the calculation of a composite score across items. With the statistical methods applied here, we identified two separate negative symptom trajectories, however, interpretation was limited by the duration of observation time. The combined results from the primary and extended analyses will add to the existing knowledge base of psychometric limitations of the PANSS and inform future clinical trials on how to evaluate efficacy data most efficiently in order not to use power when applying conventional composite score-based methods.

Keywords: PANSS Inconsistencies, Item Response Theory, Schizophrenia Negative Symptoms

Disclosure: Nothing to disclose.

W177

Abnormalities in Serine/Threonine Signaling Networks in Severe Neuropsychiatric Illness: Development of a Pipeline to Explore Abnormalities of Kinase Activity and Identify Novel Treatment Strategies for Cognitive Disorders

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Background: Abnormalities of cellular signaling are well characterized in neuropsychiatric illnesses, including schizophrenia, major depressive disorder, and Alzheimer's disease. Changes in signaling pathways reflect the underlying genetic, environmental, and epigenetic perturbations driving disease phenotypes. A shortcoming of most signaling studies is a focus on one or a few protein kinases at a time, a limitation since protein kinases work in networks with other kinases, phosphatases, and regulatory molecules to effect signaling events. We addressed this challenge by employing a kinome array platform that simultaneously measures protein kinase activity at hundreds of reporter peptide substrates. We then developed a novel bioinformatics pipeline to identify protein kinase nodes, signaling networks, upstream biological pathways, and drug candidates that "reverse" kinomic disease signatures.

Methods: Postmortem DLPFC brain samples from subjects with schizophrenia, MDD, and AD ($n = 20$ per group, 9-10 males and 10-11 females per group), were compared to age, PMI and pH matched control subjects ($n = 20$ per group, 10 males and 10 females per group) using the Pamgene12 serine/threonine kinome array chip. Samples were pooled by diagnosis and gender, and run in triplicate. The R-shiny app KRSA was created to automate assignment of kinases, perform permutation analyses, identify biological pathways, and connect to iLINCs for identification of drugs that reverse kinomic disease signatures. We also performed targeted confirmation studies using specific kinase activity assays, QPCR, and western blot analyses.

Results: We identified unique and common kinase nodes for each diagnostic group. Several of the nodes (for example AKT) are well characterized in schizophrenia, while others have not previously been identified (such as AMPK). We used AMPK knockdown cultures and AMPK KO brain tissues to demonstrate the validity of the kinome array for this protein kinase. We used standard kinase activity assays for AMPK and found decreased activity for AMPK in AD ($P < 0.05$). We also found decreased expression of transcripts for the regulatory subunits of AMPK in AD ($P < 0.05$). In contrast we found an increase in the enzyme stabilizing subunits of AMPK ($P < 0.05$). We identified several unique biological pathways, as well as candidate drugs, associated with the disease signatures in all three diagnostic groups. We are currently devising a strategy to compare kinomic signatures across diagnostic groups.

Conclusions: Our results confirm well characterized signaling defects in severe neuropsychiatric illness, and identify novel signaling nodes for further study. Confirmation studies for AMPK kinase show significant changes in expression and activity of this kinase, suggesting perturbation of energy sensing and production pathways in schizophrenia and AD, but not MDD. Bioenergetic pathways may be targeted by diverse mechanisms, and we identified several drug candidates that might help restore this pathway in afflicted persons. Overall our novel workflow and pipeline provides a promising new avenue for understanding the complex signaling perturbations found in brain diseases and may

provide new leads for developing treatments for cognitive disorders.

Keywords: Bioinformatics, Postmortem Brain Tissue, Kinome Array, Neuropsychiatric Disorders [Schizophrenia, Parkinson's Disease, Major Depressive Disorder]

Disclosure: Nothing to disclose.

W178

A Comparison of Cortico-Limbic Structural Brain Networks in Patients With Remitted Psychotic Depression to Healthy Controls

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Background: Cortico-limbic brain regions have been implicated in depression. The limited neurobiological evidence on psychotic depression also suggests cortical and limbic involvement. In contrast with a regional approach, this case-control study uses structural magnetic resonance imaging (sMRI) to compare structural brain networks in patients with remitted psychotic depression to healthy controls.

Methods: Male and female participants were recruited at four academic centers and our sample included patients with remitted psychotic depression ($n = 86$) and healthy controls ($n = 159$). Patients participated in the Study of Pharmacotherapy of Psychotic Depression (STOP-PD) II randomized controlled trial, were treated with sertraline and olanzapine, and attained remission on these two medications. We used a novel, whole-brain, unsupervised pattern analysis technique--Non-negative Matrix Factorization (NMF)--and applied it to cortical thickness data. Structural brain networks were derived from cortical thickness data using NMF. We aimed to compare patients to controls and hypothesized NMF would identify networks implicating cortico-limbic abnormalities in patients.

Results: We confirmed our hypothesis and found significant ($p<0.05$, False Discovery Rate corrected) abnormalities in structural brain networks that incorporated cortico-limbic regions in patients. Cortical thinning was observed in five structural brain networks, namely the insular-limbic ($t=3.658$, $p = 0.004$), occipito-temporal ($t=2.434$, $p = 0.044$), temporal ($t=3.160$, $p = 0.008$), parahippocampal-limbic ($t=3.290$, $p = 0.008$), and inferior frontal-temporal networks ($t=2.684$, $p = 0.027$).

Conclusions: Future studies will examine longitudinal cortical thickness changes in these structural brain networks and further examine their utility in predicting treatment outcomes.

Keywords: Psychotic Depression, Neuroimaging, Cortical Thickness

Disclosure: Nothing to disclose.

W179

Salience Network Involvement in Psychopathology in the Clinical High-Risk (CHR) for Psychosis State

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Background: The brain's Salience Network (SN) is comprised of structures such as the anterior insulae (AI) and the amygdalae, which are critical for signaling the occurrence of biologically-important (but not necessarily rewarding) stimuli or events. Given that psychosis has been linked to the abnormal processing and signaling of salience, it is not surprising that SN structures are often implicated in the development and expression of psychosis. The AI and the amygdalae have also been shown to play roles in learning and motivation, by virtue of their responsivity to salient outcomes and cues that predict those outcomes. Evidence suggests that SN abnormalities are present not only in people with established psychotic illness, but also in those at clinical high-risk (CHR) for psychotic illness, who experience a range of symptoms and are often diagnosed with disorders of mood and anxiety. We hypothesized that aberrant neural activity in SN structures could be directly involved in the expression of psychopathology in CHR individuals, with different neural abnormalities associated with specific symptom domains.

Methods: Participants were 22 individuals at CHR for psychosis and 19 demographically-matched controls. Participants were classified as being at CHR for psychosis using the Structured Interview for Psychosis-risk Syndromes (SIPS), and symptom severity was assessed using the SIPS and the Behavior Assessment System for Children (BASC). In conjunction with fMRI, participants performed two experimental tasks designed to measure overlapping aspects of reward processing. In order to distinguish neural signals evoked by reward anticipation from those associated with reward receipt, we used a modified Monetary Incentive Delay (MID) task. Participants saw cues that signaled they could either expect to gain money, lose money, or experience a neutral outcome. Responding within an acceptable time window on a given trial resulted in either a large gain or a nominal loss ($1). Failing to respond within this window on a given trial resulted in either a nominal gain ($1) or a large loss. On neutral trials, participants always received $0. In order to distinguish neural signals evoked by surprising and unsurprising gains and losses, participants performed a simple probabilistic reinforcement learning (RL) paradigm. Participants selected one out of three card decks, identified by color using a button-box, with the goal of identifying the optimal deck (the one with the highest expected value) as quickly as possible. A choice of the optimal deck led to a 100-point gain on 70% of trials (and a loss of 50 points on 30% of trials), while choices of two non-optimal decks led to 100-point gains on 50% and 30% of trials, respectively. Outcomes were classified as "Valid Wins" when participants were rewarded for choosing the optimal deck and as "Invalid Losses" when participants were punished for choosing the optimal deck. Outcomes for choices of non-optimal decks were termed either "Valid Losses" or "Invalid Wins". We tested for between-group differences in cue and outcome MRI contrasts using t-tests, and we tested for systematic relationships between symptom measures and MRI contrasts using Spearman correlation analyses.

Results: In the context of the MID Task, we observed that anterior insula (bilaterally) clearly differentiated between anticipated gains and losses, and between received gains and losses, in the entire sample (corrected for multiple comparisons across the whole-brain). Regions-of-interest analyses revealed that psychotic symptom scores correlated significantly with sensitivity to cue valence and magnitude in both left ($rho=0.538$) and right ($rho=0.588$; both $p's<0.02$) AI, with more psychotic individuals actually showing greater insular activations. Both anxiety ($rho = -0.607$) and depression ($rho = -0.595$) subscores from the BASC correlated with sensitivity to cue valence and magnitude in left amygdala, with more anxious and depressed individuals showing reduced amygdalar activations. In the context of the RL Task, we observed that CHR individuals and controls differed in [Valid – Invalid Win] contrasts in right amygdala [$t(32)=2.484$, $p = 0.018$], primarily because CHR

participants showed greater activations to surprising wins than controls did. Within the group of CHR participants, we observed significant correlations between psychotic symptom scores and [Valid Win – Loss] contrasts in left AI (rho = −0.463), left amygdala (rho = 0.584), and right amygdala (rho = 0.651). Finally, we observed significant correlations between anxiety and depression scores from the BASC (as well as multiple measures of anhedonia) and [Valid Win – Invalid Loss] contrasts in both left and right AI.

Conclusions: These findings indicate that the amygdalae and anterior insulae – critical nodes of the brain's Salience Network – exhibit abnormal signals in adolescents and young adults at clinical high risk for psychotic illness. Furthermore, we observed systematic relationships between the severity of clinical symptoms in multiple domains and the sensitivity of the amygdalae and anterior insulae to the valence and magnitude of reward predicting cues and outcomes. These results support the idea that specific aspects of salience processing can be tied to clinically-ratable psychiatric symptoms in multiple domains (psychotic, anxiety, and depressive) across the spectrum of psychotic illness.

Keywords: Schizophrenia, Insular Cortex, Amygdala, Negative Symptoms

Disclosure: Nothing to disclose.

W180

Real-World Effectiveness of Antipsychotic Treatments in 37,368 Patients With Schizophrenia in the U.S. VA System

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Background: Randomized controlled trials are considered the gold standard to compare effectiveness between different drugs; however, they are very expensive, participants often receive more care and support relative to common clinical practice, and they only include participants who consent to research. Large pragmatic studies based on comprehensive administrative data reflect the combination of clinicians' and patients' preference for antipsychotic medications in the real world, and all drugs administered to all patients can be compared. We utilized data on medication utilization on 37,368 US veterans with schizophrenia treated in the US Veteran's Administration, in order to observe the effectiveness of all antipsychotic drugs administered for all patients with schizophrenia. This study was funded by the Stanley Medical Research Institute, a non-profit, charitable organization.

Methods: We used VA administrative health services and prescription records to identify 37,368 VA patients with schizophrenia who initiated treatment with oral or long-acting injectable (LAI) first-generation antipsychotic (FGA) or second-generation antipsychotic (SGA) medications, including antipsychotic polypharmacy, between 10/2010 and 9/2015. Cox proportional hazards models compared the risk of discontinuation, defined as the first gap in prescribing of 46 days or more between prescriptions, among the antipsychotics, controlling for patient's demographic and clinical characteristics. The reference group was oral olanzapine.

Results: In multivariable analysis, clozapine (Hazard Ratio (HR): 0.425, P<0.0001), LAI aripiprazole (HR: 0.706, p = 0.0003), LAI paliperidone (HR: 0.764, p <0.0001), antipsychotic polypharmacy (0.772, p <0.0001), and LAI risperidone LAI (HR: 0.905, p = 0.0043) were associated with a reduced hazard of discontinuation compared to oral olanzapine. Oral FGAs (HR: 1.161, p<0.0001), oral risperidone (HR: 1.146, p <0.0001), oral aripiprazole (HR: 1.138, p <0.0001), oral ziprasidone (HR: 1.128, p>0.0001), and oral quetiapine (HR: 1.106, p< 0.0001) were statistically significantly associated with an increased risk of discontinuation compared to oral olanzapine.

Conclusions: These results show clearly that clozapine, long-acting injectable second generation antipsychotics and antipsychotic polypharmacy are taken for the longest period by patients and might be considered to be the most effective drugs in this study; oral antipsychotics were less effective then oral olanzapine in terms of time to discontinuation. These results are strikingly similar to those of very similar studies performed in Scandinavia and reflect what happens in clinical practice, but contradict guidelines recommending monotherapy.

Keywords: Antipsychotic Treatment, Long-Acting Injectable Antipsychotics, Pharmacoepidemiology, Schizophrenia

Disclosure: Nothing to disclose.

W181

A Structural Basis for How Ligand Binding Site Alterations can Allosterically Regulate GPCR Signaling and Engender Functional Selectivity

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Background: G protein-coupled receptors (GPCRs) represent the largest family of cellular receptors in mammals and are critical drug targets accounting for approximately one third of all FDA-approved drugs. These receptor proteins regulate multiple physiological processes by transducing extracellular stimuli into intracellular signals through activating both G protein-dependent and independent pathways, leading to second messenger generation and downstream signaling events. G protein-independent pathways are primarily mediated by β-arrestin proteins, which were originally identified as mediators of agonist-induced desensitization, but subsequently determined to also function as multi-valent scaffolding proteins that orchestrate various intracellular signaling pathways. While endogenous agonists promote GPCR signaling through the activation of both G proteins and β-arrestins, some synthetic agonists have been described to preferentially activate one pathway versus the other, a phenomenon known as functional selectivity or biased signaling. The therapeutic potential of biased signaling is high as drugs that selectively modulate clinically relevant pathways, without affecting other signaling events, may exhibit fewer side effects. While the molecular mechanisms underlying biased signaling are not known with certainty, a leading hypothesis is that GPCRs can adopt distinct active conformational states that are selectively stabilized by different signaling biased ligands. We previously described a G protein-biased D2 dopamine receptor (D2R) agonist, MLS1547, that is efficacious for G protein-mediated signaling, but relatively ineffective for recruiting β-arrestin. Structure-activity relationship analyses using MLS1547 and its analogs led to a pharmacophore model to explain the biased signaling properties of this compound. This involved the interaction of the ligand with a hydrophobic pocket comprised of residues I184, F189 and V190 at the junction of the fifth transmembrane segment (TM5) and second extracellular loop (EL2) of the D2R. Here, we identify residue F189 in the D2R (position 5.38 using the Ballesteros-Weinstein numbering system) as a micro-switch that regulates the active state for recruiting β-arrestin.

Methods: Receptor constructs encoding the D2R, D3R, D4R, and the beta2-adrenergic receptor (β2R) were mutated using standard

procedures. The WT and mutant receptors were transiently transfected into HEK293 or CHO cells and evaluated using multiple signaling outputs mediated by either G proteins or β-arrestins. To elucidate how mutation of Tyr5.38 to Ala affects β-arrestin interactions with the receptor, we comparatively simulated β2R-WT and β2R-Y1995.38A using a molecular dynamics (MD) simulation approach. The coordinates of active state β2R bound to the agonist BI-167107 (PDB code 4LDE) were used in MD simulations. The β2R-Y1995.38A model was prepared from representative frames from equilibrated β2R-WT MD simulation trajectories. For both β2R-WT and β2R-Y1995.38A, we collected 14 total trajectories with an aggregated simulation length of 21.0 μs.

Results: As position 5.38 is relatively conserved (frequently Phe or Tyr) in class A GPCRs, we introduced an alanine mutation (Phe/Tyr to Ala) at this position for all D2-like receptors (D2R, D3R, and D4R) and the β2-adrenergic receptor (β2R) to study the role of position 5.38. Strikingly, we found that the alanine 5.38 mutation negated the receptors' ability to recruit β-arrestin in response to agonists, while G protein signaling efficacy was maintained. These data suggest that the presence of a Phe or Tyr residue at position 5.38 in these GPCRs is critical for stabilizing an activate state for recruiting β-arrestin. To investigate how alterations at this position produce conformational rearrangements resulting in signaling bias, we used the β2R, for which active state crystal structures are available, to build both β2R-WT and β2R-Y199A models in complex with the full β2R agonist BI-167107, and performed extensive molecular dynamics simulations. Using this approach, we identified residues that differentially interact with BI-167107 in the β2R-WT versus β2R-Y199A leading to conformational rearrangements that propagate through the TM3-TM4-TM5 interface to the intracellular side of the receptor. These coordinated changes result in a different tilt of TM4, face shift of TM4 and TM5 on their extracellular sides, and ultimately an altered orientation of intracellular loop 2 (IL2) in the β2R-Y199A compared to the β2R-WT. Strikingly, such coordinated changes and altered IL2 conformations are reminiscent of the differences between the recently solved cryo-EM structure of the rhodopsin-Gi complex and the crystal structure of the rhodopsin-arrestin complex. As the conformation of IL2 is known to be critical for β-arrestin engagement and activation, these results may provide a mechanism for how residue 5.38 can modulate the signaling bias for many GPCRs.

Conclusions: In summary, our current study illustrates how structural perturbations in the extracellular ligand binding site can allosterically propagate to the intracellular surface of a GPCR and affect its ability to interact with signaling transducers, thus producing signaling bias. The further elucidation of structural determinants that underlie agonist-specific signaling states may assist in the rational design of novel functionally-selective agents that can serve as improved therapeutic agents.

Keywords: Dopamine, Signaling, Allosterism, Bias, Receptor

Disclosure: Nothing to disclose.

W182

Differential Resting-State Connectivity and its Relation to Spatial Gene Expression Patterns in a Mouse Model of 22q11 Deletion

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Background: 22q11 deletion is commonly regarded as the strongest known single molecular genetic risk factor for developing schizophrenia. Characterizing the effects of this CNV on neural networks offers a unique avenue towards delineating polygenic interactions in the pathogenesis of this disorder.

Methods: We here relied on a mouse model of 22q11 deletion (Df(h22q11)+) and littermate controls to identify differential resting-state functional connectivity (rsFC) patterns across networks derived from cortical, subcortical and mesencephalic seed regions. Using the Allen Mouse Brain Atlas, we analyzed the expression patterns of the genes deleted in our model (27 genes in the deletion, 24 of them available in the Allen Mouse Brain Atlas) to identify which gene expression patterns spatially overlapped with differential connectivity. To confirm the translational relevance of our findings in humans, we used machine learning to explore, whether genes implicated by our analyses were co-expressed in human tissues.

Results: We found significant associations between differential resting-state connectivity and spatial gene expression patterns for all contrasts. Genes overexpressed in a given region with differential connectivity formed functional networks above chance for all contrasts. These networks were functionally annotated with the terms "response to drug", "axon part", "plasma membrane" and "flavin adenine dinucleotide binding". Of note, two genes, COMT and Trmt2a, were consistently overexpressed in regions showing hyper- or hypoconnectivity in our model animals. Our analyses of human data sets confirmed co-expression of COMT and Trmt2a in humans but did not retrieve any different patterns between patients and controls.

Conclusions: Our findings suggest that differential resting-state connectivity patterns in our mouse model of 22q del are mediated by polygenic networks in regions with altered connectivity. COMT and Trmt2a seem to play a key role in this regard and form the core components of these networks. Co-expression patterns in humans hint at a potential interaction also in humans. Although the mechanistic interactions between the two genes have not been explored yet, but might constitute an important link between neurotransmission, transcriptional activity and functional changes on a neural systems level.

Keywords: Copy Number Variation, Functional Neuroimaging, Animal Models, Gene Expression, Gene Co-Expression

Disclosure: Nothing to disclose.

W183

Combined PET-FDG, Fractional Anisotropy, Dopamine Receptor Binding Potential and MRI Imaging in Never-Medicated Patients With Schizophrenia

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Background: Dopaminergic dysfunction, diminished frontal function, and changes in white matter integrity are among the most replicated findings in schizophrenia. A modulating role of dopamine in white matter integrity has been proposed in animal models and healthy human brain, but has not yet been systematically explored multimodal imaging in schizophrenia. We used four modes, FDG-PET, diffusion tensor imaging, 18F-fallypride positron emission tomography, and anatomical MRI in 19 healthy and 25 never-medicated schizophrenia subjects to assess the relationship between dopamine binding potential, metabolic rate, and white matter fractional anisotropy.

Methods: Study participants comprised 19 healthy and 25 schizophrenia subjects, recruited from greater Dayton, Ohio, metropolitan area (Table 1), and evaluated using the Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992). Schizophrenia subjects were diagnosed with the Structured Clinical Interview for DSM-IV Axis I Disorders and standard clinical psychiatric evaluation. Eight subjects initially diagnosed with schizophreniform disorder were followed longitudinally to confirm their progression to schizophrenia. Twenty schizophrenia subjects were neuroleptic-naïve and five were previously medicated (of which 2 subjects had a lifetime neuroleptic exposure of approximately one week, and one subject each – approximately 2 weeks, 3 months, and 3.8 years). No subjects received neuroleptic medications for the 30-day period prior to the scanning. Healthy volunteers were age- and sex-matched to the patient group and were recruited through advertisement.

Results: We found a pattern of predominantly negative correlations between white matter metabolism and fractional anisotropy in both healthy and schizophrenia subjects. The overall strength of the relationship was attenuated or reversed in subjects with schizophrenia, who displayed significantly fewer and weaker correlations. Significant differences in correlation were marked in the frontal white batter adjacent to Brodmann areas 46, 9 and 10 where we and others have found disturbed fractional anisotropy values. Logistic regression with FDG and fractional anisotropy significantly predicted diagnosis from this same area. Logistic regression combining FDG-PET, Fallypride-PET and fractional anisotropy extended these findings into gray matter areas.

Conclusions: Frontal white matter, anterior limb of the internal capsule and frontal gray matter contribute to differences between healthy volunteers and never-medicated patients with schizophrenia. Psychopharmacological approaches which target connectivity between dopamine-rich structures and prefrontal regions may be valuable.

Keywords: Fallypride Binding Potential, Prefrontal Cortex, Diffusion Tensor Imaging

Disclosure: Nothing to disclose.

W184

Postnatal Development of Glutamate and GABA Transcript Expression Across the Cortical Visuospatial Working Memory Network in Monkeys

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Background: Visuospatial working memory (vsWM), a key cognitive function impaired in schizophrenia, requires information transfer among multiple cortical regions, including visual (V1, V2), parietal (PPC) and prefrontal cortices (PFC). This information is conveyed principally via projections from layer 3 glutamatergic pyramidal neurons whose activity is regulated by GABAergic neurons. In primates, vsWM performance improves through adolescence, when multiple components of glutamate and GABA neurotransmission are being refined. In layer 3 of the adult human neocortex, we previously reported the presence of opposed caudal-to-rostral gradients of glutamate and GABA transcript levels across the vsWM network, such that glutamate transcript levels were generally lowest in V1 and highest in the PFC, whereas GABA transcript levels were generally highest in V1 and lowest in the PFC. Therefore, in this study we sought to determine 1) if these glutamate and GABA transcript gradients are present in layer 3 from homologous regions of adult monkey neocortex, 2) the developmental trajectories of layer 3 glutamate and GABA transcripts in these regions, and 3) if these developmental trajectories differ between layers 3 and 6.

Methods: Rhesus monkeys (N = 30) were assigned to 4 age groups based on developmental changes in excitatory synaptic density as follows: Group 1 (0.1-1 months), rising phase, the postnatal period when excitatory synaptic density is increasing; Group 2 (3-15 months), plateau phase, when excitatory synapse density is at a stable plateau; Group 3 (30-47 months), declining phase, the period of excitatory synaptic pruning during the peri-pubertal epoch; and Group 4 (60-144 months), mature phase, following the end of pruning when the density of excitatory synapses is at stable, adult levels. Tissue sections from V1, V2, PPC, and PFC were labeled with Nissl substance and separate samples of layer 3 and layer 6 were captured by laser microdissection from each area. Samples were then processed for real-time quantitative PCR. The expression ratio of 12 transcripts including 3 normalizers, 5 glutamate transcripts (GLS1, vGLUT1, EAAT2, GRIA2, GRIN1), and 4 GABA transcripts (GAD67, vGAT, GAT1, GABRG2) were studied. Normalized composite measures for glutamate and GABA were computed for each layer, region and age group.

Results: In the Group 4 adult monkeys, the glutamate composite measure for all 5 transcripts in layers 3 and 6 showed a caudal-to-rostral gradient of low-to-high levels, whereas the GABA composite measure of all 4 transcripts showed the opposite high-to-low gradient from caudal to rostral regions. However, for the glutamate composite measure, the magnitude of the difference between PFC and V1 was greater in layer 3 than in layer 6, whereas for the GABA composite measure the magnitude of the difference was greater in layer 6 than in layer 3. The glutamate composite measure showed significant effects for age group, region and layer, as well as for the interactions of region-by-age group and region-by-layer. The glutamate composite measure in layer 3 showed a significant effect for region, and the region effect within each age group was also significant. In layer 6, the effect for region was also significant, but the magnitude of this regional effect was smaller than in layer 3. The region effect was significant in Groups 2, 3, and 4 but not in Group 1. For both layers, the age group effect was significant in PPC and PFC, but not in V1 and V2. The GABA composite measure showed significant effects for region and layer, as well as for the interactions of region-by-age group and region-by-layer, but no significant effect of age group. The GABA composite measure in layer 3 showed a significant effect for region, and the region effect was significant in age groups 2, 3 and 4. In layer 6, there was no significant effect for region. However, the age group effect was significant in V1, but not in V2, PPC or PFC. For the glutamate composite measure, regional gradients first appeared in Group 1 in layer 3 and in Group 2 in layer 6, whereas for the GABA composite measure, the regional gradient first appeared in Group 2 in layer 3 and in Group 3 in layer 6.

Conclusions: These findings indicate that 1) glutamate and GABA caudal-to-rostral gradients are present and opposed in the monkey cortical vsWM network; 2) adult patterns of these gradients arise at different phases of cortical development and depend on the region, with the glutamate gradient formed by earlier expression increases in the PFC and the GABA gradient formed by later expression increases in V1; and 3) the rostral PFC and caudal V1 difference is more pronounced in layer 3 than in layer 6 for glutamate, but is more pronounced in layer 6 than in layer 3 for GABA. These findings suggest that in schizophrenia the altered glutamate gradient may be driven by aberrant maturation in the PFC, whereas the altered GABA gradient may be driven by aberrant maturation in V1.

Keywords: Visuospatial Working Memory, Glutamate GABA, Cortical Regions, Cortical Development, Macaque Monkey

Disclosure: Nothing to disclose.

W185

Neuroimmune Mechanisms of Psychiatric Disorders: Longitudinal Evaluation of Diffusion MRI Measures of Extracellular Free Water in a Non-Human Primate Model of Maternal Immune Activation

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Background: A key finding in developmental neurobiology is a fundamental role for immune molecules in guiding normal brain development and healthy synaptic function. Evidence has been accumulating for an immune-based developmental pathophysiology in psychiatric disorders, particularly in schizophrenia. Epidemiological studies have revealed an increased incidence of schizophrenia in offspring of mothers who had an infection during pregnancy while GWAS studies have identified genetic links to the major histocompatibility complex and peripheral changes in immune markers are widely reported in the illness. The murine maternal immune activation model system is widely used to investigate the effects of immune activation during pregnancy on brain development in behavior in offspring. Here we report findings from an ongoing study of a unique cohort on non-human primates (NHP) who underwent MIA (compared to controls) on a promising biomarker of neuroimmune perturbation in vivo--extracellular free water--a diffusion magnetic resonance imaging measure obtained with a multi-shell acquisition, which we have shown in multiple studies to be increased in young people with early psychosis.

Methods: Fourteen pregnant rhesus monkeys (Macaca mulatta) received polyICLC and 14 control animals have been scanned prospectively from both to their carrent age of 3.5 years. The offspring from both groups underwent a diffusion MRI scan on a 3 Tesla Siemens Skyra scanner in which multiple b-value shells were acquired to improve estimation of extracellular free water. Data were collected when the offspring were 1, 6, 12, 24 and 36 months to date. Diffusion images were nonlinearly aligned to individual subject MPRAGE scans, which were segmented and parcellated into regions of interest using multi-atlas techniques. For this preliminary analysis, frontal, cingulate, and temporo-limbic regions were selected as a priori ROIs in addition to whole-brain gray and white matter masks. Group differences were assessed using repeated measures ANOVA and independent samples t-tests.

Results: Results from birth to age 2 years showed a significant main effect of group in both white ($p<.05$) and gray ($p<.001$) cingulate cortex free water, with MIA-exposed offspring showing higher free water. Similar trends were also identified in prefrontal white matter free water ($p = .07$) and whole-brain white ($p = .11$) and gray matter free water ($p = .07$). No significant group by time interactions were identified. Data analysis is currently underway including the 3-year time point.

Conclusions: Despite the lack of gross behavioral abnormalities at age 2, extracellular free water values are increased in MIA-exposed offspring, particularly in the cingulate cortex. More global whole-brain free water group differences, however, did not reach statistical significance, which may indicate some regional specificity to these changes early in development. These NHP MIA model complement the human schizophrenia literature in which extracellular free water increases have been repeatedly identified. And show that changes in the brain occur early in life, well before the emergence of atypical behaviors in the NHP model. Additional more detailed developmental behavioral analyses together with an updated analysis of the free water data at age 3 will be presented.

Keywords: Neuroinflammation, Neuroimmune, Neurodevelopment, Psychosis, Diffusion MRI

Disclosure: Nothing to disclose.

W186

A Novel Animal Model for Schizophrenia: Suppression of the Presynaptic Cytomatrix Protein Piccolo in the Medial Prefrontal Cortex of Mice

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Background: Piccolo, a presynaptic cytomatrix protein, plays a role in cytomatrix organization and synaptic vesicle trafficking in the presynaptic active zone. Previous studies have revealed that mRNA expression levels and some single-nucleotide polymorphisms of the Piccolo-coding gene PCLO were associated with psychiatric disorders. However, few studies are available on the causal relationship between Piccolo dysfunction and schizophrenia symptoms.

Methods: Postmortem brains from eight patients with schizophrenia and five age- and sex-matched normal control subjects based on the Nagoya Brain Bank Consortium were used for this study.

Male C57BL/6J mice (Nihon SLC, Hamamatsu, Japan) were 7-week-old initially weighing 21–26 g. All procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Guidelines for the Care and Use of Laboratory Animals at the University of Toyama (Approval No. A2018PHA-5). Viral vectors were designed to express an antisense sequence for PCLO (TGCTGATCCCAAACTGTCACCTCCAAGTTTTGGCCACTGACTGACTTGGAGGTCAGTTTGGGAT) and enhanced GFP sequence (AAV-PCLO miRNA/EGFP vectors) based on murine miR-155 (BLOCK-iT, Invitrogen), with those containing only the enhanced GFP sequence (AAV-EGFP vectors) being used as control. All procedures were performed in accordance with the Guideline for Recombinant DNA Experiment by the Ministry of Education, Culture, Sports, Science and Technology, Japan and were approved by the Gene Recombination Experiment Safety Committee at the University of Toyama (Approval No. G2015PHA-14). All reactions were performed in duplicate using the following cycling protocol: enzyme heat activation for 10 min at 95 °C, 40 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 40 s and extension at 72 °C for 60 s. Piccolo primers used for real-time PCR were as follows: 5′-GTCAAAACAGCCAGCAGTCC-3′ (forward; 14607–14626 bp) and 5′-GTCCATGAGATCGGAGATGG-3′ (reverse; 14752–14771 bp), 5′-TGCCTGGTTTCTTCTCAGATGT-3′ (forward; 753–774 bp) and 5′-GAGTCTGATATCAAATCAAAAGGGT-3′ (reverse; 816–840 bp). A 36B4 transcript quantified using forward primer 5′-ACCCTGAAGTGCTCGACATC-3′ and reverse primer 5′-AGGAAGGCCTTGACCTTTTC-3′ was used as the internal control. The 64-channel multi-electrode dish system (Alpha MED Sciences, Tokyo, Japan) was employed. In vivo microdialysis was performed. For optogenetic stimulation, AAV-PCLO miRNA/EGFP or AAV-EGFP vectors mixed with AAV-ChIEF vectors were microinjected into the mPFC. Four weeks after microinjection, in vivo dialysis in the dSTR was performed as described above. In optogenetic stimulation, mice received blue light pulses (pulse width, 15 ms; frequency, 10 Hz; laser, 473 nm) for 15 min (ESFI-700, Eicom). Locomotor activity, PPI, novel object recognition, Y-maze and forced swimming tests were performed. All data are expressed as mean ± standard error of the mean. Statistical differences between groups were determined using Student's t-test. Statistical differences among individual values were determined using analysis of variance (ANOVA) followed by the

Student–Newman–Keuls post-hoc test when F ratios were significant ($p < 0.05$). In microdialysis analysis, statistical differences were determined repeated measures ANOVA followed by Bonferroni's post-hoc test (Prism version 5).

Results: Investigation of Piccolo expression levels in the PFC of postmortem patients with schizophrenia who had been prescribed both typical and atypical antipsychotic drugs showed that patients with schizophrenia had significantly higher Piccolo expression in the PFC compared to age- and sex-matched controls. To examine the synaptic properties of mPFC neurons, electrophysiological recordings in mPFC slices from miPiccolo mice were conducted. Analysis of paired-pulse facilitation, an indicator of presynaptic functions, revealed that miPiccolo mice had significant lower ratios at an interstimulus interval of 20 and 60 ms compared to Mock mice.

In measuring acoustic startle response and PPI, a psychometric measure of sensorimotor gating, in Mock and miPiccolo mice, highly significant impairments with the 74 dB prepulse-120 dB pulse trial were observed in miPiccolo mice without any change in startle responses against several single pulses. No differences in both the interaction time in the social interaction test and immobility time in the forced swimming test were observed between non-stress- and stress-exposed Mock mice. However, stress-exposed miPiccolo mice had significantly shorter interaction time in the social interaction test compared to non-stress-exposed miPiccolo mice.

Conclusions: Piccolo-suppressed mice could be useful animal model for schizophrenia.

Keywords: PCLO, Piccolo, Schizophrenia

Disclosure: Nothing to disclose.

W187

Unsupervised Topological Data Analysis Predicts Long-Term Functional and Social Outcome in Early Psychosis

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Background: Early intervention in psychiatry is key to improving patient engagement, response to treatment and functional outcome. In this context, clinical and biological markers that quantify disease course or response to medication play a crucial role in treatment decision. The clinical heterogeneity of patients in the early phase of psychosis hampers the identification of such markers. Stratification is therefore a key step to tailor intervention and to improve functional deficits, which critically contribute to long-term quality of life.

Topological data analysis (TDA) is a powerful approach to studying the shape of biological datasets. We applied TDA to stratify early psychosis patients according to their symptoms and assessed the predictive power of this stratification for long-term functional outcomes. We then searched for a biosignature of the groups, which may represent distinct underlying pathophysiological pathways. We studied redox markers motivated by our hypothesis of redox dysregulation/oxidative stress as a central source of micro- and macro-circuit impairments.

Methods: Subjects: Early psychosis patients (mean age 25y) were recruited within the first year of the Lausanne "Treatment and early intervention program" (Switzerland). A cohort of $n = 101$ patients was used as a test and a second cohort of $n = 93$ patients was used to replicate the results.

Stratification: We used the Mapper algorithm for stratification by TDA. The input data were the 30 item scores of the Positive And Negative Syndrome Scale.

Outcomes after 3-year follow-up: scores of global or social and occupational functioning, percentage of patients in symptomatic remission, working, or living independently.

Biomarkers: we quantified blood levels of 29 amino acids and related metabolites, and the activity of 3 antioxidant enzymes.

Results: Three TDA-derived groups were identified. Each group was determined by the shared characteristics and presented a coherent clinical profile: group A was characterized by an overall low level of symptom, group B by high positive and negative symptoms, group C by high negative symptoms.

Importantly, group A had a high predictive value for good outcomes: patients in this group functioned better at follow-up than those from group B and C. In the validation cohort, we confirmed that group memberships are associated with distinct clinical profiles at baseline and outcomes at follow-up. Moreover, TDA-derived stratification had a better predictive power than clusters defined by k-means clustering. The metabolic biosignature suggests a better regulation of the anti-oxidant defenses in patients with better outcome (group A) and a deficient redox homeostasis in groups B and C.

Conclusions: Unsupervised data-driven topological analysis allowed patients' stratification into clinically relevant subgroups. This stratification was robust and was predictive of long-term outcomes. The predictive power of TDA-derived groups surpassed the one of standard clustering approach as quantified by machine learning. This approach, combined with mechanism based metabolic profile, should pave the way to personalized functional-disability preventive strategies at early stages of the disease.

The present study revealed a new, unsupervised, stratification based on patient symptoms at baseline, which was associated with a specific metabolic signature and had a predictive value for long-term outcomes. The combination of unsupervised topological methods with machine learning algorithm is a new exciting frontier in TDA with high potential for the field of psychiatry.

Keywords: Schizophrenia Subtypes, Redox Modulation, Computational Methods

Disclosure: Nothing to disclose.

W188

Functional Brain Connectivity Improves Clinical Outcome Prediction in Youth at Risk for Psychosis

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Background: The first episode of psychosis is typically preceded by a prodromal phase with subthreshold symptoms, cognitive difficulties, and functional decline. Improved outcome prediction at this early stage is of key importance as it may contribute to targeted early intervention. This study examines whether resting-state functional connectivity data, alone or in addition to validated clinical predictors of psychosis, improves outcome prediction in the prodromal stage.

Methods: This study involves a total of 137 adolescents and young adults at Clinical High Risk (CHR) for psychosis from the Shanghai At Risk for Psychosis (SHARP) program. Based on

outcome at one-year follow-up, CHR participants were separated into three outcome categories including good outcome (symptom remission, N = 71), intermediate outcome (ongoing CHR symptoms, N = 30), and poor outcome (conversion to psychosis or treatment-refractory, N = 36). Resting-state fMRI data were acquired for each participant and processed using the Conn toolbox, including rigorous motion correction. Multinomial logistic regression analysis and leave-one-out cross-validation were used to assess the performance of three prediction models: 1) a clinical-only model using validated clinical predictors from the NAPLS-2 psychosis-risk calculator, 2) an fMRI-only model using measures of functional connectome organization and within/between-network connectivity among established resting-state networks, and 3) a combined clinical and fMRI prediction model. To assess model performance, we computed an F1 measure that reflects the harmonic mean of the positive predictive value and sensitivity for each outcome category. This measure was compared to expected chance-levels using a permutation test with 1,000 sampled permutations in order to evaluate the statistical significance of the model's prediction.

Results: The clinical-only prediction model failed to achieve a significant level of outcome prediction (F1 = 0.32, F1-chance = 0.26 +/- 0.06, p = .154). The fMRI-only model did predict clinical outcome to a significant degree (F1 = 0.41, F1-chance = 0.29 +/- 0.06, p = .016), but the combined clinical and fMRI prediction model showed the best performance (F1 = 0.46, F1-chance = 0.29 +/- 0.06, p < .001). On average, positive predictive values (reflecting the probability that an outcome label predicted by the model was correct) were 39% better than chance-level and 32% better than the clinical-only model. Analyzing the contribution of individual predictor variables to the combined model's performance showed that GAF functional decline, a family history of psychosis, and performance on the Hopkins Verbal Learning Test were the most influential clinical predictors, whereas modular connectome organization, default-mode and fronto-parietal within-network connectivity, and between-network connectivity among language, salience, dorsal attention, cerebellum, and sensorimotor networks were the leading fMRI predictors.

Conclusions: This study's findings suggest two main points. First, that functional brain abnormalities reflected by alterations in resting-state functional connectivity precede and possibly drive subsequent changes in clinical functioning. And second, that neuroimaging markers of functional connectivity may be useful for improving early identification and clinical decision-making in prodromal psychosis.

Keywords: Clinical High-Risk of Psychosis, Resting State Functional Connectivity, Clinical Outcome Prediction

Disclosure: Nothing to disclose.

W189

Successful Treatment of Clozapine-Nonresponsive Refractory Hallucinations and Delusions With a Serotonin 5HT-2A Receptor Inverse Agonist: Pimavanserin as an Alternative to Clozapine

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Background: Clozapine was the first and the widely accepted gold standard treatment for refractory or treatment-resistant psychotic symptoms (hallucinations and delusions), which can occur in up to 30% of patients with schizophrenia. Clozapine has efficacy of about 50% of treatment-refractory cases and some responding patients have to discontinue it due to serious adverseeffects like agranulocytosis or myocarditis or ileus. The search for medications to use for clozapine-non-responders continues. We hypothesize that one such possible novel agent is the non-dopaminergic antipsychotic pimavanserin, an inverse agonist of serotonin 5-HT2A receptors, which was recently approved for the hallucinations and delusions of Parkinson's

Disease Psychosis. We report here the results of using adjunctive pimavanserin in patients with refractory hallucinations and delusions who failed to respond to clozapine.

Methods: We present 30 consecutive cases of patients with schizophrenia and schizoaffective disorder with refractory hallucinations and delusions who received a trial of pimavanserin when clozapine or multiple antipsychotics failed. The subjects' ages ranged between 21 and 77 years and were followed up for several months with clinical assessments of their psychotic symptoms as well as their negative symptoms.

Results: 75% of the 30 patients with refractory hallucinations and delusions showed significant clinical response to pimavanserin 34 mg/day within 4-8 weeks, with continuation of the response for several months and marked reduction in re-hospitalization. Side effects were minimal and none of the patients stopped pimavanserin due to an adverse effect. Responders were noted to have brighter affect, increased socialization with other patients, and more cooperative with the staff.

Conclusions: This series of 30 cases of patients with refractory psychosis who responded to pimavanserin is an important new finding that has never been reported before. It may herald a new direction in the treatment of patients who have either failed to respond to clozapine or even as an option before resorting to clozapine with its many adverse events and the need for frequent blood draws to measure white blood counts, which many patients decline to do. Controlled studies comparing clozapine and pimavanserin in refractory schizophrenia are warranted.

Keywords: Treatment Refractory, Schizophrenia Novel Treatment, 5HT5A Receptor Antagonist, Clozapine

Disclosure: Acadia, Advisory Board, Acadia, Consultant, Honoraria

W190

Discovery of TAK-041: Potent and Selective GPR139 Agonist for Treatment of Negative Symptoms Associated With Schizophrenia

Abstract not included.

W191

Novel EEG Biomarkers for the A Priori Identification of Responders to the Experimental Antipsychotic Agent Pomaglumetad Methionil

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Background: Pomaglumetad methionil (LY-2140023, or "poma") is an experimental antipsychotic drug which is an agonist at metabotropic (mGluR2/3) glutamate receptors and has no known effects on dopamine receptors. This profile differs from all currently used antipsychotic agents, which act on the dopamine system. Multiple phase II and III clinical trials suggested that while this agent may not be effective for patients with schizophrenia as a whole, there may be particular subgroups for whom it is uniquely helpful. Our objective was to develop novel EEG biomarkers to identify patients with schizophrenia who are more

likely to show a positive response to treatment with poma. Previous attempts to use EEG readouts to predict antipsychotic treatment responders have largely been unsuccessful. However, these studies generally studied dopamine acting antipsychotics. Furthermore, we examined additional EEG measures—e.g., response to photic stimulation, magnitude of power law exponent (PLE)—that, to our knowledge, have not been used in prior predictive studies.

Methods: This study used data from clinical trials NCT00845026 (N = 117) and NCT01052103 (N = 196), which studied male and female patients with schizophrenia treated with poma vs. antipsychotic standard of care. EEG recordings were taken in the pre-treatment period using a standard 19-lead montage, both in the resting state and when patients were exposed to photic stimulation (flashing light), at frequencies ranging from 1 to 30 Hz. As a possible predictive measure, we subjected resting EEG data to conventional power spectrum analysis to determine strength of activity in the delta, theta, alpha, beta, and gamma bands. For the photically stimulated state, we calculated only power at the stimulated frequency. Additionally, for resting EEG signals we calculated the PLE (also known as the "fractal exponent"). All measures were calculated for each EEG electrode. Response to poma treatment was operationalized as percentage change from baseline to trial endpoint on a number of clinical outcome measures, including the Positive and Negative Symptom Scale (PANSS), and the MATRICS Consensus Cognitive Battery (MCCB) and its seven individual cognitive domain scales. We performed statistical analysis to determine whether there was a significant relationship between any of our calculated EEG metrics in the pre-treatment condition and the treatment outcomes.

Results: We identified a number of pre-treatment EEG metrics that correlated with clinical outcomes, and may represent predictors of response to treatment with poma. For example: (1) In the photically stimulated condition, there was a positive correlation between pre-treatment gamma (30 Hz) activity in EEG lead T6 and post-treatment improvement in cognition, as measured by the attention-vigilance domain score of the MCCB ($r = 0.385$, $p = 0.000001$). Correlation coefficients ranged from 0.20 to 0.39 with similar levels of significance at other occipital and inferior leads. (2) For left central electrode C3, there was a positive correlation between pre-treatment PLE and improvement in the working memory (WM) domain score of the MCCB ($r = 0.288$, $p = 0.000558$). Correlation coefficients of 0.17 to 0.31, with similar significance levels, were seen at other fronto-central leads. Taking this effect as an example and using improvement in WM performance of 50% as the definition of treatment response, receiver operator curve (ROC) analysis revealed that PLE at lead C3 could identify poma responders with a sensitivity of 0.750 and a specificity of 0.897 (AUC = 0.809, $p = 0.039$). In all, 23 lead-level relationships were judged to have relatively high effect size and robust statistical significance, and thus potential clinical utility. Notably, all response variables that we identified involved improvement in cognitive functioning, rather than positive or negative symptoms or other measures of psychopathology. Also, the positive EEG predictors did not occur in one particular cortical area. This raises the possibility that there is a patient subgroup which shows unique benefit in terms of cognitive improvement, and that may be characterized by particular EEG "spectral fingerprints".

Conclusions: We have identified a number of individual EEG-based pre-treatment effects that may serve as a priori biomarkers of treatment outcome. However, the particular brain phenotype that is uniquely responsive to the drug is likely characterized by a difficult to discern combination of these effects. We have developed an artificial intelligence (AI) approach using deep learning artificial neural nets (ANNs), as this methodology is well-suited to complex, non-linear, pattern recognition tasks with multiple inputs. The resultant "composite biomarker" takes into account all of the effects identified, and should be a much more robust predictor than any one singly. Additionally, it has long been clear that schizophrenia is a highly heterogeneous disease. It is possible that illness subcategories are not defined simply by symptom clusters, but by differences in the manner in which the brain processes information. A patient subgroup uniquely responsive to this novel agent may represent a heretofore unappreciated subcategory of the disease. Finally, we feel that the methodology described here could be applied to other psychoactive medications, as these also may result in unique patterns of EEG activity in responders vs. non-responders that may be difficult to appreciate with conventional methods.

Keywords: Predictor of Treatment Response, Novel Methods, Schizophrenia, Antipsychotics, Electroencephalography, Biomarkers

Disclosure: Nothing to disclose.

W192

Snap 101: Randomized, Crossover, Active and Placebo-Controlled, Safety, Pharmacokinetic, and Pharmacodynamic Study of 3 Ascending Doses of Olanzapine Delivered by the Novel Precision Olfactory Delivery (POD®) Device

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Background: An estimated 1.7 million acute agitation events occur annually; OLZ IM is a preferred option due to a shorter Tmax than oral, but IM administration, predominantly administered in a hospital setting, can be painful, humiliating, invasive, and requires cooperation or restraint which reduces trust, increases healthcare worker injuries and may be interpreted as an assault. Further, heavily medicated patients may require "boarding" until sedative effects have resolved. Oral administration is preferred but for OLZ, has a slower onset of effect and typically requires isolation and observation of the patient. INP105 is a drug-device combination product in development which delivers OLZ powder by the novel Precision Olfactory Delivery (POD®) device to the vascular rich upper nasal space. It is being developed for rapid control of agitation in a cooperative patient and can be self- or caregiver administered to potentially provide rapid onset of relief without a needle. INP105 may also be suitable for early use by patients who have insight into their condition and recognize early symptoms of agitation. This may avoid escalating agitation leading to more intensive management, violence, and injury to the patient, their caregivers and/or healthcare workers.

Methods: Randomized, double-blind, active and double-blind, placebo comparator-controlled, ascending-dose, 2-way, 2 period, incomplete block, crossover Phase 1 trial to compare the safety, tolerability, PK and PD of 3 doses of INP105 (5 mg, 10 mg and 15 mg) or POD-placebo with either OLZ IM (5 mg or 10 mg) or OLZ-ODT (10 mg). Period 1 was open label (the OLZ 10 mg IM dose was discontinued after dosing 2 NHVs); followed by a 14-day washout period and then a double-blind period with INP105 or POD-placebo. Dose escalation was staggered to allow safety monitoring committee assessment of tolerability of INP105 between dose levels. PK draws and PD assessments (VAS, ACES and DSST), were obtained at multiple timepoints. All subjects were observed as in-patients for at least 72 hours post-dosing with follow-up occurring 4, 5 and 14 days after dosing in both periods.

Results: 40 subjects were randomized; 37 dosed in Period 2 (Placebo=10, INP105 5 mg =10, 10 mg=9, 15 mg=8). INP105 was well tolerated with TEAEs reported in 100% IM OLZ 10 mg, 90.1% IM OLZ 5 mg, 83.3% OLZ ODT 10 mg, and 80% INP105 5 mg, 66.7% INP105 10 mg and 62.5% INP105 15 mg. The most

commonly reported TEAEs of dizziness, hypotension, presyncope and orthostasis, occurred in 75% with OLZ IM or ODT and 52% with INP105.

INP105 was rapidly absorbed and median Tmax was reached within 9.5 to 15 minutes with INP105 5 mg vs. 20 minutes with OLZ IM 5 mg and 120 minutes with OLZ ODT 10 mg. For approximately half of individuals following administration of INP105 5, 10 or 15 mg, Tmax had been achieved by the time the first blood sample was drawn at 5 minutes, which was not observed with the IM or ODT comparator. Cmax following INP105 5 mg was similar to OLZ IM 5 mg and approximately 2-fold greater than OLZ ODT 10 mg. AUC0-inf following INP105 5 mg was similar to IM 5 mg, and approximately half of AUC0-inf following ODT 10 mg. Dose-related, statistically significant PD effects with VAS ($p < 0.01$), ACES ($p < 0.001$) and DSST ($p < 0.01$) were observed for all three INP105 dose levels compared to placebo at maximum change from baseline. As early as 15 to 30 min, statistically significant from baseline effects were reported following INP105 5, 10 or 15 mg [30 min all doses: ACES $p < 0.02$; VAS $p < 0.01$; DSST $p < 0.02$].

Conclusions: INP105 may offer non-invasive delivery of OLZ with rapid absorption and is well tolerated.

OLZ exposure with INP105 was similar to IM delivery at the same dose, but achieved Tmax in half the time and exposure exceeded that of OLZ ODT at the same dose.

PD measures of sedation and attention exhibited clinically meaningful effects with INP105 compared to placebo within 15 minutes.

Keywords: Olanzapine, Nasal Delivery, Acute Agitation, Pharmacokinetics, Pharmacodynamics

Disclosure: Impel NeuroPharma, Employee.

W193

Multifaceted Role for Serine Racemase in Regulating the Development of Axonal Tracts and Cortical Interneurons in the Embryonic Forebrain

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Background: Schizophrenia is considered a neurodevelopmental disorder that is associated with pathological brain alterations, including reduced cortical thickness, ventricular enlargement, and neurochemical abnormalities, such as aberrant γ-aminobutyric acid (GABA) signaling. Within the forebrain, there are important developmental changes that contribute to the pathophysiology of schizophrenia, such as synaptic pruning and GABAergic neuronal migration and distribution, which has been shown to receive cues from vascular networks. Although there are dynamic changes in corpus callosum (CC) development, including midline crossing of axonal projections and myelination, the contribution of CC malformation to schizophrenia is still uncertain. It is therefore important to understand the spatiotemporal distribution of proteins associated with the schizophrenia across development and determine if they contribute to aberrant forebrain development. Srr, which encodes for serine racemase (SR), has been implicated in the pathophysiology of schizophrenia. SR produces D-serine by the racemization of L-serine. In addition to glutamate, N-methyl-D-aspartate receptors (NMDARs) require the binding of the co-agonist D-serine to function. Post-mortem, genetic and pharmacologic studies have shown that NMDAR hypofunction contributes to the pathophysiology of schizophrenia. Recent studies show that: brain endothelial cells express NMDARs, D-serine is important for vascular function, and SR mRNA is present in isolated periventricular endothelial cells from mouse embryonic brains. SR protein is also present in GABAergic interneurons and in the CC. We therefore hypothesize that SR is involved in the formation of cortical axonal tracts and in interneuron development. Here, we will investigate the spatiotemporal expression of SR during prenatal development, and the effect of SR knockout (SRKO) on axonal tract formation, migration and distribution of interneurons, and forebrain morphology.

Methods: Brains from E13 E15, and E17 embryos (collected from C57BL6/J or glutamic acid decarboxylase of 65kDa (GAD65)-GFP dams) will be fixed in 4% paraformaldehyde. Cryostat sections (20μM) will be prepared and incubated in primary antibody (mouse anti-serine racemase, anti-biotinylated isolectin B4, anti-CHL1, anti-GABA), at 4°C overnight, and then incubated with secondary antibody (anti-mouse Alexa 488 conjugate, streptavidin Alexa 594 conjugate, anti-goat Alexa 647 conjugate). The McLean Hospital Institutional Animal Care and Use Committee approved all animal care and experimental procedures.

Results: At E13, we detected SR specifically in the cortical hem, the embryonic organizer for the hippocampus that can also regulate the size and patterning of the neocortex. At E15, SR is expressed in the sub-cortical axonal fiber tracts, which decussate at the midline, most notably at the corpus callosum. We detected SR-positive endothelial cells using the blood vessel marker, isolectin IB4. SR also co-localized with GAD65-positive (GFP+) cortical interneurons. At E17, SR is present in the hippocampus and dentate gyrus. Next, we will expand our observations with more animals and compare the spatial profile of SR expression in E13, E15 and E17 forebrains. We will determine whether the brains of embryonic SRKO mice display abnormalities in various measures that have been associated with or could contribute to the pathophysiology of schizophrenia including: vascular densities, forebrain GABAergic neuronal migration and distribution, abnormalities in CC formation, ventricular size and cortical thickness.

Conclusions: Our results stimulate new hypotheses that SR is involved in the formation of axonal tracts and cortical interneuron migration and/or distribution that leads to changes in cognitive and behavioral outcomes. In summary, our results lay the groundwork for novel roles of SR in brain development.

Keywords: Serine Racemase, Schizophrenia, Axonal Tract, Cortical Interneurons, Neurodevelopment

Disclosure: Nothing to disclose.

W194

Computational Assessment of Excitatory Drive to Parvalbumin Interneurons and Cortical Gamma Oscillations in Development and Schizophrenia

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Background: Task-related lower power of prefrontal cortical gamma oscillations in schizophrenia is proposed to arise from altered maturation of excitatory inputs to fast-spiking parvalbumin interneurons. These alterations include disturbances in morphological and molecular indices of excitatory synaptic density and strength onto parvalbumin neurons. Here, we used a computational neuronal network model to simulate how the alterations in the density and strength of excitatory synapses on parvalbumin interneurons could affect the generation of cortical gamma oscillations. Then, we performed proof-of-concept analyses of neuroanatomical data to validate the computational prediction of changes in gamma oscillations during adolescence and in schizophrenia.

Methods: The computational model comprised a network of 50 excitatory and 20 fast-spiking inhibitory (FSI) quadratic

integrate-and-fire neurons connected via AMPA, NMDA and GABA synapses. Excitatory synaptic density and strength onto parvalbumin interneurons were modeled by AMPA synapse connectivity and conductance onto FSI neurons, respectively. Network synchrony, firing rates and gamma oscillation power were measured. The levels of VGlut1 and PSD95 within excitatory synapses onto prefrontal cortical parvalbumin-positive neurons in adolescent monkeys and in matched pairs of schizophrenia and unaffected comparison subjects were obtained from our previous studies. The synaptic strength index was calculated by summing the z-scores of mean synaptic VGlut1 and PSD95 levels. The coefficient of variation for synaptic strength index was calculated across parvalbumin interneurons to estimate the variability of synaptic strength across these neurons.

Results: At 100% connectivity, peak gamma power occurred at a narrow range of AMPA conductance onto FSI neurons that optimally balanced network firing rates and synchrony. Lowering connectivity while increasing conductance, which reflects developmental pruning, widened the peak of optimal AMPA conductance range that results in peak gamma power while preserving the amplitude of peak gamma power. Further lowering connectivity without changing conductance, which reflects findings in schizophrenia, reduced the amplitude of peak gamma power over all AMPA conductances by disrupting the firing rate-synchrony balance.

Our computational model predicts that a pruning of excitatory inputs in adolescence allows for a wider range of optimal synaptic strength onto parvalbumin neurons that can achieve maximum gamma power. Consistent with this prediction, the coefficient of variation for synaptic strength index was higher in post-pubertal relative to pre-pubertal monkeys. The coefficient of variation for synaptic strength index was also higher in schizophrenia relative to comparison subjects, in support of the modeling prediction that the peak of optimal AMPA strength is abolished with a pathological loss of excitatory inputs in the illness.

Conclusions: Our computational modeling predicts that the maturation of excitatory inputs to parvalbumin interneurons improves network resilience by widening the range of optimal synaptic strength, whereas altering this process leads to lower gamma power. This prediction is supported by the proof-of-concept analyses of changes in the variability of molecular index for synaptic strength onto parvalbumin neurons in adolescence and schizophrenia. Thus, our study provides a mechanistic link between computational measures of cortical gamma oscillations and changes in indices of excitatory inputs to parvalbumin interneurons observed in the prefrontal cortex of developing monkeys and schizophrenia subjects.

Keywords: Computational Modeling, Postmortem Brain Tissue, Schizophrenia, Adolescence

Disclosure: Nothing to disclose.

W195

Altered Rbfox1 Expression and Cortical Parvalbumin Dysfunction in Schizophrenia

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Background: In schizophrenia, alterations in prefrontal cortical GABAergic circuitry appear to be prominent in interneurons that express parvalbumin but not those that express calretinin. This cell type-specific alteration may result, at least in part, from disturbances in post-transcriptional processing that are unique to parvalbumin interneurons. An important regulator of such processing is Rbfox1, an RNA-binding protein that is enriched in interneurons derived from the medial (e.g., parvalbumin) but not the caudal (e.g., calretinin) ganglionic eminence. The binding of Rbfox1 increases the stability of transcripts in cytoplasm. Here, we tested the following two hypotheses: 1) cytoplasmic Rbfox1 is enriched in parvalbumin interneurons; and 2) in schizophrenia, lower cytoplasmic Rbfox1 expression is associated with lower levels of Rbfox1-binding transcripts in these neurons in the prefrontal cortex.

Methods: Fluorescent immunohistochemistry, confocal microscopy and post-image processing techniques were used to assess the protein levels of cytoplasmic Rbfox1 in parvalbumin-positive neurons in the dorsolateral prefrontal cortex (DLPFC) from 20 matched pairs of schizophrenia and unaffected comparison subjects. To assess the Rbfox1-binding transcripts that are differentially expressed in parvalbumin interneurons in schizophrenia subjects, the list of transcripts from a previous microarray study of laser microdissected parvalbumin neurons was compared to the transcripts that were previously shown to bind Rbfox1 in 3′ UTR.

Results: In the DLPFC from unaffected comparison subjects, Rbfox1 levels were three-fold higher in parvalbumin-positive neurons relative to calretinin-positive neurons, and the cytoplasmic-to-nuclear Rbfox1 ratio was two-fold greater in parvalbumin-positive neurons relative to calretinin-positive neurons. The level of Rbfox1 in parvalbumin-positive neurons was significantly 27% lower in schizophrenia relative to comparison subjects. This deficit was present in both nuclear and cytoplasmic compartments.

Of the 872 transcripts in parvalbumin interneurons that were differentially-expressed in schizophrenia, 77 transcripts have been reported to bind Rbfox1 in 3′ UTR. Among these transcripts, 70 transcripts had lower expression levels in the illness. Correlation analysis with correction for multiple comparisons revealed that the expression levels of three Rbfox1-binding transcripts with lower expression in schizophrenia (Kif3c, TMCC1 and PSD3) were significantly predicted by the level of cytoplasmic Rbfox1 expression in parvalbumin interneurons across subjects.

Conclusions: Consistent with our predictions, Rbfox1 protein levels were 1) preferentially enriched in parvalbumin neurons in human prefrontal cortex, and 2) lower in these neurons in schizophrenia. Lower RBfox1 protein levels in schizophrenia were associated with lower expression of key RBfox1-binding transcripts which could have important functional consequences. For example, TMCC1 is a membrane protein involved in cargo transport from endoplasmic reticulum to the Golgi apparatus, whereas Kif3c is a motor protein that transports cargos from cell body to synapse; deficits in these pathways could impair the trafficking of synaptic proteins in the illness. Moreover, PSD3 is an Arf6-specific guanine nucleotide exchange factor that regulates the recycling of synaptic vesicles and alteration in this pathway could reduce the release of GABA from parvalbumin interneurons. Thus, our findings suggest that lower cytoplasmic Rbfox1 expression may provide a cell type-specific mechanism contributing to a dysregulated post-transcriptional processing that could lead to synaptic dysfunction in cortical parvalbumin interneurons in schizophrenia.

Keywords: Schizophrenia, Post-Transcriptional Processing, Parvalbumin Interneuron, Rbfox1, Synaptic Dysfunction

Disclosure: Nothing to disclose.

W196

Polygenic Risk Scores Analyses in Antipsychotic-Induced Weight Gain

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Background: Antipsychotic drugs are widely used for the treatment of schizophrenia (SCZ). Despite clinical efficacy, antipsychotics are associated with severe side effects including antipsychotic-induced weight gain (AIWG). Specifically, significant AIWG (i.e., >7% from baseline) is observed in more than 30% of individuals treated with antipsychotics and frequently leads to metabolic disturbances such as Type-2 diabetes (T2D) and cardiovascular diseases. Although twin and family studies have pointed to high heritability for AIWG (h2=0.6-0.8), there are currently no established predictors of AIWG given that it involves a combination of genes and non-genetic factors. Given the complex phenotypic nature of AIWG, polygenic risk scores (PRS) of certain morbidities, which combines thousands of common variants weighted by their effect size, may provide a measure of genetic liability for AIWG. Therefore, we aimed to investigate whether PRSs based on the genome-wide association studies (GWAS) for SCZ, body mass index (BMI) and diabetes (Type 1 & 2) were associated with AIWG.

Methods: For our analysis, we used two samples, including (1) a subset of individuals diagnosed with SCZ with AIWG phenotyping from the Clinical Antipsychotic Trials in Intervention Effectiveness (CATIE; n = 189, see Brandl et al., 2016), and (2) the Toronto cohort, a multi-study cohort of individuals diagnosed with SCZ with AIWG (n = 151, see Brandl et al., 2014). Within these two cohorts, our phenotypes of interest included the percentage of BMI/weight change from baseline to end-of-treatment, as well as the presence/absence of significant weight gain (≥7% weight change). We investigated associations between PRSs of SCZ, BMI, and diabetes and AIWG using regression models, corrected for age, sex, risk medication for AIWG, and duration of the study, using PRSice-2. We used the Psychiatric Genomics Consortium schizophrenia GWAS to calculate PRSs for SCZ. We used GWAS summary statistics from the GWAS Catalog of BMI and diabetes in individuals of European ancestry. For BMI, we used one dataset for BMI (i.e., GCST006900: 2,336,269 variants across up to 700,000). For Type-1 diabetes (T1D), we used one dataset from the GWAS catalog (ID: GCST005536) which included 123,130 variants across 6,683 cases, 12,173 controls, 2,601 affected sibling-pair families, and 69 trios. Likewise, we used three datasets forT2D (i.e., GCST006801: 8,404,432 variants across 4,040 cases and 113,735 controls, GCST007517: 133,871 variants across up to 48,286 cases and up to 250,617 controls, and GCST007518: 133,586 variants across up to 48,286 cases and up to 250,617 controls).

Results: The demographics and characteristics of the subjects were as follows: 1) CATIE sample (N = 189) [age=41.4±12, male: 151 (79.9%), baseline BMI: 28.83±5.43, mediations: olanzapine n = 63; quetiapine n = 67; risperidone n = 59]; and 2) our local sample (N = 156) [age=36.7±11.2, male: 98 (63.8%), baseline weight: 78.97 ±15.60, mediations: clozapine n = 68; risperidone n = 27; olanzapine n = 18; aripiprazole n = 16; others = 24]. We observed no significant associations of PRS for SCZ or BMI with AIWG. We observed significant associations of PRS for T1D with percentage BMI change from baseline at PT=0.0031 (R2=0.04, p = 0.01), as well as weight gain at PT=0.00015 (R2=0.09, p = 0.004) in the CATIE sample only. Furthermore, we observed significant associations of PRS for T2D status with percentage weight change from baseline at PT=0.49 (R2=0.03, p = 0.03), as well as weight gain at PT=0.0001 (R2=0.06, p = 0.02) in the Toronto cohort only.

Conclusions: To the best of our knowledge, this is the first genetic association study evaluating whether PRSs for SCZ, BMI, or diabetes are associated with AIWG in patients with SCZ. We found that there was a genetic overlap between the risk of diabetes and risk for developing AIWG. Limitations of this study include a small sample size of the target data and that only patients of European ancestry were analyzed. Nonetheless, our suggestive results highlight the importance of studying metabolic-related disorders to unravel the mechanism of AIWG. Further studies with larger sample sizes and individuals of various ethnic ancestries are required.

Keywords: Antipsychotic-Induced Weight Gain, Polygenic Risk Score, Schizophrenia

Disclosure: Nothing to disclose.

W197

Role of GRK3 in Brain Immune Activation and Psychosis

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Background: Kynurenic acid (KYNA) is an endogenous antagonist of N-methyl-D-aspartate and α7 nicotinic acetylcholine receptors that is derived from the kynurenine pathway of tryptophan degradation. Evidence suggests that increased central KYNA levels are involved in the pathophysiology of schizophrenia. However, the mechanism of action for aberrant levels of KYNA in these patients is still under investigation.

The G protein-coupled receptor (GRK) family comprises a number of isoforms designated GRK1 - GRK7. Although GRK3 is the least abundant of all GRKs the kinase is widely expressed in the brain, including limbic regions. Growing evidence suggests that this protein is related to mental processes, and genetic studies implicate this kinase in psychotic disorders. Expression, as well as protein levels of GRK3, are reduced in post-mortem prefrontal cortex of schizophrenia subjects, and recent studies suggest that the GRK3 receptor controls P2X7-receptor-induced secretion of IL-1β. Here, we investigated functional behavior and KYNA related to immune activation and psychosis using the GRK3 mouse knock-out model (GRK3-/-).

Methods: Male Grk3-/- mice and their corresponding age-matched wild-type C57BL/6J male were used. Cytokines were analyzed using a mice Ultra-Sensitive 7-Plex Kit (MesoScale Discovery). KYNA was estimated by microdialysis, followed by HPLC detection.

Prepulse inhibition (PPI) was analyzed by SR-LAB™ system, San Diego Instruments.

In vivo single cell recordings were done from dopamine neurons in the mouse ventral tegmental area. We performed Western blotting to analyze P2X7 receptor protein levels in fractions of brain internal and plasma membranes, and GFAP immunoreactivity to evaluate the degree of astrogliosis.

Results: Compared to WT controls the GRK3 KO mice showed a number of aberrations, including increased turnover of KYNA, elevated brain levels of IL-1β (p = 0.003, t-test) and a hyper-reactive response to D-amphetamine (p < 0.001, 2-way ANOVA, post-hoc Bonferroni). Also, these mice showed an elevated spontaneous firing of midbrain dopamine neurons (p = 0.0012, Mann-Whitney U-test), as well as a disruption in PPI (p<0.0001, 2-way ANOVA), and attentional deficits.

We also found a decrease in brain P2X7-receptor protein levels in the internal cell membrane fractions (p = 0.017 Mann-Whitney U-test), suggesting a disrupted internalization of the receptor. Immunohistochemical experiments showed astrogliosis in GRK3-/- mice.

Conclusions: We have previously found that IL-1β induces the production of KYNA, a compound elevated in patients with psychotic disorders. KYNA acts as a messenger, transmitting information to neuronal circuits from immune signalling mechanisms in glial cells. Here we show that enhanced P2X7-receptor signalling in GRK3 KO mice activates the same immune driven pathophysiological pathway. Elevated levels of KYNA may thus, underlie the behavioral, and electrophysiological aberrations presently observed in GRK3 KO mice.

To conclude, our analyses emphasize a pivotal role of KYNA to signal processes of inflammatory origin, induced by GRK3 deficiency, to neuronal circuits of the brain.

Our biochemical and functional data obtained from GRK3-/- mice bear striking similarities to clinical findings in patients with psychotic syndromes.

Keywords: PPI, P2X7, Kynurenic Acid, IL-1b, Locomotor Activity

Disclosure: Nothing to disclose.

W198

Effectiveness of Pharmacological Therapies for Delusional Disorder

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Background: Delusional disorder is a serious chronic mental disorder. It is characterized by delusions of often paranoid nature, which can cause anxiety and fear in the patients and even guide their life decisions towards ones that lower their overall quality of life, such as secluding themselves from society or in the most severe cases even committing crimes. A challenge for treatment is that patients often do not have insight into the origin of their delusions and thus building sufficient rapport for long term medication adherence can be very difficult. Another challenge is that very little is known about the effectiveness of pharmacotherapies for delusional disorders and often standard treatment with dopamine blocking agents can be of little help, although it often remains unclear whether this is due to inefficacy of the medication or poor adherence.

We aimed to study the comparative effectiveness of pharmacological agents for delusional disorder using Swedish national registries to observe a national cohort of patients with delusional disorder (n = 9,076).

Methods: We studied the risk of work disability and hospitalization due to psychosis among all patients who had received a diagnosis of delusional disorder (ICD-10: F22, equates to DSM-V: 297.1) in Sweden during the years 2005 – 2016 in either inpatient or specialized outpatient care (N = 9,076; 53.3% men, mean age 45.1 years [std 12.5 years], mean follow-up time 4.9 years) using prospectively gathered nationwide databases for specialized outpatient care, hospitalization, work disability (sickness absence and disability pension) and dispensed medications. The corresponding ICD-8 and -9 codes were used to calculate time since first diagnoses (ICD-9 297B–297C, 297W, 297X, ICD-8: 297). Persons with previous diagnoses of schizophrenia, schizoaffective disorder or bipolar disorder (F20, F25, F31) were excluded and the follow-up censored if any study subject received any of these diagnoses during follow up. Individuals with a hospitalization due to psychosis during the follow-up period (n = 2074) were analyzed for rehospitalization risk. Patients not already on disability pension at start of follow-up (n = 5025) were analyzed for risk of work disability. The primary analysis was a within-individual Cox proportional hazards model. Analyses were adjusted for the effects of time since cohort entry, order of treatments, and current use of other treatments. Long-acting injectables were pooled into a single therapeutic group. Results are reported as hazard ratios (HRs) with 95 % confidence intervals (95% CI).

Results: In comparison between use and no use among specific pharmacological agents reaching nominal statistical significance, use of clozapine (HR 0.10, 95% CI 0.02 to 0.50, p = 0.005), any long-acting injectable (HR 0.23, 95% CI 0.11 to 0.48, p < 0.0001), olanzapine (HR 0.30, 95% CI 0.13 to 0.66, p = 0.0032) or polytherapy with any two or more antipsychotics (0.48, 95% CI 0.27 to 0.85, p = 0.012) were associated with a reduced risk of rehospitalization due to psychosis. Almost half (n = 4051, 45%) of the patients were already on disability pension at the time of cohort entry. Among those whom were not, in comparison between use and no use among specific pharmacological agents reaching nominal statistical significance, use of clozapine (HR 0.05, 95% CI 0.01 to 0.404, p = 0.005) or use of risperidone (HR 0.61, 95% CI 0.39 to 0.96, p = 0.031) were both associated with a decreased risk of work disability.

Conclusions: Although delusional disorder has been thought to be a disorder affecting mainly the dopaminergic system, use of clozapine was associated with a reduced risk of hospitalization due to psychosis and work disability in this cohort of Swedish patients with delusional disorder. Clozapine treated patients are often seen or screened with blood work more regularly than patients with other per oral medications, due to the possible side-effects of clozapine, which might translate into better adherence or closer follow-up by the physician. Also long-acting injectables were associated with a reduced risk of rehospitalization due to psychosis, which might be again be due to better adherence and more regular follow ups required by the injectables being only administered by healthcare staff. These results indicate that clozapine and long-acting injectables might be effective in the treatment of delusional disorder patients at high risk for hospitalization due to psychosis. Clozapine might also be beneficial in preventing work disability.

Keywords: Delusions, Hospitalization Risk, Disability, Delusional Disorder

Disclosure: Sunovion ltd, Honoraria, Orion Pharma ltd., Honoraria

W199

What You See is What You Get: Visual Scanning Failures of Naturalistic Social Scenes in Schizophrenia

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Background: Impairments in social cognition are a major cause of disability in schizophrenia patients (SzP). A key component of social cognition is visual scanning for social cues, such as facial expressions. While SzP are known to have visual processing deficits, no comprehensive account has emerged linking these deficits to visual scanning and social cognition. The temporoparietal junction/posterior superior temporal sulcus (TPJ-pSTS) is a region thought to underlie these abilities in humans, and has been implicated in social cognition deficits previously, but the relationship between these deficits and social cognition remains unclear.

Methods: In 39 SzP and 27 healthy controls (HC), we used eye-tracking to examine the relationship between performance on The Awareness of Social Inference Test (TASIT), which tests social cognition using naturalistic video clips of social situations, and visual scanning, measured for each individual versus the mean performance of HCs. We then examined the relative contributions of specific visual features (motion, contrast, luminance, faces) within each video frame to visual scanning.

In a subset of these groups (27 SzP and 21 HC), we collected fMRI data while they watched a cinematic movie ("The Good, the Bad, and the Ugly"). Intersubject correlation (ISC) was used to assess the engagement of cortex by the movie in both groups by performing the pairwise correlation of the movie-evoked timecourse of BOLD activity in each individual's brain to each of the HC subjects, and

then averaging these maps within each group. A series of localizer task fMRI scans were also performed in the HC to functionally separate the TPJ-pSTS into regions of interest (ROIs).

Results: Visual scanning (p<10-7) and TASIT performance (p<10-5) was significantly impaired in SzPs, especially in sarcasm clips containing sarcasm, conveyed through exaggerated facial expression and prosody. While TASIT sarcasm performance depended on visual scanning in HCs ($r = 0.46$), SzP performance instead correlated with measures of cognitive abilities ($r = 0.5$). In addition, SzPs were less likely to be looking at faces overall ($p = 0.0001$) and less likely to orient to facial motion in peripheral vision ($p = 0.035$).

In the subset of subjects who performed the movie-watching task, voxels within the TPJ-pSTS showed to strongest engagement deficit in either hemisphere. This cluster of voxels fell within a region at the center of the TPJ-pSTS (TPJm), which at the ROI level demonstrated the strongest failure to engage of all TPJ areas in SzP versus HC ($p = .0062$).

Conclusions: SzP show highly significant deficits in naturalistic social cognition that reflect differential reliance on visual scanning versus cognitive abilities. Alterations in visual scanning may reflect impaired processing of facial motion, particularly within peripheral vision. Failure to engage the TPJ-pSTS may underlie these deficits. Overall, these results highlight the utility of naturalistic stimuli in the study of social cognition deficits in schizophrenia.

Keywords: Functional MRI (fMRI), Social Cognition, Attention, Social Attention, Facial Emotion Processing

Disclosure: Pfizer, Inc, Employee (Spouse)

W200

Young Adults With Psychotic Disorders Have Similar Access to Technology and Social Media but May be Less Active in Posting Than Clinical Risk and Psychosis-Free Peers

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Background: Digital technology, the internet and social media are increasingly investigated as promising means for monitoring and delivering mental health treatment. Previously, there were concerns that decreased access to technology would limit implementation in individuals with psychosis. Studies show that when access is available, internet and social media-based interventions for improving functioning in individuals with psychosis are acceptable and feasible. In this study, we aimed to evaluate access and use of technology and social media in young adults with psychotic disorders (PD), subthreshold psychotic symptoms/clinical risk for psychosis (CR) and without psychosis symptoms ("psychosis-free" PF). We hypothesized that among young people with psychotic disorders, access and use of technology would be high.

Methods: Fifty-five young adults (PD $n = 21$, CR $n = 22$, PF $n = 12$) were surveyed regarding their access to technology and use of social media, specifically Facebook and Twitter, as a part of a larger effort to investigate social media language and usage in individuals with psychosis. Participants were aged 18-32 years, included 24 females (44%), and was 55% Caucasian. Categorical variables were compared among groups using R with Fisher's exact test and continuous variables were compared using one-way ANOVA. Significance was two-tailed with alpha=0.05.

Results: There was a high level of access to technology across the groups. There were no differences among groups in access to mobile phones (100% for all groups, $p = 1.00$), smartphones (100% PF, 95% CR, 95% PD, $p = 1.00$), computers (83% PF, 91% CR, 95% PD, $p = 0.52$), or the internet (100% PF, 100% CR, 95% PD, $p = 0.60$). The majority of young adults used Facebook (92% PF, 73% CR, 71% PD, $p = 0.40$) but not Twitter (25% PF, 23% CR, 10% PD, $p = 0.48$). Individuals with psychotic disorders were similarly likely to view social media at a weekly or greater frequency (58% PF, 73% CR, 62% PD, $p = 0.62$) but less likely to actively post at a weekly or higher frequency compared to both CR and PF (25% PF, 41% CR, 5% PD, $p = 0.02$).

Conclusions: Overall, the results encourage further development of internet and social media-based interventions and treatment monitoring for young people with psychosis. Young people with psychotic disorders have a similar level of access to technology and social media compared to those with subthreshold symptoms and psychosis-free youths. However, they may be less actively engaged and post at a lower frequency despite accessing social media at a similar rate. Lower active engagement may reflect impairments in social cognition and functioning in general. This may present both a challenge as well as an opportunity for online and social media-based interventions in psychosis. Interestingly, there was no difference in active engagement between individuals with clinical risk vs. psychosis-free. Future studies should evaluate whether this finding generalizes to other online platforms.

Keywords: Psychosis, Intervention, Digital Phenotyping, Social Media Use, Social Functioning

Disclosure: Nothing to disclose.

W201

Efficacy and Safety of Lumateperone 42 Mg in the Treatment of Schizophrenia: A Pooled Analysis of Randomized Clinical Trials

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Background: Lumateperone (lumateperone tosylate [ITI-007]) is in late-phase clinical development for schizophrenia and other disorders. Lumateperone has a unique mechanism of action that simultaneously modulates serotonin, dopamine, and glutamate neurotransmission. Lumateperone was evaluated in 3 randomized, double-blind, placebo (PBO)-controlled studies in patients with an acute exacerbation of schizophrenia. In 2 studies, lumateperone 42 mg (ITI-007 60 mg) showed significant reduction vs PBO in the Positive and Negative Syndrome Scale (PANSS) Total score. In 1 study, statistically significant differences were not observed for lumateperone 42 mg vs PBO, due to a high PBO response; however, the magnitude of improvement in PANSS Total score for lumateperone was similar to that in the two positive studies. In all 3 studies, lumateperone was well tolerated. Herein, the efficacy, safety, and tolerability of lumateperone 42 mg (QAM) is evaluated via pooled analyses of the late phase studies.

Methods: Efficacy data were pooled from the 2 positive studies; safety data were pooled from all 3 studies. The primary efficacy endpoint was change from baseline to Day 28 in PANSS Total score. Additional assessments included change in PANSS subscale scores and percent of patients meeting various PANSS response criteria. Safety assessments included treatment-emergent adverse events (TEAEs), laboratory parameters, and extrapyramidal symptoms (EPS)/movement disorder scales.

Results: The intent-to-treat population (efficacy analyses) comprised 520 patients (221, PBO; 224, lumateperone 42 mg; 75, risperidone 4 mg). Lumateperone 42 mg significantly reduced PANSS Total score (least squares mean difference versus PBO [LSMD] = −4.76; P<.001) with efficacy similar to risperidone 4 mg (LSMD = −4.97; P=.014). Lumateperone showed significant improvement vs PBO on change in CGI-S score (LSMD = −0.29;

P<.001) and on multiple PANSS subscales. Lumateperone 42 mg was associated with significantly higher PANSS response rates than PBO. Negative results from the third study did not impact the ability of lumateperone 42 mg to significantly separate from PBO when the 3 studies were pooled.

The safety population comprised 1,073 patients (412, PBO; 406, lumateperone 42 mg; 255, risperidone 4 mg). The only TEAEs that occurred in the lumateperone 42 mg group at a rate of ≥5% and twice PBO were somnolence/sedation (24.1% vs 10.0%) and dry mouth (5.9% vs 2.2%); rates for these TEAEs in the risperidone group were 23.9% and 4.7%, respectively. Weight increase TEAE occurred in more risperidone (6.3%) than placebo (2.7%) or lumateperone 42 mg (2.0%) patients. Mean change in weight was small for lumateperone 42 mg (1.6 kg) and PBO patients (1.3 kg) but larger in risperidone patients (2.6 kg); the percent of patients with ≥7% weight increase was similar for lumateperone 42 mg (9.1%) and PBO groups (9.2%) and greater for risperidone (22.0%). Mean change in metabolic parameters were similar or smaller for lumateperone 42 mg vs PBO and generally higher in risperidone patients. Risperidone but not lumateperone 42 mg or PBO increased mean prolactin levels. Rates of EPS-related TEAEs were similar for lumateperone 42 mg and PBO and higher for risperidone.

Conclusions: In these pooled analyses, lumateperone 42 mg significantly reduced schizophrenia symptoms and provided clinically meaningful improvement. Lumateperone 42 mg showed good tolerability with a favorable safety profile, especially with respect to metabolic, prolactin, and EPS measures. The only TEAE that occurred in >10% of lumateperone patients and twice the rate of placebo was somnolence/sedation, which was impacted by morning administration; in subsequent studies that administered lumateperone in the evening, somnolence/sedation rates were markedly reduced. These results suggest that lumateperone may be a promising new treatment for schizophrenia.

Keywords: Antipsychotic, Efficacy and Safety, Schizophrenia-Novel Treatment

Disclosure: Intra-Cellular Therapies, Employee

W202

fMRI Study of Intrinsic Motivation Impairment in Psychosis Risk

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

Methods: Preliminary analysis was conducted on data from 14 individuals with PS and 8 typically developing controls (TD) age16-26. Final target samples for Time1 are 100 PS and 40 TD, and participants will return for longitudinal imaging 2 years later. During fMRI, participants performed a visual fractal memory task, under three counterbalanced feedback conditions: 1) none 2) accuracy information 3) monetary. On each trial, participants identified which of two presented fractals was the one viewed previously during a pre-scan encoding session. Trials included three phases: choice, confidence rating, and feedback, separated by temporally-jittered delays. fMRI analysis focused on VS, together with exploratory whole-brain analyses. Additional study measures include clinical negative symptom ratings with the CAINS, prodromal symptoms (SOPS), self-report of trait and task IM, a behavioral free-choice measure of task IM, and a behavioral effort-discounting task. Additional MRI data include structural MPRAGE and resting state BOLD (ABCD sequences).

Results: As expected, the PS group showed greater clinical amotivation (CAINS) than TD ($t=2.1$, $p=0.045$). Across all participants CAINS amotivation correlated more strongly with self-reported trait IM ($r=-0.32$) than EM ($r=0.05$). Mean memory task accuracy was 68%, indicating the task was challenging but achievable, conditions designed to elicit IM. Across-trial confidence ratings correlated with correct/incorrect outcome on average $r=0.26$ across the sample, indicating that confidence ratings are an (imperfect) indicator of actual memory accuracy. There were no group differences in task accuracy, confidence, or confidence-accuracy correlation (p's >0.5). fMRI revealed trend VS activation to the fractal stimulus (memory choice) phase ($t=1.7$), and there was a significant relationship between across-trial confidence ratings and VS activation during the choice phase ($t=2.2$, $p=0.03$). Interestingly, the strongest confidence-correlated activations were in the orbitofrontal cortex, an important region for explicit confidence evaluations. There was no VS activation to the outcome phase overall ($t=0.24$), but as expected, VS showed significantly greater activation to correct vs. incorrect feedback ($t=2.7$, $p=0.01$). A simple prediction error (PE) metric (outcome – confidence) correlated significantly with VS activation ($t=2.3$ $p=0.03$). The analyzed fMRI sample was too small to detect group or dimensional correlates. In the post-scan task operationalizing IM as continued voluntary performance of the memory task, PS trended lower ($p=0.14$), completing an average of 6.7 of a maximum possible 10 blocks, while TD completed 8.4 blocks.

Conclusions: Findings are consistent with the hypothesis that VS responses during a cognitive task reflect internally-generated reinforcement signals related to accuracy and confidence. These preliminary results support the utility of our fMRI paradigm as a neurobehavioral probe of intrinsic motivation. Ongoing work will compare feedback conditions, and examine group effects and dimensional relations to symptoms and self-reported IM/EM both cross-sectionally and longitudinally. Our ultimate goal is to characterize the neural mechanisms of amotivation and develop biomarkers for neurobehaviorally-defined amotivation dimensions or subtypes. These biomarkers will be tested for prognostic utility and as moderators/mediators for early interventions in at-risk youth.

Keywords: Intrinsic Motivation, Psychosis, Ventral Striatum, Functional MRI (fMRI), Motivation

Disclosure: Nothing to disclose.

W203

Additional Results From a 12-Month Open-Label Safety Study of Lumateperone (ITI-007) in Patients With Stable Symptoms of Schizophrenia

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Background: Lumateperone (lumateperone tosylate, ITI-007) is an investigational drug for the treatment of schizophrenia, bipolar depression, and other neuropsychiatric disorders. Lumateperone has a unique mechanism of action that simultaneously modulates serotonin, dopamine, and glutamate neurotransmission. This may provide advantages in the treatment of the broad symptoms associated with schizophrenia, including negative and depression symptoms and improve tolerability relative to current standard of care (SOC) antipsychotics. In 2 previous placebo-controlled trials in patients with acute schizophrenia, lumateperone 42 mg (ITI-007 60 mg) demonstrated statistically significant improvement in the Positive and Negative Syndrome Scale (PANSS) Total score compared with placebo. In these studies, lumateperone was well tolerated with a safety profile similar to placebo.

A 2-part open-label long-term study was conducted to evaluate the safety and effectiveness of lumateperone 42 mg in patients with schizophrenia and stable symptoms. The first part of the open-label study demonstrated a favorable safety profile when patients were switched from SOC antipsychotics to lumateperone 42 mg for 6 weeks of treatment. These safety benefits were lost when patients were switched back to SOC. The current presentation reports on the safety and effectiveness of lumateperone 42 mg from the completed 1-year open-label study; a post-1–year extension analysis is currently ongoing. The primary objective of the study was to determine the long-term safety of lumateperone 42 mg; secondary and additional objectives included determining the effectiveness of lumateperone 42 mg on PANSS and Calgary Depression Scale for Schizophrenia (CDSS) scores.

Methods: Patients with stable schizophrenia were switched from SOC antipsychotics to lumateperone 42 mg for up to 1 year of treatment. None of the patients in this long-term study were directly rolled over from a previous lumateperone study. Safety assessments included treatment-emergent adverse events (TEAEs), body weight, laboratory parameters, and extrapyramidal symptoms (EPS)/motor symptom assessments. Efficacy analyses included evaluation of changes in PANSS Total score and in depression symptoms, as measured by the CDSS.

Results: In the 1-year open-label study, 602 patients received at least 1 dose of lumateperone 42 mg and were included in the safety population; 239 patients completed 1 year of treatment. The most common SOC antipsychotics that were used in order of frequency were risperidone, quetiapine, aripiprazole, and olanzapine, which is consistent with current US clinical practice. Only 4 TEAEs occurred in ≥5% of patients including weight decrease (10.1%), dry mouth (7.6%), diarrhea (7.3%), and headache (7.0%); the majority of these TEAEs were mild or moderate in intensity. Mean cholesterol (total and low-density lipoprotein) and prolactin levels significantly (P<.05) decreased from SOC baseline, as did waist circumference, and BMI. Notable improvements in mean body weight were seen at Day 368 (mean change from baseline: −2.1 kg; 95% confidence interval [CI]: −3.3, −0.9; P<.001). There was potentially clinically significant (PCS) weight loss (≥7% decrease) from SOC baseline in 25.0% of the population, with PCS weight gain (≥7% increase) in 9.1% of the population. Based on AE reporting and EPS/motor symptom scales, lumateperone treatment was associated with minimal EPS risk.

In the overall population, mean PANSS score was 62.5 at SOC baseline; in patients with moderate-to-depression symptoms at baseline (CDSS>6), mean baseline PANSS score was 70.5. Lumateperone 42-mg treatment was associated with significant reductions in PANSS Total score from SOC baseline, with continuing PANSS improvement throughout the study (Day 368 mean change from baseline: −4.0; 95% CI −5.8, −2.1; P<.001). Relative to the overall population, reduction in PANSS Total score was greater in patients with moderate-to-severe depression symptoms at baseline (Day 368 mean difference: −11.6; 95% CI −20.2, −3.0; P=.01). Mean CDSS scores were 2.2 at baseline in the overall population and significantly decreased during treatment (Day 368 mean change from baseline: −0.6; 95% CI −1.1, −0.1; P=.01). In patients with moderate-to-severe depression symptoms at baseline, mean CDSS score improvements were more pronounced, improving from a mean CDSS score of 7.9 at baseline to 2.0 at Day 368 (P<.001).

Conclusions: Consistent with the previous interim analysis, long-term treatment with lumateperone 42 mg was associated with minimal metabolic, EPS, and cardiovascular safety and tolerability issues relative to current SOC antipsychotic therapy. Discontinuation rates were similar to other long-term studies with SOC antipsychotics. Lumateperone improved schizophrenia symptoms from SOC baseline with continued long-term treatment. In patients with moderate-to-severe depression symptoms at baseline, lumateperone treatment was associated with greater PANSS improvement relative to the overall population and significantly improved CDSS scores from baseline. These data, taken together, are consistent with and extend previously reported data from placebo-controlled and open-label studies in patients with schizophrenia treated with lumateperone.

Keywords: Schizophrenia, Antipsychotics, Open Label Trials, Clinical Trial, Long-Term Safety

Disclosure: Intra-Cellular Therapies, Inc, Employee

W204

Safety and Effectiveness of SEP-363856 in Schizophrenia: Results of a 6-Month, Open-Label Extension Study

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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 at clinical meaningful concentrations and has shown efficacy in animal models of psychosis. In a previous double-blind (DB), placebo-controlled study, SEP-363856 (in flexible doses of 50 or 75 mg/d) demonstrated efficacy in the treatment of an acute exacerbation of schizophrenia. We now present results of a 6-month extension study whose objective was to evaluate the safety and effectiveness of longer-term treatment with SEP-363856.

Methods: Patients with an acute exacerbation of schizophrenia who completed a 4-week, DB, placebo-controlled, flexible-dose (50 or 75 mg) study of SEP-363856 were given the option to enroll in an extension study in which they were treated, open-label (OL), with flexible doses (25/50/75 mg/d) of SEP-363856 for 26-weeks. To maintain the blind in the initial placebo-controlled trial, patients enrolled in the extension study were started on a SEP-363856 dose of 50 mg/d for 3 days, regardless of initial treatment assignment. Patients were assessed at weekly intervals for the first 4 weeks, then every 4 weeks thereafter. The primary outcome measures were overall incidence of adverse events (AEs), AEs leading to discontinuation, and serious AEs. Secondary safety outcomes included change in weight, laboratory tests, ECG, and measures of extrapyramidal symptoms. Suicidality was assessed using the Columbia – Suicide Severity Rating Scale (C-SSRS). The Pittsburgh Sleep Quality Index (PSQI) was obtained. Effectiveness outcomes included the PANSS total and subscale scores, CGI-Severity score, and the Brief Negative Symptom Scale (BNSS) total score.

Results: A total of 193 patients completed the 4-week DB study, and 156 (80.8%) entered the OL extension study and received at least one dose of SEP-363856 (safety population); 52 patients

(33.3%) discontinued, and 18 (11.5%) discontinued due to an AE. A total of 15 patients (9.6%) experienced an SAE; schizophrenia was the only SAE to occur in more than one patient (n = 7, in the group switched from DB placebo to SEP-363856; n = 4, in the group continuing SEP-363856). There were no deaths in the study. Suicidal ideation (assessed using the C-SSRS) was reported by 3 patients, and 1 patient made an attempt. A total of 88 patients (56.4%) experienced at least one AE; individual AEs with an incidence ≥2% were schizophrenia (12.2%), headache (11.5%), insomnia (8.3%), anxiety (5.1%), somnolence (4.5%), nasopharyngitis (4.5%), nausea (3.8%), irritability (3.2%), influenza (3.2%), weight decreased (3.2%), and prolactin increased (2.6%). Extrapyramidal (EPS)-related symptoms were reported by 5 patients. Mean month 6 change from DB/OL baseline in PSQI global score was -4.2/-2.0. Mean month 6 change from DB/OL baseline in weight was -0.26/-0.32 kg. No clinically meaningful median changes were observed at week 26 in metabolic laboratory parameters (total and LDL cholesterol, triglycerides, hemoglobin A1c) or in prolactin levels. During 6 months of OL treatment, no patient had an increase in QTcF ≥60 msec and a prolonged QTcF interval (≥450 msec in men; ≥475 msec in women). Treatment with SEP-363856 was associated with significant improvement from OL baseline to week 26 (observed/LOCF-endpoint) in the PANSS total score (-22.6/-13.8), positive subscale score (-7.3/-4.5), negative subscale score (-5.2/-3.5), and general psychopathology score (-10.2/-5.8); and in the CGI-Severity score (-1.0/-0.6) and BNSS total score (-11.3/-8.0).

Conclusions: In this 6 month extension study, OL treatment with SEP-363856 was generally safe and well-tolerated, with a relatively high completion rate, minimal effects on weight, lipids, glycemic indices, prolactin, and a very low incidence of EPS measures. During 6 months of SEP-363856 treatment, sustained improvement was noted on the PANSS total score and other effectiveness measures.

Keywords: Schizophrenia Novel Treatment, TAAR 1, Long-Term Safety

Disclosure: Sunovion Pharmaceuticals Inc, Employee

W205

Variations in Brain Regulatory Profiles of Chinese and European Populations Influence the Risk for Schizophrenia

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Background: Genome-wide association studies (GWAS) have identified different risk variants for schizophrenia (SCZ) in populations of East Asian than in populations of European descent. Expression quantitative trait loci (eQTLs) map the genomic loci that regulate gene expression. Used in conjunction with GWAS, eQTL can explain GWAS signals and identify risk genes. However, samples of European descent comprise the majority of available postmortem brain data, impeding interpretation of non-European GWAS signals. Therefore, developing brain regulatory profiles to compare East Asian and European populations will facilitate a better understanding of racial variations evident in SCZ GWAS signals.

Methods: We included 151 post-mortem prefrontal cortex samples of East Asian origin from the China Human Brain Banking Consortium, applying whole-genome and RNA sequencing to obtain corresponding genotyping and gene expression data. We also included 407 prefrontal cortex samples of European descent from the PsychENCODE/BrainGVEX project with both DNA and RNA sequencing data. After quality control, 145 East Asian samples with 18,939 genes and 6,045,349 single nucleotide polymorphisms (SNPs) and 397 European samples with 16,099 genes and 8,108,028 SNPs remained. We applied probabilistic estimation of expression residuals (PEER) to remove covariates and mapped eQTL with FastQTL.

Results: We detected 604,244 eQTLs in East Asian samples with 439,509 eSNPs and 8,739 eGenes, and 2,790,193 eQTLs in European samples with 1,614,824 eSNPs and 15,574 eGenes (q value < 0.01).

To compare the eQTL results of the two populations, we first calculated $\pi 1$ to estimate the proportion of true positives eQTLs in one group that were also detected in another group. $\pi 1$ are 0.73 and 0.86 when using eQTLs from European and East Asian populations as discovery data respectively, suggesting moderate replication between the two eQTL results.

We next defined race-shared eQTLs (common to both populations with the same direction) and race-specific eQTLs and found a low race-shared eQTL ratio (0.39). To explore whether differences in allele frequency contribute to race-specific eQTLs, we compared the distribution of Fst (fixation index, measuring population differences due to genetic structure) value for race-specific eQTLs. We found that race-specific eQTLs were significantly enriched for population-divergent SNPs (Fst > 0.25) in population comparisons (P < 2.2e-16). In contrast, the race-shared eQTLs are significantly enriched SNPs with Fst < 0.05 (OR=15.04, P < 2.2e-16). These results suggest that differences in allele frequency drive race-specific eQTLs. To identify specific risk genes, we integrated eQTL results with PGC2 (Psychiatric Genomics Consortium) and East Asian SCZ GWAS data. Using Summary-data-based Mendelian Randomization, we identified 91 SCZ risk genes in the European descent cohort and 62 SCZ risk genes in the East Asian cohort. Only three of the genes were shared. Most of the causal SNPs detected were race-specific SNPs. These results indicated that racially specific SCZ risks genes are related to allele frequency.

To understand the impact of genetic variation factors on co-expression networks across the two populations, we employed gene co-expression network analysis. Although little SCZ risk genes were shared, we found all co-expression modules preserved in both populations (z-score > 2), indicating similar co-expression patterns in these diverse populations. Shared risk genes enriched in the same preserved co-expression modules, and their functions were enriched in glial cell differentiation, neuronal and axonal ensheathment (adjusted enrichment p < 0.05).

Conclusions: Collectively, we provided the non-European brain expression resources and conducted the first brain eQTL analysis in Chinese population. Through comparison of eQTLs between Chinese-European populations and integration of GWAS results, we observed race-specific SCZ risk genes, which primarily due to differences in allele frequency. However, those race-specific genes contributed to a consistent gene network and to similar brain-related functions, suggesting convergent etiology of SCZ in diverse populations.

Keywords: Schizophrenia, Expression Quantitative Trait Loci (eQTLs), Diverse Populations

Disclosure: Nothing to disclose.

W206

Mitochondria Encoded Gene Expression in Schizophrenia and Bipolar Disorder

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Background: Previous studies of schizophrenia (SZ) and bipolar disorder (BD) have implicated mitochondria-associated pathways

as plausible mechanisms in the pathophysiology of these disorders. Only a few studies have investigated transcripts encoded in the mitochondria genome in either SZ or BD, limiting the complete understanding of the mitochondria and nuclear genome interactions in these serious mental illnesses. The impact of antipsychotic drugs (APDs) on the mitochondria pathway has been controversial in that some reports indicate a normalization of the transcriptome differential gene expression signature in SZ, while others suggest APD treatment likely increases the differential gene expression in SZ. The purpose of this study is to analyze the mitochondria transcriptome consisting of both mitochondria- and nuclear-encoded genes in SZ and BD, to contrast the overlap int hose results with the signature of APD treatment in a non-human primate model.

Methods: Differential gene expression in the prefrontal cortex was investigated focusing on the unreported mitochondria-encoded genes and nuclear-encoded genes using RNA-seq datasets obtained from Capstone Collection. Freeze 1 and Freeze 2 counts of gene quantification from 13 different studies were combined using a linear effects model of SZ, control, and BD subjects. The analysis was restricted to single subjects >17 years of age, to restrict effects due to early postnatal neurodevelopmental changes in predominantly control subjects since the youngest psychiatric patient was 17. The resulting cleaned dataset consisted of 802 controls, 563 schizophrenia, and 221 bipolar disorder from brain regions identified as BA9, BA6, BA9/46. Both demographic model fit and surrogate variable model fit were run separately and overlapping to evaluate outcomes on differential gene expression. Genes passing Benjamini-Hochberg false discovery ($p < 0.05$) are reported.

Results: Analysis of Capstone data and controlling for biological and technical variables showed 4,843 genes passed FDR threshold for SZ and 96 genes passed FDR threshold for BD. When applying 7 surrogate variables and study in the linear model, the significant number of differentially expressed genes doubled for SZ to 9955 and to 2319 genes for BD. There was a significant correlation between fold change when using either the demographic variables and SVA analysis linear models if the study were included in both. We also found positive correlations between the fold changes for a high dose of haloperidol treatment and SZ differential gene expression (DGE) using both linear models. This positive correlation remained for clozapine effects compared to SZ DGE. Further, when restricting the analysis to 1,643 MitoMiner genes representing the nuclear and mitochondria genomes and using 7 surrogate variable analysis model there was a positive correlation between SZ DGE and high haloperidol treatment. The overlap of SZ DGE (nominal p-value < 0.05) with 1,643 MitoMiner genes was 69%, while the same overlap with BD was 31% -- attributable to a larger number of SZ subjects among all of the 13 studies creating greater power. The BD MitoMiner gene set overlapped with the SZ MitoMiner gene set was 40%. The number of MitoMiner genes differentially down-regulated in SZ compared to overall SZ DGE was enriched by over 2-fold.

Conclusions: The Capstone data represents a rich resource to test specific hypotheses. Our initial analyses have shown that traditional demographics analysis may need to be supplemented by hidden variables inclusion. When using surrogate variables, the actual number of 'significant' genes increases leading to the question of which analysis yields a representative snapshot of the SZ transcriptomic architecture. Secondly, the effect of the typical APD haloperidol is associated with an increase in the SZ DGE signal positively, across the global transcriptome, including the mitochondria genomes indicating the need to conduct transcriptomic analysis with actual APD exposure rather than estimates. Finally, this study reveals differential expression, with down-regulation, across a large part of the mitochondria genome and that signal overlaps in SZ and BD.

Keywords: Transcriptome, Mitochondria, Antipsychotic Drug

Disclosure: Nothing to disclose.

W207

Generation of Human-Derived Surface Autoantibodies for Use as Tools to Probe the Pathobiology of Psychiatric Disorders

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Background: The development of research tools that invoke disease-relevant molecular, neurophysiological, and behavioral dysfunction will help to elucidate the underlying mechanisms of psychiatric symptoms. Anti-NMDA receptor encephalitis is an autoantibody-mediated neurologic syndrome with prominent psychiatric symptoms. Antibodies cloned from subjects with anti-NMDA encephalitis recapitulate behavioral symptoms in vivo and have been used to understand the underlying molecular mechanism of disease in vitro. As such, this approach serves as a model for utilizing patient-derived anti-neural autoantibodies as research tools to invoke and study mechanisms underlying human psychiatric symptoms.

Herein we describe a framework for combining anti-neural autoantibody discovery with recombinant antibody technology to yield novel disease-relevant monoclonal antibodies from individuals with prominent psychiatric symptoms. As a proof of principle, we have cloned two monoclonal anti-NMDA antibodies from a single symptomatic subject and demonstrated their biological reactivity in vivo. In a separate study we have utilized an unbiased autoantibody discovery approach to identify a novel, disease-associated autoantibody against ankyrin G.

By combining these methodologies, we expect to produce novel anti-neural antibodies that can used as tools to study the molecular, circuit, and network basis of psychiatric symptoms.

Methods: Cerebrospinal fluid (CSF) samples were obtained from symptomatic subjects under an IRB-approved protocol and divided into fractions for antigen discovery and recombinant antibody cloning. Our anti-neural autoantibody discovery approach combines anatomic mouse brain immunostaining and complementary immunoaffinity assays.

Antigen Discovery: First, sagittal mouse brain sections are incubated with cerebrospinal fluid and counterstained with a fluorescently tagged anti-human IgG secondary antibody. Positive immunostaining indicates the presence of an anti-neural antibody and anatomic data is recorded by capturing and annotating panoramic images obtained using a Zeiss Axioscan imaging system.

In parallel, CSF is screened on a phage display immunoprecipitation sequencing platform (PhIP-Seq) that encodes all known human proteins as overlapping 49 amino acid peptides. Because conformational and post-translationally modified epitopes are not well-represented in PhIP-Seq, immunoprecipitation mass spectrometry (IP-MS) is used as a complementary antigen discovery method in some cases. Candidate antigens identified by PhIP-Seq and/or IP-MS are validated using orthagonal cell based overexpression, immunoblotting, or knock out tissue immunostaining assays.

Antibody cloning: From the same CSF sample from which anti-neural antibodies have been identified, B-cells are printed into 96-well plates using a CellenOne single cell printer. Full length RNA is obtained from single B cells utilizing the Smart-Seq2 protocol and transcribed into cDNA. Using the Tiller et al. protocol, immunoglobulin sequencing of single cell cDNA templates is used to generate paired heavy and light chain sequences from single B cells. Using Gibson assembly V, D, J immunoglobin sequences are reassembled and cloned into a human IgG backbone vector.

Recombinant antibodies are overexpressed in Expi293 cells, purified using protein A/G beads and screened for reactivity against validated antigens from the same subject. Auto-reactive anti-neural antibody clones targeting validated autoantibodies are then tested in downstream pathobiological studies.

Results: In a validation of our unbiased PhIP-Seq autoantibody discovery approach, we identified a novel autoantibody targeting ankyrin G in the cerebrospinal fluid of an individual with steroid-responsive meningioencephalitis and a change in personality. The ankyrin G autoantibody was validated on knockout tissue.

Separately, we identified a subject with recurrent NMDA-encephalitis and verified the presence of anti-NMDA receptor 1 antibodies (NR1) in the CSF using anatomic immunostaining and IP-MS. From the same patient we generated four recombinant monoclonal autoantibodies, two of which demonstrated reactivity against NR1 by immunoprecipitation. Injection of the subject's CSF or subject-derived recombinant anti-NR1 antibodies into the lateral ventricles of developing mice demonstrated an in vivo tropism for the hippocampus that was not observed with the two non-NR1 recombinant antibodies derived from the same patient.

Conclusions: Combing autoantibody discovery and recombinant antibody technology will allow for the development of human-derived, anti-neural autoantibodies that can be used in in vivo and in vitro assays as biologically active tools to probe the underlying pathobiology of psychiatric symptoms.

Keywords: Autoimmune Encephalitis, Psychoneuroimmunology, Neuropsychiatric Symptoms (NPS), Antibody

Disclosure: NowRX, Stock / Equity

W208

Motor Learning in Schizophrenia Over Sleep and Wakefulness

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Background: Studying motor functioning and learning may help elucidate neural circuits involved in neuropsychiatric disorders (Mittal et al., 2017) and guide treatment development. Motor task performance is impaired in schizophrenia (Walther and Mittal, 2017), but whether and how motor learning in schizophrenia is also impaired in schizophrenia is not as clear (Adini et al., 2015; Cornelis et al., 2016; Green et al., 1997). There is evidence that sleep-dependent consolidation of motor learning is impaired in schizophrenia (Manoach et al., 2010), so the degree of motor learning impairment may depend upon the motor task, the timescale of learning, and whether consolidation over sleep occurs. To further characterize motor learning deficits in schizophrenia, we investigated whether motor learning on two tasks over periods of sleep and wake is impaired in schizophrenia.

Methods: 14 stable outpatients with schizophrenia and 14 age- and gender-matched healthy controls between the ages of 18 and 55 participated in 3 administrations of 2 motor tasks, the Motor Sequence Task (MST) and the Grooved Pegboard Task (GPT). These tasks were chosen because prior studies have identified improvement in performance with repeated administrations in healthy volunteers (Solana et al., 2010; Walker et al., 2002). There were 12 hours between task administrations for each participant, and participants were randomly assigned to have overnight sleep versus daytime wakefulness in between the first two task administrations. Repeated measure ANOVAs were performed to determine whether there were group by session by time interactions for each task.

Results: Though participants with schizophrenia performed more poorly than healthy controls on both the MST and GPT, both groups had clear improvement on both tasks. There were no significant differences in learning between the groups on either task. Learning rates in both groups on both tasks were unaffected by sleep versus wake intervening between task administrations.

Conclusions: These results suggest that at least some motor learning may be intact in schizophrenia, despite deficits in performance on motor tasks. Further study of what drives learning to seem spared in the presence of performance deficits is warranted. Understanding which types of learning are intact in schizophrenia will also help create better therapeutic interventions.

Keywords: Schizophrenia, Motor Learning, Sleep

Disclosure: Nothing to disclose.

W209

Gene Co-Expression Networks Offer Unique Insight Into the System-Level Functions of miR-137 Target Genes

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Background: Risk for Schizophrenia (SCZ) is polygenic and can be cumulated in Polygenic Risk Scores (PRSs). However, PRSs do not provide insight into risk biological pathways. We hypothesized that miR-137, a gene expression regulator located in a SCZ risk locus, represents a node of convergence of multiple SCZ risk genes and is associated with system-level phenotypes of SCZ via gene co-expression.

Methods: We compared a direct measure of genetic risk with a co-expression-mediated measure of SCZ risk loci regulation on working memory (WM) and Emotion Processing (EP) brain activity to assess the specific phenotypic contribution of miR-137-associated co-expression.

i) we identified and functionally characterized a gene co-expression module enriched for SCZ-associated genes targeted by miRNA-137. We validated the module-miRNA association using miRNA-137 CRISPR/Cas9 Knock-out and Overexpression in mouse neuroblastoma cell lines.

ii) we computed a) an index of co-expression (Polygenic Co-expression Index, or PCI) of genes in the module, accounting for miR-137 downstream effects via co-expression; b) a PRS of variants harbored in miR-137 target genes, accounting for the portion of risk involving miR-137.

iii) to investigate downstream intermediate phenotypes of SCZ, we used PRS and PCI as predictors of brain activity during WM and EP assessed with fMRI in two independent samples (N1=486; N2=360) of healthy volunteers, free of SCZ-related confounders.

Results: A single module out of 51 was enriched for miR-137 and also for SCZ-associated genes (Bonferroni $p < .05$). Genes expressed in glutamatergic and GABAergic neurons contributing to cell-cell signaling, synapses, and neurodevelopment were overrepresented (Bonferroni $p < .05$). Differentially expressed genes in the mouse cell lines were also over-represented in this module ($p = .011$). fMRI supported prior evidence that miR-137-parsed PRS is associated with PFC activity during WM (FWE-corrected $p < .05$) and not EP. Instead, the PCI was associated with PFC activity during EP (FWE-corrected $p < .05$) and not WM.

Conclusions: Results suggest that miRNA-137 is a node of convergence of polygenic risk for SCZ via regulation of risk genes co-expression. Importantly, while risk-weighted genetic variants in miR-137 target genes support a link between miR-137 and WM, miR-137-related co-expression offers insight into the physiological function of these genes during EP in healthy controls.

Keywords: Coexpression Network, Functional MRI (fMRI), Polygenic Scores, Facial Emotion Processing, Working Memory

Disclosure: Nothing to disclose.

W210

Specific Relationship of Extreme Prior Bias to Hallucination-Like Phenomena in the General Population: An M-Turk Study

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Background: Bayesian inference models of perception posit that percepts are formed by combining information about prior beliefs and the stimulus likelihood to produce a current (updated) belief which corresponds to the resulting percept. Recent studies utilizing novel computational psychiatry approaches have developed the hypothesis that hallucinations arise from an unwarranted increase in the reliance on prior information during perception (i.e. an extreme prior bias). Findings from behavioral studies in clinical hallucinators demonstrate a link between extreme prior bias and hallucinations and the neurobiological basis for this seems to depend on striatal dopamine. This study uses a robust perceptual decision-making paradigm to investigate the relationship between extreme prior bias and hallucination-like phenomena in the general population.

Methods: This study was approved by the NYSPI IRB. General population subjects (N = 50; 25 males, age = 38 ± 8 years) were recruited online through Amazon Mechanical-Turk. Subjects completed self-report questionnaires including the Cardiff Anomalous Perception Scale (CAPS) and Peter's Delusion Index (PDI). Subjects performed a two-tone frequency discrimination task similar to Lieder et al. (Nat Neurosci 2019) to investigate prior bias and its modulations as a function of stimulus distribution. In this task, the subject has to compare the frequency of two auditory tones of short duration and separation (first tone [f1] vs. second tone [f2]). Performance on this well-studied task reflects a prior bias that manifests as a shift in the representation of the first tone towards the mean of prior information in previous trials. The task consisted of 1 set of 300 trials with a broad stimulus distribution and 1 set of 300 trials with a narrow stimulus distribution. For the broad distribution, the f1 were sampled from a Gaussian distribution in the log scale with a mean frequency of 800 Hz and standard deviation of 0.56 octaves. For the narrow distribution, f1 were sampled from a Gaussian with a mean of 800 Hz and standard deviation of 0.31 octaves. For both distributions, f2 were sampled such that the proportional absolute frequency separation between the two tones was log-uniformly sampled from 0.5% – 20%. Data from all 600 trials were analyzed using a logistic regression fit to each individual subject: $P(f2) \sim \beta0 + \beta1 \cdot \delta + \beta2 \cdot d1 + \beta3 \cdot d\infty + \beta4 \cdot d1 \cdot \text{distribution} + \beta5 \cdot d\infty \cdot \text{distribution}$. Where P(f2) is the probability of the participant choosing f2 having a higher frequency; δ is the frequency difference between f1 and f2 on the current trial; d1 is the frequency difference between f1 of the current trial and the mean of f1 and f2 one trial back; d∞ is the frequency difference between f1 of the current trial and the mean of tones across all previous trials; 'distribution' is either broad or narrow. β5 was our parameter of interest as it reflects the adjustment in prior bias between the broad and narrow conditions. This study had two hypotheses: 1) The narrow-distribution condition, relative to the broad-distribution condition, would in general elicit a greater prior bias (positive β5) due to greater certainty about the prior expectations (i.e., a more peaked prior distribution) in the narrow-distribution condition; 2) Based on our previous study in hallucinating patients with schizophrenia (Cassidy et al., Curr Biol 2018), we hypothesized that subjects with higher CAPS scores (i.e., more severe hallucination-like phenomena) would have a smaller adjustment in the prior bias in the broad- relative to the narrow-distribution condition (i.e., smaller β5), reflecting the overreliance on uncertain prior information.

Results: Three subjects were excluded from analysis due to self-report of diagnosed neurological problems. A wide range of CAPS and PDI scores were reported in our general population sample: CAPS = 29 ± 39 (mean ± SD, range = 0 – 126); PDI = 24 ± 21 (range = 0 – 74). The distribution of β5 was significantly greater than zero (one sample t-test: $p = 0.02$, $d = 0.36$), confirming our first hypothesis. CAPS and PDI scores were strongly correlated (Pearson correlation: $r = 0.53$, $p = 0.0002$). Multiple regression was used to test for relationships between β5 and CAPS or PDI while controlling for the other variable (e.g. $\text{CAPS} \sim \alpha0 + \alpha1 \cdot \text{PDI} + \alpha2 \cdot \beta5$). Where α0-α2 are the estimated regression coefficients. We observed a significant effect of β5 on CAPS ($\alpha2 = -6.5$, $p = 0.04$) and no effect of β5 on PDI ($\alpha2 = 0.15$, $p = 0.94$).

Conclusions: We applied a robust perceptual decision-making paradigm that implicitly elicits prior bias to test whether extreme prior biases similar to those observed in clinical hallucinators also underlie hallucination-like phenomena in the general population. The degree of prior bias was modulated by manipulating the distribution of prior information. As hypothesized, more certain prior information (i.e., a narrower stimulus distribution) resulted in greater prior bias. Most importantly, the adjustment in prior bias in the broad- compared to the narrow-distribution conditions correlated with CAPS, but not PDI scores, suggesting that extreme –and less contextually adaptable– prior biases are specifically relevant to hallucinations and related phenomena in the general population.

Keywords: Psychosis Continuum, Computational Psychiatry, Auditory Perception, Decision Making

Disclosure: Nothing to disclose.

W211

Negative Symptoms and Striatal Dopamine Synthesis Capacity in Schizophrenia

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Background: Negative symptoms in schizophrenia pose a major clinical challenge given their debilitating and enduring nature, yet their neurobiological foundations have not been fully defined. Potentially consistent with longstanding hypotheses of underlying dopaminergic dysfunction, diminished striatal activation during reward anticipation has been a replicated finding in schizophrenia that has been linked with negative symptom severity. However, corroborative evidence from neurochemical assays is needed. Striatal presynaptic dopamine synthesis capacity measured by PET has proved an important illness phenotype, but studies in medication-free individuals have been difficult to obtain.

Methods: Two cohorts of patients with schizophrenia spectrum illness were studied with [18F]-FDOPA PET (cohort 1: N = 25, mean age 27+/-9, nine women; cohort 2: N = 11, mean age 29+/-7, two women). PET scans and PANSS ratings for this work were conducted approximately four weeks into the medication-free

period of a blinded medication withdrawal protocol at the NIH Clinical Center. A 6-hour fast and 4-hour abstinence for caffeine and nicotine were required, and an oral dose of carbidopa was given one hour before injection. Each individual's separately-collected T1-weighted anatomical MRI scan was segmented for a cerebellar reference region, which excluded both medial regions surrounding the vermis and lateral regions abutting the transverse sinuses, and was coregistered to each participant's native space, attenuation-corrected, and realigned PET data. After extraction of the reference region's time activity curve, PET data were normalized to MNI-space using ANTS software. Voxelwise modeling using PMOD software yielded maps of the specific uptake constant, Ki (Gjedde-Patlak model), across the striatum, which were then interrogated for association with PANSS factor symptoms, controlling for age and sex, in SPM. A significance threshold of $p<0.05$, FDR corrected, was adopted.

Results: In cohort 1, there was an inverse association between total symptom burden and presynaptic dopamine synthesis, localized to a large cluster in the left post-commissural putamen. Negative symptoms correlated inversely in two large clusters occupying the bilateral putamen. No other symptom factors showed associations that met our statistical threshold. Results were similar in cohort 2, where four clusters throughout bilateral putamen and portions of the caudate showed an inverse association with negative symptoms, and, again, no other symptom factor yielding significant correlational results.

Conclusions: Variability in negative symptom severity may be related to striatal dopaminergic systems in schizophrenia, in accord with prior hypotheses. The inverse association identified here may be consistent with findings of striatal hypofunction during rewarded contexts, but it also adds to the growing literature on heterogeneity in dopaminergic phenotypes among patients and merit additional investigation in relation to reward-related neurophysiology.

Keywords: Dopamine, Schizophrenia, PET

Disclosure: Nothing to disclose.

W212

Behavioral Profile of Zaleplon in Rats Assessed by Active Avoidance Test and Elevated Plus Maze

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Background: Zaleplon is a pyrazolopyrimidine hypnotic, structurally distinctive from benzodiazepines and other hypnotics. Its high affinity to benzodiazepine site on GABA-A receptor containing alpha1 subunit, results in specific effects like shortening of sleep latency, recruitment of sleep continuity and increase in slow-wave phase duration, with ultra-short elimination half-life. Due to specific pharmacological profile, zaleplon is commonly used in pharmacotherapy of sleep disorders. However, as its other effects are less clear, the aim of present study was to investigate the behavioral profile of zaleplon in rats using active avoidance (AA) test and elevated plus maze (EPM).

Methods: Adult male Wistar rats received intraperitoneal injections of either zaleplon in various doses (0.625, 1.25, 2.5, 5 mg/kg) or saline ($n = 6$-7 per each treatment group), 30 min prior to testing. The AA test was performed in automated two-way shuttle-boxes, with training and test sessions procedurally identical. During the first 5 s of each trial, a sound signal was presented, allowing the animal to avoid shocks by moving to other compartment (avoidance response). If the animal did not respond within this period, a foot shock of 0.3 mA (7 s duration) was applied. Each animal was submitted to two, 24 h-separated 100-trial sessions and could move freely in the apparatus between trials. During the test, the number of successful active avoidance responses and inter-trial crossings (ITC) was counted. The EPM apparatus consisted of two open (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm), connected by a junction area (10 × 10 cm). Each rat was placed in the center of the maze, facing one of the enclosed arms, and its behavior was recorded for 5 min. Indicators of anxiety (total time spent in open arms and frequency of entries to open arms) were obtained, as well as the parameters of exploratory and locomotor activity. The data were assessed by ANOVA, and if significant differences were detected, each treatment group was compared with control by a Dunnett's test.

Results: In AA, zaleplon treatment significantly affected retrieval of avoidance responses on the second day of shuttle box testing ($F(4,25)=3.001$, $p<0.05$), without causing major variations in locomotor activity assessed through ITC. Dunnett's test applied after ANOVA indicated that the zaleplon avoidance-facilitatory dose was 0.625 mg/kg. Moreover, ANOVA indicated significant effects of zaleplon on rat behavior in EPM ($F(8,54)=3.431$, $p<0,05$). Post-hoc Dunnett's test revealed that zaleplon administered to rats in a 0.625 mg/kg dose significantly increased the total time and frequency of entries to open arms, in comparison to saline-treated group, suggesting anxiolytic-like effects. Exploratory activity, expressed as the number of rearings, was significantly increased in 0.625 mg/kg zaleplon-treated group compared to control, without influencing locomotor activity. However, 5 mg/kg zaleplon exerted hypolocomotor effects.

Conclusions: These findings suggest that very low doses of zaleplon facilitate retrieval-based learning and produce anxiolytic-like effects, without causing sedation. The molecular and neuronal substrates of observed zaleplon effects remain to be further elucidated. Although, so far, we have only investigated the effects of acute zaleplon administration, the studies of zaleplon chronic treatment are of particular importance.

Keywords: Zaleplon, Active Avoidance, Elevated Plus Maze, Behavioral Pharmacology, Animal Research

Disclosure: Nothing to disclose.

W213

Effects of Circadian Phase on the Relationship Between Kynurenic Acid and Sleep-Wake Behavior in Male and Female Rats

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Background: Tryptophan metabolism via the kynurenine pathway (KP) may represent a key molecular link between sleep loss and cognitive dysfunction. Modest increases in the KP metabolite kynurenic acid (KYNA), which acts as an antagonist at N-methyl-D-aspartate (NMDA) and α7 nicotinic acetylcholine (α7nACh) receptors, and an agonist at the aryl hydrocarbon (AhR) receptors, result in cognitive impairments. We have shown that these disruptions may be causally related to impairments in sleep-wake behavior with acute KYNA elevation (Pocivavsek et al. Sleep, 2017).

Methods: Presently, we further explored the novel hypothesis that elevated KYNA adversely impacts sleep quality in adult cohorts of both male and female Wistar rats. In consideration of the circadian rhythm, animals were treated with either vehicle or kynurenine (100mg/kg; intraperitoneally), the direct precursor to KYNA, at the beginning of the light cycle, zeitgeiber time (ZT) 0, or at the beginning of the dark cycle, ZT 12. In vivo microdialysis, was

collected from the dorsal hippocampus in 30-min fractions and analyzed for de novo KYNA formation (N 6-8 per sex per treatment). Separate animals (N = 10 – 11 per sex, within animal treatment design) were implanted with telemetric devices to acquire polysomnographic recordings that combine electroencephalogram (EEG) and electromyogram (EMG). Analysis of vigilance state-related parameters categorized as wake, rapid eye movement (REM) and non-REM (NREM) were assessed for 24 h after treatment.

Results: In vivo microdialysis, collected from the dorsal hippocampus, confirmed significant formation of KYNA with kynurenine challenge in both sexes and also during both the light phase and dark phase. Area under the curve analysis revealed a significant sex x phase interaction with kynurenine challenge; specifically, male rats produced significantly more de novo KYNA during the dark phase vs light phase (**P<0.01). Interestingly vehicle treatment, we found a phase x time interaction (**P<0.01); extracellular KYNA levels decreased across time in the light phase and increased across time in the dark phase. Analysis of vigilance state-related parameters categorized as wake, rapid eye movement (REM) and non-REM (NREM) were assessed for 24 h after treatment. Kynurenine treatment at ZT 0 significantly reduced REM duration compared to vehicle treatment (*P<0.05) during the 12 h of the light phase in both male and female rats. Conversely, kynurenine treatment at ZT 12 did not immediately impact vigilance state duration during the 12 h of dark phase, but significantly affected REM duration, in males only, during the subsequent light phase (*P<0.05).

Conclusions: Taken together, our results suggest differences in de novo KYNA synthesis in the light phase versus the dark phase in the hippocampus with kynurenine stimulation in vivo that may have functional consequences on sleep-wake behavior. The present and future complementary experiments provide mechanistic value to understanding the role of KYNA in modulating a relationship between sleep, circadian systems and cognition.

Keywords: Tryptophan, Kynurenine Pathway, REM Sleep, Alpha7 Nicotinic Acetylcholine Receptor, Kynurenic Acid

Disclosure: Nothing to disclose.

W214

Brown Adipose Tissue Activation, Sleep Restriction and Obesity

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Background: Diabetes and obesity are growing healthcare concerns throughout the world. Current strategies have not proven effective to stem the epidemic. Individuals with short sleep duration are at increased risk for obesity, while evidence is incomplete as to how sleep affects all aspects of energy expenditure (EE). Brown adipose tissue (BAT) has recently emerged as a thermogenic tissue that may be of importance in preventing the development of obesity and diabetes-related disorders. Animal studies have suggested that adequate, high-quality rebound sleep after sleep restriction (SR) may be dependent upon functional BAT. The goal of the proposed study is to evaluate the impact of SR on BAT activation and to assess the role of BAT in recovery sleep (RecS) after 3 nights of SR in healthy adults.

Methods: After a 3-d period of habitual sleep (HS, 8 h) and sleep restriction (SR, 4 h) under identical, controlled, weight-maintenance feeding conditions, BAT activities were assessed by a PET/MR combined scanner with simultaneous acquisition. The SUVmean and SUVR (ratio of SUV BAT/muscle) of 18F-FDG were calculated. The fat fraction (FF) and R2*(=1/T2*) in BAT were quantified using a new, improved MR sequence. Blood samples were assayed to determine circulating levels of metabolic hormones, and correlated with BAT thermogenesis.

Results: Ongoing results have shown an increase in FDG-SUVR in 5 (avg. 29%±16) out of 7 subjects after SR, compared to HS. A similar trend of increase in FF in BAT was also observed after SR (avg. 13%±8), supporting our hypothesis that BAT activation was greater after sleep loss as compared to HS. There were good correlations between BAT activation and various metabolic markers: SUVR_HS and BMI (Pearson's r = −0.85), SUVR_SR and insulin (r = −0.99), SUVR_SR and HOMA-IR (r = −0.98). It was noted that the subject who had least BAT activation had highest insulin, HOMA-IR and leptin levels, and worst RecS.

Conclusions: These ongoing results suggest that BAT activation may play an important role in maintaining a healthy metabolic state. Inadequate BAT function may increase the risk for obesity and diabetic disorders.

Keywords: Obesity, Sleep Deprivation, PET Imaging, Brown Fat Activation

Disclosure: Nothing to disclose.

W215

Relative Contribution of Drug Re-Exposure and Salient Cues in the Reinstatement of Cocaine and Heroin Seeking; Implications for the Treatment of Substance Use Disorders

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Background: The rates of stimulant and opiate abuse have been steadily rising in the USA over the last decade (The Guardian, 2018). Treating drug dependence and addiction are critical areas of unmet clinical need. Persuading subjects to quit substances of abuse is a first step, but helping them to maintain abstinence from drug taking after quitting is a major problem when treating substance use disorders. It is known that reinstatement of drug seeking can be initiated in humans and experimental animals by salient cues associated with abuse, drug re-exposure, or a combination of both (Gardner, 2011; Bossert et al., 2013). What is less clear is (i) the relative contribution of these triggers to initiating relapse, and (ii) whether their contributions are the same for different types of substance of abuse.

Methods: Experiments were conducted in mildly food-restricted, singly-housed, adult, male, Sprague-Dawley rats. The investigation was conducted in 2 parts. In part 1 we explored heroin (15µg/kg/inj [injection]) and cocaine (0.29 and 0.45mg/kg/inj) as positive reinforcers by intravenous self-administration (IVSA) on a fixed ratio (FR) schedule in 2hr sessions and then determined their relative reinforcing effects by break-point determination on a progressive ratio (PR) schedule in a single 4hr session. In part 2, we used an additional 2 cohorts of rats and trained them to self-administer heroin (15µg/kg/inj; Group 1) or cocaine (0.36mg/kg/inj; Group 2) on a FR5 reinforcement schedule in 2hr sessions. Tone + light cues were briefly presented contingently with each drug injection. After robust stable self-administration had been established (≥12inj/session), the responding of the rats was extinguished with saline on FR5 without cues (≤6infusions/session). Reinstatement of drug seeking was initiated by drug priming (cocaine 1mg/kg i.v. or heroin 0.25mg/kg s.c.), presentation of tone/light cues, or drug priming + cues. Results are mean ±SEM.

Results: Part 1: Heroin (15µg/kg/inj) and cocaine (0.29 and 0.45mg/kg/inj) maintained significantly greater levels of self-administration than saline (inj/session [n]: heroin = 19.2±0.6 [9] vs 4.3±0.4 [9], $p < 0.001$; cocaine 0.29mg/kg/inj = 19.5±0.4 [10] vs 3.7 ±0.3 [25], $p < 0.001$; cocaine 0.45mg/kg/inj = 19.0±0.6 [10] vs 3.7 ±0.3 [25], $p < 0.001$). The break-points for reinforcement of heroin (15µg/kg/inj) and cocaine (0.29 or 0.45mg/kg/inj) were not significantly different from one another (lever presses/inj [n]: heroin = 87.1±33.1 [9] vs cocaine 0.29mg/kg/inj = 74.8±23.7 [10] or cocaine 0.45mg/kg/inj = 84.9±22.7 [10]). Therefore, these 2 substances of abuse were tested at equally reinforcing doses in Part 2.

Part 2: Heroin (15µg/kg/inj) and cocaine (0.36 mg/kg/inj) maintained significantly greater levels of "active" drug-paired lever-presses than saline (active lever-presses [n]: heroin = 240.6±50.1, [12] vs saline = 25.7±2.4, [12], $p < 0.001$; cocaine = 142.4±7.7 [18] vs saline = 16.7±1.2 [18], $p < 0.001$). Reinstatement of drug-seeking was initiated by drug priming + cues (active lever-presses: cocaine = 104.4±15.1 [8]; heroin = 166.6±25.7 [6]; both $p < 0.001$ vs respective saline extinction values), drug priming alone (active lever-presses: cocaine = 59.7±11.8 [9]; heroin: 131.2±39.6, [6]; $p < 0.001$ and $p < 0.01$ vs saline, respectively). However, the presentation of tone/light cues alone induced reinstatement to cocaine seeking, but not heroin seeking (active lever-presses: cocaine = 60.0±14.9 [10]; heroin = 39.3±7.1 [6]; $p < 0.001$ and non-significant versus saline, respectively). The effects of the contingent cues and drug re-exposure were approximately additive for the reinstatement of cocaine and heroin seeking. However, their relative contributions were very different. Thus, the effects of drug re-exposure and salient cues were equal for the reinstatement of cocaine seeking, but in the case of reinstatement heroin seeking the effect of drug re-exposure was 3x greater than that of the salient cues ($p<0.05$).

Conclusions: The findings reveal that cocaine and heroin both served as powerful reinforcers. At equivalent reinforcing doses which induced a high degree of psychological dependence, re-exposure to the reinforcing effect of the drug is a much more important factor in the reinstatement of heroin seeking than it is for cocaine. Substitution therapy is effective for treating dependence on opiates, but not cocaine. Our results suggest that one reason may be because contextual cues play a much more important role in triggering relapse to cocaine abuse.

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Keywords: Reinstatement, Heroin, Cocaine, Drug prime, Contextual Cue

Disclosure: RenaSci Ltd, Employee

W216

The Role of Interleukin-1 Receptor-Associated Kinase 4 in Opioid Addiction

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Background: Opioid addiction remains a challenging and costly disease. Apart from neuronal adaptation, growing recognition arises that glial proinflammatory activation importantly contributes to the rewarding effects of opioid abuse. Recent studies suggest the essential role of Toll-like receptor 4 (TLR4), a candidate neuroimmune therapeutic target, in drug addiction. While Interleukin-1 receptor associated kinase 4 (IRAK4) plays a crucial role in TLR4 mediated innate immunity, there is no further studies support its functioning in opioid addiction. We hypothesized that opioid use activates IRAK4 which contributes to the reinforcing effects, and disruption of IRAK4 signaling attenuates the addictive-like behaviors.

Methods: In the present study, IRAK4 and IRAK1 phosphorylation after morphine and cocaine self-administration was evaluated using western blotting. The role of IRAK4 in morphine self-administration and cue-induced reinstatement was examined with its inhibitor, PF06650833. Moreover, local pharmacological manipulation was conducted to determine the role of IRAK4 in the nucleus accumbens (NAc) core in the cue-induced reinstatement of morphine seeking.

Results: We found that morphine self-administration significantly increased the phosphorylation of IRAK4, but not IRAK1, both in NAc and VTA. However, neither cocaine short access nor long access self-administration had any effect on the phosphorylation of IRAK4. Systemic administration of PF06650833 significantly attenuated cue-induced reinstatement of morphine seeking without affecting the spontaneous locomotion in rats, and microinjection of PF06650833 into NAc core sufficiently decreased cue-induced morphine reinstatement.

Conclusions: These results demonstrated that modulation of IRAK4 activity regulates the cue-induced morphine reinstatement, and suggested it as a potential novel drug target in treating opioid addiction.

Keywords: Morphine, Intravenous Drug Self-Administration, Reinstatement, Proinflammatory Cytokines, Rats

Disclosure: Nothing to disclose.

W217

Activation of Astrocytes in the Dorsomedial Striatum Differentially Regulates Medium Spiny Neurons and Reward-Seeking Behaviors

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Background: Shifting between goal-directed and habitual actions enables effective decision-making processes for reward-seeking behaviors. Consequently, disruption of reward-value-dependent shifting may underlie addicted behaviors. Dorsomedial striatum (DMS) is the primary brain region in regulating goal-directed reward-seeking behaviors. Recently, we demonstrated that pharmacological and optogenetic activation of adenosine A2AR-expressing indirect medium spiny neurons (iMSNs) in the DMS reduces reward-seeking behaviors in mice. However, it remains unknown how astrocytes, the major modulator of adenosine signaling, contribute to DMS neuronal activity and the reward-seeking behaviors.

Methods: To investigate the astrocytic activity-driven neuronal synaptic events and behavioral consequences, we chemogenetically activated astrocytes in the DMS using GFAP promoter-driven expression of hM3Dq, the excitatory designer receptors exclusively activated by designer drugs (DREADDs). First, we employed ex vivo and in vivo calcium imaging to examine chemogenetically induced cellular activity in the DMS astrocytes of ALDH1L1-GCaMP6s calcium fluorescence indicator-expressing mice. Then, we recorded electrophysiological changes in the synaptic activity of the two types of MSNs, direct MSNs (dMSNs) and iMSNs. To evaluate the behavioral consequences, we trained mice in nose-poke operant chambers that developed either habitual or goal-directed reward-seeking behaviors. Then, we assessed whether

the mice were able to distinguish the value of the reward after the astrocytic activation, and whether this effect depended on pre-existing habitual or goal-directed behaviors.

Results: Chemogenetic activation of the astrocytes in the DMS increased the intensity and frequency of calcium signaling. Importantly, our results showed that the chemogenetic activation of the DMS astrocytes decreased the dMSNs excitability, whereas it increased the iMSNs excitability. Specifically, it reduced the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in the dMSNs, whereas it increased the amplitude of the sEPSCs and decreased the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in the iMSNs. The changes in sEPSCs and sIPSCs induced by astrocytic activation were significantly inhibited by the pretreatment of DPCPX, A1R antagonist, and genetic deletion of the astrocytic adenosine transporter, equilibrative nucleoside transporter 1 (ENT1, slc29a1). NBTI, an ENT1-specific blocker, also reduced the chemogenetic-evoked changes in the synaptic events, suggesting that ENT1 is, at least partly, required for astrocytes-induced changes in the neuronal activity.

In the evaluation tests of goal-directed and habitual reward-seeking, the chemogenetic activation of the DMS astrocytes shifted the habitual behaviors to goal-directed actions in sucrose reward-seeking for WT, but not ENT1 KO mice. In contrast, the astrocytic activation-induced shifting was rescued, when ENT1 expression was normalized in the DMS astrocytes of the ENT1 KO mice. On the other hand, when mice were trained to have goal-directed behaviors, the astrocytic activation in the DMS did not change the reward-seeking behavioral pattern.

Conclusions: Together, our results indicate that astrocyte-mediated adenosine signaling in the DMS determines goal-directed and habitual actions by selectively regulating synapses in the MSNs. The interaction between astrocytes and neurons via adenosine signaling could be a potential therapeutic target for maladaptive reward-seeking behaviors.

Keywords: Reward-Based Decision-Making, Astrocyte, Adenosine Signaling, Dorsomedial Striatum

Disclosure: Nothing to disclose.

W218

Chemogenetic Inhibition of Accumbens Cholinergic Interneurons Inhibits Cue-Induced Nicotine Seeking

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Background: Nicotine is the primary addictive substance in tobacco and is widely abused. Due to the recent surge in e-cigarette use especially adolescents and young adults, it is essential to understand underlying neurophysiological mechanisms of nicotine addiction. While previous studies have shown that increased glutamate release from prelimbic afferents targeting the nucleus accumbens core (NAcore) contributes to cue-induced reinstatement, the role cholinergic interneurons (ChIs) within the nucleus accumbens in mediating nicotine-seeking behavior is unknown. The principle hypothesis for the conducted studies is that cue-induced glutamate release from prefrontal cortical projections into the NAcore activates ChIs, and this induces acetylcholine (ACh) release through activation of nicotinic acetylcholine receptors (nAChRs). We hypothesize that ACh, released from ChIs is a feedforward mechanism that promotes additional glutamate release from prelimbic afferents, which exacerbates relapse of nicotine seeking. In this way, ChIs modulate glutamatergic signaling, transitioning drug craving to drug seeking.

Methods: Using choline acetyltransferase (ChAT)-Cre transgenic rats, ChIs were bi-directionally manipulated prior to cue-induced reinstatement using chemogenetics. Prior to self-administration, cannulae were placed into the NAcore, and Cre-dependent inhibitory, excitatory and control DREADD vectors packaged in AAVs were then bilaterally infused into the NAcore allowing for chemogenetic control of NAcore ChIs. Rats underwent nicotine self-administration (0.02 mg/kg/infusion), in which an infusion was paired with a compound stimulus (discrete lights + tone) for 10 sessions. Following nicotine self-administration, rats were placed into daily extinction sessions, where no nicotine or cues were delivered upon active lever presses, for a minimum of 14 days. Prior to cue-induced reinstatement, intra-NAcore clozapine-N-oxide (CNO) was administered. Following reinstatement, whole-cell electrophysiology was conducted from medium spiny neurons (MSNs) within the NAcore to identify changes in synaptic plasticity (measured via AMPA/NMDA ratio).

Results: Chemogenetic inhibition of ChIs inhibits cue-induced reinstatement as well as rapid, transient synaptic plasticity in accumbens MSNs, which is a biomarker of drug relapse motivation. Chemogenetic activation of ChIs did not inhibit nicotine seeking, however plasticity was decreased compared to control conditions, indicating that overactivation of ChIs due to chemogenetic activation inhibits NAcore MSNs.

Conclusions: ChIs, which represent a small subset of neurons within the NAcore, have outsized control over relapse-associated rapid, transient synaptic plasticity. These results indicate that ChIs drive the circuit to promote nicotine seeking behavior. The findings of this study lay the groundwork for identifying new treatment strategies that target cholinergic interneuronal activity to treat nicotine addiction.

Keywords: Nicotine Addiction, Cholinergic System, Relapse, Glutamate Homeostasis, Synaptic Plasticity

Disclosure: Nothing to disclose.

W219

Metabotropic Glutamatergic Receptor 5 (MGLUR5) at the Interaction of Stress and Substance Use: Analysis of In Vivo PET Imaging in Humans

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Background: The metabotropic glutamatergic receptor 5 (mGluR5) has emerged as a candidate treatment target for a variety of psychiatric disorders through mostly preclinical investigations. Technological advances now allow us to quantify or measure density of this target in vivo in humans using positron emission tomography (PET). We previously reported on the involvement of mGluR5 in major depressive (MDD) and posttraumatic stress (PTSD) disorders. Given that individuals with these disorders are at greater risk for substance use and misuse, we examined for the first time the effects of nicotine and cannabis use on mGluR5 availability in individuals with MDD and/or PTSD.

Methods: Three groups of people participated in magnetic resonance and [18F]FPEB PET brain imaging scans with clinical assessments: (1) 33 individuals with MDD (mean age = 38.0, 21 female, 9 current tobacco smokers, 6 former smokers); (2) 31 individuals with PTSD (mean age = 37.6, 13 female, 11 current tobacco smokers, 6 former smokers); and (3) 62 healthy individuals

(mean age = 36.7, 29 female, 17 tobacco current smokers, 3 former smokers). Sixteen individuals in the total sample were cannabis users. Volume of distribution (Vt) was the main outcome measure and primary analyses were completed in the hippocampus, amygdala, and frontal cortical regions. These regions have been shown to be implicated in mood and substance use disorders. In addition to descriptive and correlational analyses, three comparisons of mGluR5 availability were performed in the noted regions of interest using multivariate ANOVA. Specifically, we compared: (1) smokers vs. non-smokers across groups; (2) smokers vs. non-smokers within groups; and (3) cannabis users vs. non-cannabis users across groups.

Results: In the overall sample, there were no significant differences in mGluR5 availability between tobacco smokers and nonsmokers, even after adjustment for cannabis use. However, stratification of analyses by clinical diagnosis showed that MDD-smokers exhibited significantly higher mGluR5 availability compared with MDD-non and MDD-former smokers in the hippocampus ($F=5.9$, $p = 0.02$) and posterior cingulate cortex ($F=7.9$, $p = 0.01$). Furthermore, in MDD, smokers had a significantly higher depression scan-day score than non-smokers. Interestingly, in the MDD non-smokers, depressive symptoms negatively correlated with mGluR5 availability across all ROIs ($r's = -0.56$ to -0.70; $p's=0.005$-0.049). This was not observed in MDD smokers. There were no significant differences in mGluR5 availability by smoking status in the PTSD and HC groups. When we evaluated the relationship between cannabis use and mGluR5 availability in the overall sample, cannabis users had significantly higher mGluR5 availability across regions of interest ($p's < 0.05$). The relationship between mGluR5 availability and mood symptoms did not differ as a function of cannabis use.

Conclusions: The relationship between mGluR5 and tobacco smoking appears to differ as a function of psychiatric diagnosis, such that a differential relationship was seen in the MDD group only where smoking appeared to affect the association between mGluR5 and mood in MDD. We also observed higher mGluR5 availability in cannabis users regardless of psychiatric status, although these data are preliminary. A larger sample of cannabis users is required to examine within group differences. Overall, these novel findings point toward mGluR5 involvement in the comorbid interplay between mood and addictive disorders, and suggest a potential therapeutic target to decrease smoking addiction in these populations.

Keywords: Smoking, mGluR5 Receptors, Marijuana, Depression

Disclosure: Nothing to disclose.

W220

Investigating Sex as a Moderator of Acute Objective and Subjective Intoxication After Ad Libitum Cannabis Concentrate Use

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Background: This novel study looks at sex-dependent differences in the acute effects of high potency cannabis products. While data on low potency cannabis products has suggested that sex differences may emerge in the effects of acute cannabis use, results are mixed, often lack ecological validity, and may not generalize to high potency products. Using a naturalistic design that relies on self-administration and assessments of acute concentrate use in a mobile pharmacology laboratory, we examined sex as a moderator of the effects of ad libitum administration of cannabis concentrates on cannabis intoxication and impairment outcomes: THC blood levels, subjective intoxication, and cognitive performance.

Methods: We used naturalistic methods to assess the effects of commonly-used commercially-available concentrates (70% or 90% THC) after ad libitum use. Regular concentrate users were asked to self-administer one of two legal market concentrate products (70% or 90% THC) that they purchased from a dispensary. 65 participants ($N = 34$ men, $N = 31$ women) were assessed in a mobile pharmacology lab before, immediately after, and 1-hour after cannabis self-administration. Plasma cannabinoids levels (THC, 11-OH-THC, and THC-COOH), subjective intoxication, and cognitive performance were assessed at all three time points.

Results: Male and female participants reported using similar amounts of cannabis concentrate [Males = .12 (.16) mg; Females -.11 (.13) mg, $p = .92$] during ad libitum administration. Further, across participants, plasma THC levels exhibited a quadratic effect of time, $b = 623.15$, $t(59) = 5.64$, $\eta 2 = .35$, $p < .001$, such that THC levels peaked at the acute post-use assessment and dropped an hour after use. Thus, to test the prediction that sex would moderate the quadratic change in THC levels, we ran a regression model with quadratic change over time as the dependent variable (pre-use THC $= -1$, immediate post-use THC $= +2$, one-hour post-use THC $= -1$), and sex (Men $= -1$, Women $= +1$) and baseline plasma THC as predictors. Controlling for THC levels at baseline, there was a significant sex by quadratic change interaction on THC levels, $b = -280.99$, $t(58) = -3.00$, $\eta 2 = .13$, $p = .004$, with men displaying a stronger quadratic effect for THC. Sex-dependent differences were also found in THC related metabolite (11-OH-THC, and THC-COOH) plasma levels after concentrate use, with men displaying higher levels both acutely and across all assessment time points ($p<.01$). Despite these striking sex differences in THC-related plasma concentrations, sex-dependent differences in subjective and cognitive effects did not emerge.

Conclusions: Sex-dependent differences emerged in THC and related metabolite plasma levels following ab libitum use of high potency cannabis concentrate products. More specifically, although men and women consumed the same amount of cannabis concentrate, men displayed higher levels of plasma THC, 11-OH-THC, and THC-COOH both acutely and across time relative to women. This may be due to biological differences between the sexes in THC metabolism or due to differences in the ways the men and women titrate or inhale cannabis concentrates (Cooper & Craft, 2018). Despite these differences in THC related blood levels, men and women did not display differences in the effects of THC on either subjective intoxication or cognitive performance. This incongruence between objective intoxication and other cannabis impairment metrics (e.g. subjective intoxication and cognitive performance) suggests that men may have a greater tolerance to the subjective and cognitive effects of THC than women or have pre-existing differences that impact the way men experience cannabis. These results contribute to a relatively lacking body of literature examining the risks associated with higher potency forms of cannabis and cannabis concentrates, and the potential to inform public health policies to reduce the harms of cannabis.

Keywords: Gender, Marijuana, Abuse Liability, Cognition

Disclosure: Nothing to disclose.

W221

Epigenetic Priming Underlies Transcriptional Disruption Linked to Drug Relapse

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Background: Drug addiction is a major public health crisis that inflicts tremendous psychological and financial costs on patients, their families, and society at large. Current pharmacological therapies target neuronal receptors or transporters upon which drugs of abuse act initially, yet these treatments remain ineffective for most individuals and do not prevent disease relapse after abstinence. Drugs of abuse, in addition to their acute effects, cause persistent plasticity after repeated use, involving dysregulated gene expression in several central brain regions of reward, including the nucleus accumbens (NAc). Permanent changes in chromatin structure are hypothesized to underlie the transcriptional dysregulation linked to the persistent behavioral abnormalities that characterize drug addiction; yet, there is no direct link between drug-induced epigenetic alterations and the aberrant gene regulation linked to relapse. A fundamental challenge is to determine which neuronal subtypes are responsible: the NAc is composed of two opposing types of medium spiny neurons (MSNs), the D1 and D2 dopamine receptor-expressing subtypes, which exhibit dramatic differences in activity and effects on drug reward. Our goal is to identify the precise epigenetic mechanisms that establish and preserve the molecular pathology in these distinct striatal subpopulations. Based on recent evidence, we hypothesize that chromatin 'scarring' by drugs of abuse alters the inducibility of key neuronal genes involved in plasticity, referred to as gene priming and desensitization. Such epigenetic priming of gene transcription, however, is virtually unexplored in the context of psychiatric disease and the molecular mechanisms responsible remain unclear.

Methods: The NAc is largely composed (>90%) of two functionally distinct subtypes of MSNs, therefore making the cell-type specific identification of epigenetic changes critical. In recent years, the assay for transposase-accessible chromatin using sequencing (ATAC-seq) has become a fundamental tool of epigenomic research and is used to assess chromatin structure genome-wide to detect "open" chromatin regions, which can be indicative of active gene transcription or priming. Here, we defined chromatin accessibility genome-wide in D1 and D2 MSNs using fluorescence-activated cell sorting (FACS) coupled to ATAC-seq in two transgenic mouse lines (Drd1a/ Drd2a::EGFP-L10a), and distinguished immediate versus long-term alterations in combination with unbiased histone modification profiling by mass spectrometry and ChIP-sequencing. Specifically, persistent alterations in subtype-specific chromatin structure were investigated following prolonged withdrawal after chronic cocaine (30d withdrawal after 10d of cocaine injections i.p.), with focus on gene-regulatory promoter and enhancer elements. All experimental protocols in animal studies were approved by Mount Sinai's Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health.

Results: We found that cocaine-induced changes in chromatin accessibility discriminate D1 from D2 MSNs, and that chromatin is overall less accessible in D1 MSNs than in D2 MSNs at baseline; however, a genome-wide "opening" of chromatin occurs selectively in D1 MSNs upon acute exposure to cocaine. Intriguingly, this D1-specific chromatin opening is sustained even after prolonged periods of withdrawal. Further, following withdrawal from chronic cocaine, the histone variant H2A.Z – a recently identified memory suppressor – is evicted from important gene-regulatory promoter and enhancer regions. Curiously, this chromatin 'scarring' occurs at key neuronal genes that have been linked to the long-lasting transcriptional dysregulation in the NAc, in particular, priming of neuronal gene expression upon relapse.

Conclusions: Together, our studies investigate an emerging view of epigenetic adaptation and gene dysfunction that may contribute to drug addiction, providing novel insight into epigenetic priming as an important mechanism whereby drugs of abuse alter brain function and behavior. Specifically, our data implicate persistent remodeling of the D1 MSN epigenome in the long-lasting effects of cocaine-induced changes in gene regulation. We hypothesize that the altered gene-regulatory chromatin landscape in D1 MSNs and priming of cocaine-related gene expression programs ultimately promote the enduring changes in neuronal plasticity and behavior that characterize addiction. Importantly, since these epigenetic aberrations may be reversible, an improved mechanistic understanding of chromatin 'scarring' by drugs of abuse could pave the way to novel epigenetic interventions to treat drug addiction.

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Keywords: Epigenetics, Drug Addiction, Gene Priming, Chromatin Modification, Single-Cell RNA Sequencing

Disclosure: Nothing to disclose.

W222

Intratelencephalic and Pyramidal Tract Neurons Differentially Mediate Cocaine Sensitization and Taste Aversion

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Background: Intratelencephalic (IT) and Pyramidal Tract (PT) are two classes of glutamatergic cortical projection neurons that exhibit unique morphology, firing patterns, and connectivity. However, little is known about their functional role in mediating behavioral outputs. Of interest is their common projection to the striatum, which is frequently implicated in processing and coordination of reward, aversion, learning, and motivated behaviors. Furthermore, the cortico-basal ganglia circuit comprising cortex and striatum is also known to be dysregulated in substance use disorders. We have recently found distinct roles of IT and PT neurons in anterior cingulate cortex (ACC) in the appetitive and aversive components of cocaine use, using conditioned place preference and conditioned taste aversion (CTA) assays. Specifically, inactivation of IT neurons blunted a cocaine-induced conditioned taste aversion to sucrose whereas PT inactivation increased the rewarding value of cocaine and transiently altered cocaine sensitization. Nonetheless, how IT neurons influence additional dimensions of substance use, including the locomotor sensitization to cocaine, has yet to be elucidated.

Methods: In order to determine how IT neurons contribute to sensitization, as well as if IT inactivation inherently alters aversive processing, IT neurons were virally targeted for chemogenetic modulation in ACC. To achieve selective expression in IT neurons, male Sprague Dawley rats received unilateral infusions of AAVrg-CRE and hEF1α-IRES-FLPO into different hemispheres of dorsomedial striatum (DMS), while the complementary CRE- and Flp-dependent AAV-hM4Di was injected into the contralateral ACC. A minimum of two weeks following surgery, rats were pseudorandomly divided into one of four groups: pretreatment with CNO (5 mg/kg, i.p.) or vehicle (5% Dimethyl sulfoxide (DMSO) in sterile water, i.p.) 30 minutes prior to treatment with either cocaine (15 mg/kg, i.p.) or saline (0.9%, i.p.) for seven sessions across 14 days (one sessions every other day). Ambulations were recorded in locomotor activity boxes over 60 minutes. Two weeks following the final session, all groups were given a challenge injection of vehicle 30 minutes prior to an injection of cocaine (10 mg/kg) and locomotor activity was recorded for 60 minutes to test for sensitized responding. To assess the inherent effects of inactivating IT neurons on aversive responses, rats were divided into treatment groups receiving either CNO or vehicle. Rats were given

access to a 15% sucrose solution for 30 minutes in a novel cage, followed by i.p. injections of CNO (5 mg/kg) or vehicle (5% DMSO in sterile water) and subcutaneous injections of 0.9% saline 15 minutes after i.p. injections. Conditioning occurred every other day for four total sessions. Sensitization and sucrose-drinking data were analyzed using two-way RM ANOVA in Graphpad Prism 8.2. All experimental protocols were approved by Seattle Children's Research Institute Institutional Care and Use Committee and conducted in accordance to National Institute of Health guidelines.

Results: Rats given i.p. injections of cocaine following IT inactivation produce significantly greater ambulations compared to vehicle treated groups and saline controls, in contrast to the effects previously observed in PT neurons, where chemogenetic inhibition reduced ambulations in early training sessions (Session 2: Treatment x Time: $F(177, 413) = 1.64$, $p<0.0001$). This effect, however, did not persist in later training sessions (6-7) nor to challenge cocaine injections following two weeks of withdrawal. During challenge sessions without CNO, cocaine injections produced significantly higher ambulations in both CNO and vehicle groups, but did not significantly differ between treatment conditions. For the sucrose experiment, both groups developed a sucrose preference and there were no differences in volume of sucrose solution consumed between vehicle and CNO groups (Main effect of session: $F(2.61, 23.46 = 157.6$, $p<0.0001$)).

Conclusions: Inactivation of IT neurons in ACC heightens locomotor activity during early exposure to cocaine, in contrast to PT inactivation. Furthermore, IT neuronal inhibition is not in of itself aversive, suggesting that alterations of a cocaine-induced conditioned taste aversion to sucrose was not just a result of loss of IT activity. Ongoing work will confirm these initial findings. Additionally, examining the role of these cell types in contingent cocaine administration and relapse behaviors will provide greater understanding of the role of these neurons in processing the motivational and effort-based facets of substance use.

Keywords: Cocaine Use Disorder, Corticostriatal Circuit, Cortical Neurons

Disclosure: Nothing to disclose.

W223

CRISPR/Cas9 Editing of Neuropeptide Receptor Signaling Reveals an Extended Amygdala Circuit Mechanism Modulating Alcohol Drinking, Anxiety, and Avoidance

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Background: Negative emotional states linked to alcohol addiction are thought to arise from detrimental neuroplasticity within limbic brain systems. In particular, the effects of stress on addiction are proposed to occur via long-lasting adaptations within complex neuronal networks of the hypothalamus and amygdala. These circuits encompass an enormous diversity of cell types that display specialized connectivity patterns and innumerable forms of communicative signaling.

Specifically, lateral hypothalamus (LH) neurons containing the neuropeptide Hypocretin (Hcrt; orexin) profoundly influence arousal (wakefulness) and motivated behavior. We previously identified connectivity between Hcrt-LH neurons and "extended amygdala" neurons of the bed nuclei of stria terminalis (BNST) containing the prototypical stress neuropeptide corticotropin-releasing factor (Crf). Those studies characterized Hcrt-LH and Crf-BNST neurons as tightly coupled nodes in a stress-promoting circuit, suggesting their involvement in addiction.

Methods: Here we investigated Hcrt-LH neurocircuits in free-choice binge alcohol drinking and addiction-related behaviors by performing genetically defined physiological monitoring, optical manipulations, and molecular perturbations in neurons of freely-behaving mice.

Results: First, we identified Hcrt-LH activation during alcohol withdrawal-enhanced anxiety behavior, and used in vivo $Ca2+$ recordings to reveal withdrawal-heightened sensitivity of Hcrt-LH neurons to aversive stimuli. We next uncovered the necessity of Hcrt for behavioral avoidance driven by Crf-BNST stimulation, and focused on BNST-projecting Hcrt-LH neurons with the hypothesis that BNST Hcrt receptors drive negative affective consequences and excessive alcohol drinking. We developed a CRISPR/Cas9 gene editing system to reveal that disruption of HcrtReceptor1 (hcrtr1) in Crf-BNST neurons dramatically reduced alcohol intake, anxiety, and avoidance behavior.

Conclusions: These studies advanced prior work by identifying the precise cells and mechanisms through which LH→BNST circuits mobilize physiological and behavioral changes promoting excessive alcohol consumption. We posit an essential role for Crf-BNST-HcrtR1 signaling that maintains alcohol addiction through negative emotional states and dysregulated hyperarousal. These outcomes have considerable implications for addressing challenges faced in developing effective strategies to treat addiction.

Keywords: BNST, Orexin, CRISPR

Disclosure: Nothing to disclose.

W224

SGK1 Activity in VTA Dopamine Neurons Regulates Drug Reward

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Background: Drugs of abuse are known to regulate activity of the mesolimbic dopamine (DA) system. Specifically, drug-induced changes in ventral tegmental area (VTA) cellular activity and gene regulation contribute to behavioral outputs associated with addiction. Our previous work has determined that serum- and glucocorticoid-inducible kinase 1 (SGK1) catalytic activity is increased by chronic, but not acute, administration of cocaine or morphine. Furthermore, we have shown that viral overexpression of SGK1 mutants in the VTA of adult mice produce behaviorally relevant effects on drug reward, assessed by cocaine conditioned place preference (CPP) and voluntary morphine intake using a two-bottle choice task. Specifically, intra-VTA infusion of a catalytically inactive SGK1 mutant (K127Q) significantly decreases cocaine CPP and morphine preference, suggesting that decreasing VTA SGK1 activity is sufficient to decrease drug reward. To more fully understand the role of VTA SGK1 in behaviors relevant to addiction, are now manipulating SGK1 expression in a cell type-specific manner to determine whether SGK1 activity in DA or GABA neurons drives the observed behavioral effects.

Methods: In order to determine whether VTA SGK1 activity mediates drug-related behavior in a cell type-specific manner, Cre-dependent AAV constructs (AAV-DIO) constructs were used to overexpress an SGK1 kinase-dead mutant (K127Q) or wild-type SGK1. Vectors were stereotaxically injected into the VTA of dopamine transporter (DAT)- or vesicular GABA transporter (VGAT)-Cre male mice using established coordinates and procedures and behavioral analysis began 2 - 3 weeks following surgery. Floxed SGK1 mice were crossed with DAT-Cre for dopaminergic KO studies. For CPP, mice underwent twice-daily conditioning for two days, receiving saline in one chamber and cocaine (12.5 mg/

kg) in the opposite chamber. A 20 min post-test was conducted the following day and time spent in the paired – unpaired chamber was calculated. To assess morphine consumption, we used a two-bottle choice paradigm. Following acclimation to bottles filled with just water, singly-housed mice were given bottles filled with 0.2% sucrose and 0.05 mg/ml morphine sulfate or 0.01 mg/ml quinine sulfate, which were measured daily for 4 days. For cocaine self-administration studies, mice underwent instrumental training for food reward for 7 days, followed by jugular catheter surgery and recovery, and then daily 2 hr cocaine sessions (0.5 mg/kg/infusion, FR1 schedule).

Results: Intra-VTA infusion of a catalytically inactive SGK1 mutant (K127Q) into DAT-Cre mice significantly decreased cocaine CPP (unpaired t-test, ($t(28)=3.4$, $p=0.002$) while this same manipulation in VGAT-Cre mice had no effect. This suggests that decreasing SGK1 activity in VTA DA, but not GABA, neurons is sufficient to alter cocaine reward. Moreover, we have completed preliminary cocaine self-administration studies and our data suggest that decreasing SGK1 activity in VTA DA neurons impairs acquisition of cocaine self-administration, but not operant sucrose response. We are currently assessing whether SGK1 activity in VTA DA cells similarly alters opioid-associated behaviors. Future studies look to determine a potential mechanism for these behavioral effects using ex vivo slice electrophysiology.

Conclusions: Our studies highlight the role of a novel protein, SGK1, in drug-associated behaviors. The current work seeks to identify the specific cells and circuits that are critical for SGK1-mediated effects on drug reward and intake. Altogether, this work will increase our understanding of the role of VTA SGK1 activity in drug-related behaviors, a necessary step in assessing the feasibility of SGK1 inhibition as a novel therapeutic avenue for addiction. SGK1 is a promising target given the limited knowledge of its biology in general and in the brain specifically. Despite sparse literature, our data suggest SGK1 could act as a key modulator of stimulus-induced changes in neuronal function.

Keywords: Ventral Tegmental Area (VTA), Cocaine, Dopamine

Disclosure: Nothing to disclose.

W225

Subpopulations of Nucleus Accumbens Astrocytes Regulate Cued Heroin Seeking Through Distinct Mechanisms

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Background: Projections from the prefrontal cortex to the nucleus accumbens critically regulate opioid relapse, and relapse initiated by heroin-associated cues requires transient postsynaptic potentiation in accumbens medium spiny neurons. We previously showed that heroin use induces an enduring reduction in synaptic proximity by astroglial processes that insulate synapses and recover synaptically released glutamate. Exposure to heroin-associated cues transiently increases synaptic insulation by astrocytes, a process that requires phosphorylation of ezrin, a protein selectively expressed in astroglial peripheral processes. This re-association serves to suppress active lever pressing during reinstatement, since ezrin knockdown enhances heroin seeking induced by cues. Along with synaptic proximity, the glutamate transporter GLT-1 on astroglial processes strongly contributes to the ability of astroglia to regulate synaptic activity and repeated administration of all addictive drugs examined to date (cocaine, heroin, nicotine, alcohol) reduces the amount and/or function of GLT-1. We sought to determine whether synaptic re-association by astrocytes serves to suppress lever pressing by restoring GLT-1 levels at the synapse.

Methods: Male Sprague Dawley rats were trained to self-administer heroin for 10 days. Control rats received passive infusions of saline. Active lever pressing was extinguished over 10-14 days prior to reinstatement of drug seeking induced by 15-min of exposure to heroin-associated cues. Astrocytes expressing a virally transduced membrane reporter were imaged using confocal microscopy and synaptic proximity of GLT-1 was quantified using co-localization between immuno-labeled GLT-1 and Synapsin I.

Results: We found that, consistent with previous studies, heroin use induced a down-regulation of GLT-1 and produced an enduring reduction in its synaptic proximity. Moreover, we found that the transient synaptic re-association of astroglial processes during 15-minutes of heroin seeking was associated with increased surface expression of GLT-1 on astrocytes. However, the expression was not directed toward synapses with increased astroglial insulation, since co-localization of immunoreactive GLT-1 and Synapsin I was not elevated by cue exposure and increased surface GLT-1 was observed on astrocytes with low synaptic proximity.

Conclusions: Our data support a dynamic role for astrocytes in modulating drug-seeking behavior and demonstrate that sub-populations of astrocytes within the nucleus accumbens may act in distinct ways to limit drug seeking initiated by drug-conditioned cues.

Keywords: Astrocytes, Heroin Self-Administration, GLT-1

Disclosure: Nothing to disclose.

W226

Similar Distribution Patterns of AMPA Receptors Between Alcohol Dependence and Amphetamine Dependence: The First In-Vivo PET Study

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Background: Evidence on physiological roles of AMPA receptors in substance dependence has been accumulated, which mainly derives from disease model animals and post-mortem brain tissues. Its clinical translation was limited due to lack of any tool to visualize AMPA receptors in living human brain. Here, we used the first positron emission tomography (PET) probe that specifically binds to AMPA receptors and successfully visualized these receptors in living human brain of patients with alcohol or amphetamine dependence.

Methods: We developed a novel PET probe for AMPARs. We radio-labelled a derivative of 4-[2-(phenylsulfonylamino)ethylthio]-2,6-difuluoro-phenoxyacetamide with 11C, named [11C]K-2. Male patients aged 30-49 with alcohol use disorder according to Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5) and alcohol dependence according to DSM Fourth Edition (DSM-IV), those with amphetamine use disorder (DSM-5) and amphetamine dependence (DSM-IV), and age-matched healthy individuals were recruited (registration number: UMIN000025132). They 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 Addiction Severity Index (ASI).

[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. 9 patients with alcohol dependence (age, 41.7 ±6.0 years; duration of illness, 6.9±4.2 years; duration of abstinence, 2.9±3.5 years; ASI Alcohol composite score, 0.20 ±0.11), 10 patients with amphetamine dependence (age, 41.4±5.9 years; duration of illness, 10.7±7.7 years; duration of abstinence, 2.9±3.5 years; ASI Drug composite score, 0.13±0.07), and 5 age-matched healthy individuals participated in this study.

Results: According to voxel-wise analysis, standardized uptake value ratio (SUVR)30-50 min with the white matter as a reference was lower in the anterior cingulate cortex, insular cortex, occipital lobe, and basal ganglia in patients with alcohol dependence, compared to healthy individuals (FDR $p<0.05$). In patients with amphetamine dependence, the voxel-wise analysis found lower SUVR30-50 min in the anterior cingulate cortex, insular cortex, amygdala, and occipital lobe than healthy individuals (FDR $p<0.05$). Thus, both disorders showed lower SUVR30-50 min in the same regions, including anterior cingulate cortex, insular cortex, and occipital lobe, than healthy individuals.

Conclusions: [11C]K-2 has revealed lower AMPA receptor availability in some specific regions both in alcohol dependence and amphetamine dependence. Thus, these alterations may underlie the pathophysiology of these disorders and could lead to the elucidation of the molecular mechanisms of substance dependence.

Keywords: AMPA, AMPA Receptors, Alcohol Dependence, Amphetamine, PET Imaging

Disclosure: Nothing to disclose.

W227

Dynamics of Striatal Acetylcholine Release Related to Synaptic Plasticity and Actions of Abused Drugs

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Background: Acetylcholine (ACh) in the striatum has important roles in the induction of synaptic plasticity, as well as in action control, decision making and effects of abused substances. The large cholinergic interneurons (CINS) that display tonic activity both in vivo and in brain slices are the predominant source of striatal ACh. While we know a great deal about the physiology of these neurons and their roles in behavior, little is known about real-time changes in extracellular ACh associated with synaptic plasticity induction, behavioral changes or effects of drugs of abuse.

Methods: To address these questions in dorsal striatal slices and the in vivo striatum we are performing photometric recordings using the newly-developed intensity-based ACh sensing fluorescent reporter (iAChSnFR), a periplasmic binding protein-based genetically-encoded sensor. The sensor is expressed by injection of an AAV-based construct in the dorsal striatum, and experiments are performed 3-12 months after injection.

Results: In dorsomedial striatum slices, single pulse electrical stimuli (200 microsec to 1 msec duration) elicit increases in ACh detected with the sensor that persist for over 20 sec. These increases are prevented by tetrodotoxin, reducing extracellular calcium and blocking the vesicular ACh transporter. Lesioning CINs using Cre recombinase-based caspase3 expression greatly reduces the amplitude and duration of the stimulus-induced ACh increases. Responses to intrastriatal micropressure application of ACh are much shorter in duration, as are responses to electrical stimulation measured in motor and prefrontal cortex. In vivo fiber photometry experiments have revealed a number of fluctuations in striatal ACh levels during behavior. Pharmacological experiments showed that ACh levels are enhanced by peripheral cocaine treatment but inhibited by peripheral injection of ethanol.

Conclusions: Our findings in striatal slices indicate that brief electrical stimulation activates a network of striatal cholinergic interneurons. Our in vivo findings indicate that drugs of abuse, including alcohol, have prominent effects on striatal ACh levels that likely affect striatal circuit function. We are currently examining changes in striatal ACh during behaviors that are thought to involve CIN activity.

Keywords: Alcohol, Cocaine, Basal Ganglia, Photometry, Interneuron

Disclosure: Elsevier Inc., Honoraria

W228

Using Human Brain Organoids to Model Prenatal Opioid Exposure

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Background: The misuse of opioids has reached epidemic proportions over the last decade, with over 1.5 million people in the U.S. suffering from substance use disorders related to prescription opioid pain relievers. This increase in opioid misuse affects all demographics of society, including women of child-bearing age, which has led to a rise in opioid use during pregnancy. Infants exposed to opioids in utero show an increased risk for neurological and behavioral deficits, including neonatal abstinence syndrome, a cluster of withdrawal symptoms that include tremors, diarrhea, fever, irritability, and seizures. Currently, opioid use disorder in pregnant women is treated with long-acting opioid agonists, including buprenorphine. Although buprenorphine is widely considered safe for the developing fetus, few long-term studies have been conducted. The goal of the current experiments was to examine the developmental consequences of both opioid use (oxycodone) or buprenorphine treatment on the developing human brain.

Methods: While prenatal opioid exposure can be modeled in rodents, species differences limit our ability to recapitulate all aspects of human nervous system development. Therefore, we used human induced pluripotent stem cells to grow 3D brain cell cultures that model the cellular diversity, connectivity, and activity of the developing human brain. Specifically, we used patterning factors to generate dorsal forebrain progenitors (cortical organoids) or ventral forebrain progenitors (subcortical spheroids). RNA Sequencing and immunohistochemistry were used to confirm cortical or subcortical specificity. To then measure the effect of buprenorphine exposure on neuronal network properties, cortical spheroids were treated with oxycodone (20ng/ml) or buprenorphine (2 ng/ml) at various time points during development and calcium imaging or multielectrode arrays were used to record neuronal activity. qPCR or immunohistochemistry were used to measure markers of cortical and subcortical development. Finally, to model the developmental process by which inhibitory interneurons migrate tangentially into the cerebral cortex, cortical and subcortical spheroids were fused in the presence of buprenorphine. Interneuron progenitors were labelled with a GFP tag and migration was monitored for 24 hours using a confocal microscope.

Results: Preliminary data suggest that buprenorphine produces more dramatic and robust effects, which are likely attributable to the NOP receptor as opioid receptors are sparsely expressed in the organoids (and the developing human cortex).

Conclusions: Together, our results will provide new information about the developmental consequences of buprenorphine exposure during development and may ultimately lead to new therapeutic strategies to treat infants exposed to opioids in utero.

Keywords: Brain Organoids, Opioids, GABAergic Interneurons, Brain Development

Disclosure: Nothing to disclose.

W229

Effects of Alcohol Use Disorder on Splicing in Brain and Liver

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Background: Alcohol use disorder (AUD) is a widespread disease, but molecular pathogenesis of AUD is incompletely understood. Current medicines such as acamprosate and naltrexone are relatively inefficient. That these medications have a single target suggests that AUD may trigger broader changes in molecular circuits. Splicing is a nuclear mechanism of RNA processing responsible for removal of introns and transcriptomic diversity. Splicing is mediated by the spliceosome which consists of small nuclear RNAs (snRNAs) and splicing factors. We tested the hypothesis that AUD causes genome-wide posttranscriptional changes due to changes in splicing.

Methods: RNA sequencing files were generated from the brain samples provided by the Australian Brain Bank. Superior frontal cortex, basolateral amygdala, central nucleus of amygdala, and nucleus accumbens were studied. Alignment was performed with the STAR aligner (v2.5.2a) against the hg19 human genome. Raw counts were processed by edgeR in Bioconductor and normalized using CPM values. Resulting bam files from the STAR alignment were indexed with samtools for use by rMATS for the analysis of missplicing events. RNA sequencing files were generated from liver biopsies from patients with different stages of alcoholic liver disease: early alcoholic steatohepatitis (eASH), non-severe alcoholic hepatitis (nsAH), and severe alcoholic hepatitis (sAH); furthermore, explants were collected from patients who underwent liver transplantation due to liver failure (exAH). The same computational pipeline was used to analyze data from the liver. Expression of small nuclear RNAs (snRNAs) and splicing factors in brain and liver was measured by real-time PCR and/or determined based on RNA sequencing data.

Results: We observed significant alcohol-induced changes in gene expression in superior frontal cortex, basolateral amygdala, central nucleus of amygdala, and nucleus accumbens (20-100 genes per brain region). Unexpectedly, we found that alcohol caused much more severe changes in hepatic transcriptome in all alcohol-related liver diseases: ~4,900 were altered in eASH, ~9,100 – in nsAH, 14,100 – in sAH, and ~14,300 – in exAH. In the brain, alcohol caused hundreds of missplicing events, with exon slipping (ES) being most common and mutually exclusive exons (MEE) – second most common. Likewise, we observed hundreds of missplicing events in the liver, with MEE being most common event and ES – second most common event. To determine the mechanism by which alcohol perturbs splicing, we subjected rats to alcohol vapor for 7 weeks and interrogated the spliceosome by measuring snRNAs and splicing factors. In the brain, we found that alcohol decreases the expression of splicing factor 1 (SF1), a key spliceosomal protein responsible for the recognition of the branch point of introns. In the liver, in contrast, expression of splicing factors was not affected, while expression of such snRNAs as snU1, snU2, snU6atac, and snU11 was drastically decreased.

Conclusions: Alcohol causes genome-wide changes in gene expression and splicing in brain and liver. Alcohol-induced missplicing events appear to be different between these two organs, likely due to SF1 being affected in the brain and snRNAs affected in the liver.

Keywords: Alcohol Use Disorder, Splicing, Transcriptome

Disclosure: Nothing to disclose.

W230

N-Alkyl Chain Length is a Critical Determinant for the Pharmacological Activity of Cumyl-Pinaca and Related Synthetic Cannabinoids

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Background: Synthetic cannabinoids (SCs), often known as "spice" or "K2", are an evolving class of abused drugs that pose public health risks. SCs exert their psychoactive effects by stimulating cannabinoid-1 receptors (CB1) in the brain, yet few studies have examined structure-activity relationships for newly-emerging compounds.

Methods: Here, we investigated the pharmacology of SCs which possess a fixed cumyl 'head group' but variable N-alkyl chain length in the 'tail group'. CUMYL-PINACA, which contains a 5-carbon pentyl chain, served as a reference compound. In vitro CB1 binding was assessed by displacement of [3H]SR141716 in mouse brain membranes. In vivo effects of subcutaneously (sc) administered drugs were examined in male C57Bl/6J mice bearing surgically-implanted telemetric temperature transponders. Mice were subjected to a 'triad' test battery which assessed temperature, catalepsy, and analgesia every 30 min post-injection for 2 h. Temperature was measured via a handheld receiver. Catalepsy was measured using the bar test, with a cut off of 60 sec, whereas analgesia was measured using a hot plate set at 52oC, with a cut off of 45 sec.

Results: CUMYL-PINACA was the most potent inhibitor of CB1 binding with an IC50=1.8 nM. Reducing the N-alkyl chain to a 3-carbon propyl markedly decreased binding potency (IC50=44.8 nM), as did increasing the chain to a 7-carbon heptyl (IC50=124.0 nM). THC, the active ingredient in marijuana, weakly inhibited CB1 binding with an IC50=54.2 nM. Consistent with in vitro findings, CUMYL-PINACA was the most potent compound to induce cannabinoid-like effects in the triad test battery (EC50s ranging from 0.06-0.10 mg/kg, sc). Importantly, all cumyl compounds induced hypothermic, cataleptic, and analgesic effects with a rank order of potency that matched IC50s for inhibition of CB1 binding. THC exhibited EC50s in the triad test ranging from 12.7-30.3 mg/kg, sc.

Conclusions: Overall, our data reveal that CUMYL-PINACA and related compounds are more potent than THC. Furthermore, N-alkyl chain length is a critical determinant for the pharmacological effects of cumyl-containing synthetic cannabinoids, with a 5-carbon pentyl chain conferring optimal activity.

Keywords: Cannabinoid, Hypothermia, Catalepsy, Analgesia, Receptor Binding

Disclosure: Nothing to disclose.

W231

Silent Synapses Dictate Cocaine Memory Destabilization and Reconsolidation

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Background: Cocaine-associated memories are persistent, but, upon retrieval, become temporarily destabilized and vulnerable to disruptions, followed by reconsolidation. To explore the synaptic underpinnings for these memory dynamics, we studied AMPA receptor (AMPAR)-silent excitatory synapses, which are generated in the nucleus accumbens by cocaine self-administration, and subsequently mature after prolonged withdrawal by recruiting calcium-permeable AMPARs (CP-AMPARs), echoing acquisition and consolidation of cocaine memories

Methods: To established cocaine-associated memories, male rats were trained to self-administer cocaine with light cues paired with each cocaine infusion. After 45 days of withdrawal we used slice electrophysiology and confocal microscopy to determine how a brief re-exposure to cocaine-associated cues altered the functional state (silent vs mature) of excitatory synapses on medium spiny neurons (MSN) in the nucleus accumbens (NAc). In addition, we utilized interference peptides and photoactivatable Rac1 methods in vivo to determine how AMPAR trafficking and Rac1 signaling mediated cue re-exposure-induced alterations in NAc synaptic state. Lastly, we utilized cue-induce reinstatement of cocaine seeking to determine how changes in the synaptic state regulated the expression of cocaine-associated memories.

Results: We show that, upon cue-induced memory reactivation after prolonged withdrawal, the matured silent synapses become AMPAR-silent again through internalization of CP-AMPARs, followed by re-maturation ~6 hr later, defining the onset and termination of a destabilization window of cocaine memories. Furthermore, these synaptic dynamics are controlled by Rac1, with decreased and increased levels of active Rac1 destabilizing and stabilizing cocaine-generated synapses to control the onset and termination of the memory destabilization period. Lastly, preventing the re-maturation of cocaine-generated synapses within the destabilization window by manipulating either AMPAR trafficking or Rac1 activity compromised cue-induced cocaine seeking.

Conclusions: These results demonstrate cocaine-generated synapses constitute a discrete synaptic ensemble, whose functional state dictates the dynamics of cocaine-associated memories. Furthermore, they highlight they highly dynamic nature of synaptic function underlying complex learned behaviors.

Keywords: Silent Synapses, Cocaine, Memory Reconsolidation, Nucleus Accumbens (NAA)

Disclosure: Nothing to disclose.

W232

Short- and Long-Term Effects of Adolescent Alcohol and Cannabis Co-Use on Reward, Impulsivity and Cognition

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Background: Cannabis and alcohol co-use is prevalent in adolescence, but its long-term effects on adult behaviour and neural function remain largely unexplored. Therefore, the aim of this study is to investigate the long-term effects of adolescent alcohol and Δ9-tetrahydrocannabinol (THC) co-exposure on behaviour. We hypothesize that co-exposure will produce more pronounced behavioral and cognitive deficits in adulthood compared to either drug exposure alone.

Methods: Male Sprague Dawley (n = 24) rats were divided into 4 groups (THC+Alcohol, THC+Water, Vehicle+Alcohol, Vehicle +Water). Rats received vaporized THC (10 mg/pad) or vehicle every other day and had continuous access to 10% ethanol in a two-bottle choice design during adolescence (post-natal day 28-42). Alcohol intake was measured during the exposure period to assess the acute effects of THC on alcohol consumption. In adulthood, a battery of behavioural tests (i.e., novel object preference, elevated plus maze, delay discounting and two-bottle choice test) was performed.

Results: Adolescent rats showed higher alcohol preference and consumption on days in which they were not exposed to THC vapour ($p<0.05$, main effect of day and THC group). In adulthood, rats with alcohol-only access showed short-term memory deficits ($p<0.05$, main effect of delay, alcohol-only not significantly different from DR=0) while those exposed to both drugs showed potential long-term memory deficits. No differences in anxiety-like behaviour were found. In the delay discounting task, there was a main effect of THC with the THC-only and THC and alcohol groups exhibiting increased impulsive-like behaviour (preference for smaller non-delayed reward; $p<0.05$, main effect of THC group). Finally, in the two-bottle choice, there was a main effect of alcohol with the alcohol-only group showing significantly decreased alcohol preference ($p<0.05$, main effect of alcohol group).

Conclusions: These results indicate that alcohol and cannabis use in adolescent can have lasting effects on adult behaviour, and cannabis use may also modulate alcohol drinking during adolescence. Future studies will uncover the causal mechanisms underlying these behavioral consequences.

Keywords: Drug Addiction, Adolescent Alcohol Use, Cannabis Use, Vapor

Disclosure: Nothing to disclose.

W233

DNA Methylation Biosignature in Blood Predicts Alcohol Consumption in Two Distinct Populations

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Background: The process of diagnosing of hazardous alcohol drinking (HAD) is based on self-report and thereby vulnerable to bias. There has been interest in developing epigenetic biomarkers for HAD that might complement clinical assessment in the diagnostic process. Alcohol consumption has been previously linked to alteration of DNA methylation (DNAm) in the human methylome. In this study, we aimed to select DNAm signatures in blood to predict HAD from two demographically and clinically distinct populations (Ntotal =1,530). We compared the utilities of phosphatidylethanol (PEth)-associated DNAm signatures and self-report alcohol consumption-associated DNAm signatures on the prediction of HAD.

Methods: We first conducted an epigenome-wide association study (EWAS) for, PEth, an objective measure of alcohol consumption, and for self-report alcohol consumption separately in Cohort 1. In Cohort 1, serum PEth was measured from the same blood draw for DNA methylation profiling. The self-report alcohol

consumption was measured by using Alcohol Use Disorders Identification Test-Consumption (AUDIT-C) in the same individuals of Cohort 1. HAD was defined as PEth >=20ng/ml or AUDIT-C>=4 in the cohort 1. In Cohort 2, only self-report 10-item AUDIT was measured and HAD was defined and AUDIT>=8 for men and >=7 for women. A polygenetic methylation score was constructed by the epigenome-wide significant (EWS) CpG sites to test the correlation between collective effects of DNA methylation and alcohol consumption. In the second stage, we applied a newly developed regularization machine learning method, Elastic Net Regularization (ENR), to select CpGs from EWAS on PEth and on AUDIT-C for separately predicting HAD. The pre-selected CpG features from EWAS in Cohort 1 were optimized to predict HAD defined in Cohort 2 to avoid overfitting. Area Under Curve (AUC) from Receiver operating characteristic analysis and accuracy were used to evaluate the performance of the model. Finally, we compared the performances of the same panel of CpG sites to predict AUDIT-C and Alcohol Use Disorder (AUD).

Results: We identified EWS 102 CpGs on PEth (False Discovery Rate < 0.05), including 32 CpGs previously associated with alcohol consumption or alcohol use disorder. In contrast, we found no CpG site reached EWS for the self-report alcohol consumption. The polygenic methylation score from PEth-associated CpG sites was highly correlated with self-reported alcohol consumption ($r = 0.40$, $p < 2.00E-16$) and explained 10.02% variations of the variance in of HAD in an independent sample, Cohort 2. Using a machine learning ENR approach, we selected a subset of 130 CpGs that showed the best performance to predict full 10-time AUDIT in the training set of Cohort 2 with AUC 91.31% and moderate prediction of full AUDIT with 70.6% AUC in the testing set of Cohort 2. In contrast, the AUC from self-report-associated CpGs predicting on HAD was 70.1% in the training set and only 57% in the testing sample.

Conclusions: Our results demonstrate that the objective measure for alcohol consumption is a more informative phenotype than self-reported data in revealing peripheral epigenetic mechanisms of alcohol consumption. The DNAm signature associated with PEth shows greater utility on prediction hazardous alcohol drinking in comparison with self-report phenotype. These findings suggest that DNA methylation in blood is a robust biomarker for alcohol consumption.

Keywords: DNA Methylation, Alcohol Consumption, Machine Learning Classification, Alcohol Epigenetic Marks

Disclosure: Nothing to disclose.

W234

The Effects of Ovarian Hormones on Heroin Intake in Ovariectomized and Intact Female Rats

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Background: Relatively few studies have examined the effects of ovarian hormones on opioid intake, and the existing studies have reported equivocal results. The purpose of this study was to examine the effects of chronic administration of estradiol, progesterone, and their combination on heroin intake in ovariectomized female rats (Experiment 1) and to determine whether chronic administration of an ovarian hormone could serve as a potential pharmacotherapy to reduce opioid intake in intact female rats (Experiment 2).

Methods: Ovariectomized ($n = 85$; Experiment 1) and intact ($n = 55$; Experiment 2) female rats were surgically implanted with intravenous catheters, trained to self-administer heroin on a fixed ratio (FR1) schedule of reinforcement, and chronically administered ovarian hormones using a between-subjects design. Once responding stabilized, dose-effect curves for heroin (Experiment 1) or heroin and the synthetic opioid remifentanil (Experiment 2) were determined. Area under the curve (AUC) estimates were determined for each drug, and ANOVA were used to detect between-group differences in both the dose-response and AUC data.

Results: In Experiment 1, chronic estradiol significantly decreased heroin intake relative to chronic progesterone in ovariectomized rats. In Experiment 2, chronic estradiol significantly decreased both heroin and remifentanil intake relative to vehicle control in intact female rats.

Conclusions: Collectively, these data indicate that the effects of ovarian hormones on heroin intake differ from that of other drugs (e.g., stimulants) and that estrogen-based pharmacotherapies may decrease opioid intake in women with opioid use disorder.

Keywords: Intravenous Drug Self-Administration, Estradiol, Progesterone, Heroin, Remifentanil

Disclosure: Nothing to disclose.

W235

Inhibitory and Reward Activation Subtypes Associated With Risk for and Protection Against Substance Use: A Latent Profile Analysis of Brain Imaging Data

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Background: Dual-systems models of brain development posit that the earlier maturation of the reward system in relation to the cognitive control system contributes to risk behaviors in youth; and yet, these models often fail to account for within-age group heterogeneity in both brain function and substance use behaviors. Latent profile analysis (LPA) is a person-centered approach used to identify heterogeneous subgroups but has never been applied to brain imaging data. The present study identified latent classes of youth based on brain activation during Go/No-Go (GNG; inhibitory control) and Monetary Incentive Delay (MID; reward anticipation) tasks and then examined subgroup predictors in order to better understand individual differences in both brain circuitry and substance use.

Methods: One hundred forty-five right-handed participants (18-21 years old, M=19.80 (1.22), 40.0% female, 73.8% with parental alcohol use disorder (FH+)) were recruited from the Michigan Longitudinal Study. Self-report behavioral measures were cumulative substance use (alcohol: drink volume; marijuana: no, low/moderate, high use; cigarette: no, low/moderate, high use) up to the date of the scan and externalizing behavior from 9-14 years old. LPA was conducted using beta weights from regions of interest identified through whole-brain blood oxygen-level dependent (BOLD) activation during GNG (correct inhibition vs. go contrast) and MID (reward anticipation vs. neutral contrast) tasks. Logistic regression models were then used to examine predictors of class membership.

Results: Eight regions from the GNG and seven from the MID displayed significant task activation. Using BOLD activation from these regions, the four-class model had the best fit (BIC:5396.71, Entropy:91.90): (1) low inhibition/moderate reward (39.7%); (2) moderate inhibition/low reward (22.7%); (3) moderate inhibition/high reward (25.2%); and (4) high inhibition/high reward (12.4%). To account for potential multicollinearity in substance use, logistic regression models were conducted separately by substance use

type. In the alcohol-only model, Class 2 was older (OR=1.58, p<0.05), less likely to have parental AUD (OR=0.35, p<0.05), and less likely to use alcohol (OR=0.84, p<0.05); Class 4 was younger (OR=0.56, p<0.05) and had higher alcohol use (OR=1.27, p<0.05). In the marijuana-only model, Class 2 was older (OR=1.44, p<0.05) and had lower rates of high marijuana use (OR=0.32, p = 0.05); Class 3 had significantly less low/moderate use (OR=0.28, p<0.05); Class 4 was younger (OR=0.57, p<0.05) and more likely to have low/moderate (OR=6.14, p<0.05) and high (OR=9.46, p < 0.01) levels of use. In the cigarette-only model, there were no class differences in cigarette use but Class 2 was less likely to have parental AUD (OR=0.36, p<0.05). There were no significant differences in any predictors between Class 1 and all other classes across models.

Conclusions: Findings from the present study highlight the complexity of studying brain-behavior associations and provide evidence that heterogeneity exists in brain function associated with inhibitory control and reward responsivity, even among a fairly homogenous group of similarly-aged youth. A better understanding of how inhibitory control and reward circuitry interact may help uncover individual differences in neural function as a risk or protective mechanism underlying substance use.

Keywords: Inhibitory Control, Reward Functioning, Latent Class Analysis, Substance Abuse

Disclosure: Nothing to disclose.

W236

Family History of Alcohol Use Problems and Reward-Related Activation in the ABCD Study

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Background: The offspring of individuals with an alcohol use disorder are more likely to develop an alcohol or other substance use disorder themselves. The neurobiological correlates of this elevated risk are not well understood, however. Reward system functioning has been identified as an important target in the study of neurobiological risk due to its role in incentive processing and the reinforcing properties of drugs of abuse. Studies are mixed, however, with regard to the association between family history and reward system functioning. Here we utilize a large, multi-site imaging dataset of 9 and 10 year-olds to investigate whether children with a family history of alcohol use problems (FH +) demonstrate a different pattern of reward-system activation than family history negative (FH-) children. We also test whether reward-system activation is associated with experimentation with alcohol.

Methods: Participants were from the Adolescent Brain Cognitive Development (ABCD) Study Release 2.0 (baseline data: 9–10 years old) who had high-quality imaging data (N = 7,164). 14.9% (n = 1,065) were FH+. The family history positive group was significantly more likely to be female (p = .038), non-white (p = .016), and have a household income of less than $100,000 (p<.001). We used a monetary incentive delay task to measure brain functioning during reward anticipation and feedback. We used generalized linear mixed models with the following variables to test the association between family history of alcohol use problems and reward system functioning: FH+/- as the independent variable; 22 reward-related anatomical regions of interest as dependent variables (nucleus accumbens and prefrontal and limbic cortical regions); site and family as random effects; and sex, race/ethnicity, household income, and mean framewise displacement as fixed-effect covariates. To assess the association between reward-system activation and experience with alcohol, we used the presence/absence of sipping that occurred not as part of a religious ceremony ("non-religious sipping"), as rates of full-blown substance use in this sample were very low. 17.0% (n = 1218) of the present sample reported non-religious sipping.

Results: Family history was not significantly associated with left or right nucleus accumbens activation during reward anticipation or feedback. There was, however, an association between family history groups and the right entorhinal cortex during reward anticipation (p = .002). Non-religious sipping was not associated with reward-system activation in left or right nucleus accumbens or right entorhinal cortex.

Conclusions: In this large, nationally-representative dataset, family history of alcohol use problems was not associated with nucleus accumbens activation during a monetary incentive delay task. In contrast, family history was significantly associated with right entorhinal cortex activation. The entorhinal cortex is part of the limbic system and has projections to the ventral striatum, and as such it has been identified as part of the circuitry that contributes to addictive behavior. It has been associated with negative urgency, which is defined as acting in rash, impulsive ways when experiencing strong negative emotions. Post-hoc analyses demonstrated a significant association between right entorhinal cortex activation and negative urgency (p = .015), as well as a significant association between negative urgency and non-religious sipping (p<.001). These results suggest the presence of a vulnerability pathway, whereby family history of alcohol use problems may impact entorhinal cortex activation during reward, which in turn may impact levels of negative urgency, which may then impact early experimentation with alcohol. Longitudinal data from ABCD will allow for the mapping of these associations over time.

Keywords: Functional MRI (fMRI), ABCD Study, Monetary Reward, Alcohol

Disclosure: Nothing to disclose.

W237

Proteomics Analysis Suggests Demyelination as a Mechanism of Cocaine-Induced Neurotoxicity: Postmortem Brain Analyses of Cocaine Use Disorder

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Background: Cocaine dependence continues to be a significant public health problem with more overdose deaths in the US in 2015-2018 than any other period since 2000, a ~70% increase in new users from 2013-2018, and no FDA-approved options to facilitate abstinence or prevent relapse. Chronic cocaine use leads to changes in brain structure and function by causing alterations in brain proteins. However, the assessment of protein modifications in human brain has been limited by the difficulty in obtaining brain tissue. Here we present preliminary data in our investigation of brain proteome alterations in the dorsolateral prefrontal cortex (BA9) of subjects with cocaine use disorder (CUD, n = 10) who died of a cocaine overdose, and controls (n = 12) from the UTHealth brain collection.

Methods: Approximately 50 mg of tissue was lysed in RIPA buffer and extracted proteins were reduced, alkylated, delipidated and digested. Nanoflow liquid chromatography-tandem mass spectrometry (NanoLC MS/MS) was performed using a nano-LC chromatography system, coupled on-line to a mass spectrometer through a nanospray ion source. LC-MS/MS data were

acquired using XCalibur and the raw mass spectrometry data files were processed using MaxQuant. Peptide identifications were accepted if they could be established at greater than 95.0% probability. MS/MS spectra were searched against the Swiss-Prot human database. In total, 4584 unique proteins were identified in all the samples. Data was normalized and differential expression analyses were performed in CUD vs. controls using the protein-wise linear models combined with empirical Bayes statistics implemented in the DEP (Differential Enrichment analysis of Proteomics data) R package and the limma R package.

Results: We identified 49 proteins differentially expressed between the CUD and control groups (p.val < 0.05 and |logFC| > log2(1.5). Specifically, myelin oligodendrocyte glycoprotein (MOG), myelin associated glycoprotein (MAG) and and immunoglobulin domain-containing Nogo receptor-interacting protein 1 (LINGO-1) were significantly decreased in CUD subjects compared to controls. Pathway enrichment analyses also identified protein localization to synapse and myelination pathways to be enriched in CUD.

Conclusions: Our results suggest demyelination as a mechanism of neurotoxicity induced by cocaine. These results could shed light on the neurobiological mechanisms of CUD and could lead to development of novel therapeutic approaches to minimize damage induced by cocaine abuse.

Keywords: Cocaine Addiction, Postmortem Brain Tissue, Proteomics, Myelination

Disclosure: Nothing to disclose.

W238

Synaptotagmin 1 Downregulation in the Medial Prefrontal Cortex is Involved in Alcohol-Related Behaviors

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Background: Alcohol use disorder is characterized by broad and persistent changes in gene expression. Our previous results suggest that DNA methylation may drive part of the alcohol-induced neuroadaptations that occur in the brain and more specifically the medial prefrontal cortex (mPFC). We previously found that inhibition of the DNA methyltransferase DNMT1 in the mPFC, inhibited escalation in alcohol intake. Moreover, DNMT1 inhibition not only restored basal levels of DNA methylation but also prevented alcohol-induced mRNA expression changes of genes that play a major role in neurotransmission. Among those genes, we found that synaptotagmin 1 (SYT1) was down-regulated in the mPFC of alcohol post-dependent compared to non-dependent rats. SYT1 is a Ca2+ sensor in the membrane of the pre-synaptic axon terminal. It interacts with the synaptic protein of the SNARE complex to induce neurotransmitter exocytosis. Given the important role of SYT1 in neurotransmitter release, we hypothesized that SYT1 may participate to the dysregulation of the mPFC function observed in alcohol use disorder.

Methods: To functionally assess the role of SYT1 in alcohol-related behaviors, we injected an adeno-associated viral (AAV) vector expressing a shRNA to Syt1 in the mPFC. Alcohol intake was measured using operant self-administration under an FR2 schedule while aversion-resistant behavior was assessed by adding increasing concentration of quinine (10, 25, 50 and 75 mg/L).

Results: We found that Syt1 KD in the mPFC significantly increased alcohol self-administration. Repeated measure ANOVA showed a significant main effect of group ($F(1,72) = 21$; $p = 0.0006$) suggesting a persistent effect as Syt1-dependent increased alcohol self-administration lasted for at least 9 days. Furthermore, our analysis also indicated that Syt1 KD rats increased their alcohol consumption compared to their baseline which was measured by the average reward over a week of self-administration prior to surgery (Two way ANOVA: $F(1,14)=9.8$; $p = 0.007$; Newman-Keuls post-hoc: $p = 0.003$). We found that down-regulation of Syt1 in the mPFC also increased motivation to consume alcohol as indicated by an increase in breakpoint for alcohol (one way ANOVA: main effect of group scrambled vs. Syt1 KD; $F(1,13)=6.1$; $p = 0.02$). Rats with downregulation of Syt1 also showed compulsive-like behaviors as indicated by a greater tolerance to quinine adulteration when increasing concentration of quinine were added to the alcohol solution (repeated measure ANOVA: significant main effect of group (scrambled vs. Syt1 KD; $F(1,13)=0.3$; $P=0.02$).

Conclusions: Altogether, these findings demonstrated that syt1 downregulation is sufficient to mimic alcohol-related behaviors observed in alcohol post-dependent rats, suggesting that SYT1 may play an important role in motivation and compulsivity, two main features of alcohol addiction.

Keywords: Alcohol dependence, mPFC, Synaptotagmin 1

Disclosure: Nothing to disclose.

W239

Females are More Sensitive Than Males to Enhanced Sensitivity to Dopaminergic Drugs Caused by Eating a High Fat Diet

Abstract not included.

W240

Multimodal Signatures of Externalizing Behaviors

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Background: Externalizing problems often precede the initiation of alcohol use and are associated with a more severe and persistent course of alcohol use disorder. An externalizing risk pathway is characterized by problems with self-regulation (e.g. aggression, impulsivity), with the primary deficit being one of behavioral undercontrol or disinhibition. There is evidence that individual differences in traits underlying externalizing problems differentiate substance abusing subgroups on the basis of age of onset, patterns of use, and susceptibility to co-morbid psychopathology; however, neural underpinnings of these traits may represent more robust predictors of distinct trajectories of alcohol and other substance use problems than the traits themselves. Furthermore, neurodevelopmental processes that contribute to substance abuse are unlikely to affect a single region or modality. The goal of the current study is to identify a multimodal signature of externalizing behavior which may aid in prediction of risk for problem alcohol use.

Methods: Participants were 158 8–26 year olds (mean age 18.2 ±4.8 yrs; 40% female; 65% had parent with substance use disorder) recruited from the Michigan Longitudinal Study, a prospective study of families with high levels of parental alcohol use disorder and contrast families without alcohol use disorder. Externalizing behaviors were measured using the Achenbach scales (Child Behavior Checklist at ages 8–10; Youth Self Report at ages 11–17; Adult Self Report at ages 18+). Contrasts from Go/No-Go task data (false alarm vs. correct reject), resting state connectivity data, and gray matter tissue probability maps from high resolution

T1-weighted structural data were entered into a predictive model called Brain Basis Set (BBS). BBS is a validated multivariate predictive method that uses dimensionality reduction in order to produce a basis set of components to make phenotypic predictions. Principal components analysis was conducted on data from each modality and the top 15 components (based on eigenvalues) for each modality were retained for a total of 45 components. Prediction of the externalizing phenotype was assessed using a 10-fold cross validation procedure. In each fold of the cross-validation, data from one tenth of the sample served as the held-out test dataset and data from the remaining sample served as the training dataset. On each fold, the training fold was used to learn new principle component analysis components and then estimate regression coefficients for BBS modeling. Predictions for held out phenotypes were then made, and correlations between actual phenotype and predicted phenotype on the held out fold were averaged across the ten folds. The significance of cross-validation-based correlations was assessed with 10,000 nonparametric permutation tests to address elevated variance of estimates.

Results: BBS modeling resulted in significant multimodal prediction of externalizing scores. The correlation between actual versus predicted externalizing scores, averaging across folds of the cross validation, was 0.29 (permutation p-value = 0.003). Consensus component maps were constructed to visualize overall patterns across the BBS predictive model. In task fMRI maps, higher externalizing scores were associated with reduced activation during correct inhibition in medial prefrontal, inferior frontal, and parietal cortices associated. In structural maps, higher externalizing was associated with less gray matter in the anterior and subgenual cingulate, and more gray matter in the striatum associated. In resting connectome maps, higher externalizing was associated with increased connectivity between the cingulo-opercular network and the fronto-parietal, salience, auditory and subcortical networks associated. We calculated mean T-values averaged over folds of the cross validation for each modality as an estimate of relative contribution of each modality to the predictive model. Resting connectomes had the lowest mean T-value of the three modalities. BBS models were also run on single modalities separately. Only task fMRI data was a significant predictor of externalizing scores in these analyses (average $r = 0.20$; permutation $p = 0.038$). Structural data alone approached significance ($r = 0.16$, permutation $p = 0.085$), whereas resting connectomes alone did not approach significance ($r = 0.01$, permutation $p = 0.484$).

Conclusions: Utilizing BBS modeling, which utilizes low-rank representations of the data for multimodal analysis, multimodal brain signatures associated with externalizing were identified in a relatively small sample. These brain signatures included less task activation in the inhibitory control network, less gray matter in the cingulate, and more gray matter in the striatum. Multiple modalities did a better job predicting individual differences in externalizing behaviors than single modalities, and task activation and gray matter from structural data appear to contribute more to this prediction than resting state connectivity.

Keywords: Externalizing Disorders, Alcohol and Substance Use Disorders, Functional Magnetic Resonance Imaging, Multimodal Data

Disclosure: Nothing to disclose.

W241

Transcriptional Signatures of Opioid Dependence in the Human Post-Mortem Brain

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Background: In the United States, rates of opioid use disorder (OUD) and deaths from overdose are unprecedented. More than 2 million people are clinical diagnosed with OUD with an estimated 200,000 new cases each year. Despite the enormous public health impact of OUD, we lack a basic understanding of the mechanisms contributing to OUD. Current pharmacotherapies are largely aimed at symptomatic relief (e.g., mitigating 'flu-like' symptoms during acute withdrawal) rather than targeting pathways underlying craving and relapse. Our urgent need to develop novel, more effective pharmacotherapies for opioid dependence requires in-depth investigations into the molecular and cellular adaptations that occurs in the brains of patients who suffer with OUD. Human neuroimaging studies have consistently associated marked dysfunction within corticostriatal circuits with opioid dependence including in the dorsolateral prefrontal cortex (DLFPC) and nucleus accumbens (NAcc). Targeting these areas has been suggested as a viable therapeutic approach for OUD. However, little is known about the transcriptional alterations in these two major neural substrates of opioid dependence in the human brain.

Methods: In the current study, we collected DLPFC and NAcc from post-mortem tissue from the brains of OUD and control subjects, matched for sex, age, post-mortem interval, pH, and RNA quality ($n = 40$ subjects, 20 control and 20 OUD). We performed RNA-seq on bulk DLPFC and NAcc tissue (~60-80 million reads per sample). We examined differential expression (DE) of genes between OUD and control ($p<0.05$; fold change >-1.2 and 1.2<). Gene Ontology (GO) annotated databases were used to uncover significantly enriched gene pathways in control and OUD subjects. We then used rank-rank hypergeometric overlap (RRHO) analyses to assess the coherence of transcriptional alterations across brain regions associated with OUD. We also used an integrative network approach consisting of Weighted Gene Co-expression Network Analysis (WGCNA) followed by module differential connectivity (MDC), and hub gene network detection, to identify gene modules specific to OUD in the DLPFC and NAcc.

Results: We discovered 402 DE genes (148 upregulated and 254 downregulated) in the NAcc and 326 DE genes (248 upregulated and 78 downregulated) in the DLPFC of OUD subjects (corrected $p < 0.05$). In the NAcc of OUD subjects, GO analyses of DE genes revealed enrichment for pathways involved in oxidative stress, metabolism, circadian rhythms, and immune macrophage signaling ($p < 0.05$). In the DLFPC of OUD subjects, pathways were enriched for metabolism, ubiquitin ligase activity, glutamatergic signaling, and immune signaling, particularly inflammatory cytokine response and nuclear factor kappa-B signaling ($p<0.05$). The RRHO analyses revealed robust discordant patterns of transcriptional alterations between the DLPFC and NAc of OUD subjects (-log10pvalue > 160), suggesting opioid dependence is associated with opposing transcriptional signatures in major nodes of corticostriatal circuits. WGCNA identified modules specific to OUD and controls within the DLPFC and NAcc, and MCD analyses showed that there 10 significant gene co-expression modules that are gained in OUD across brain regions relative to controls and 4 modules that are lost. Hub gene network analyses within these modules revealed potential upstream regulators specific to OUD in the DLPFC and NAcc.

Conclusions: Our findings begin to uncover the transcriptional alterations linked to OUD in corticostriatal neural circuits previously associated with opioid dependence, craving, and relapse. Pathways enriched for various immune-related genes were found in both the DLPFC and NAcc of human subjects with OUD. Additional pathways were also enriched, including those involved in the regulation of circadian rhythms, which are of particular interest since among the most common symptoms and complaints of patients with OUD are severe and persistent

disruptions to sleep and rhythms. We are currently conducting additional analyses to predict potential master regulators of transcriptional pathways associated with OUD and cell-type specific enrichment analyses to determine whether the transcriptional signatures from bulk tissue reveal any additional information regarding affected cell-types.

Keywords: Opioids, Addiction, Opioid Use Disorder, Human Post-Mortem Brain, RNA-seq

Disclosure: Nothing to disclose.

W242

Effects of Metformin on Cue-Induced Cocaine Seeking in Male and Female Rats

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Background: Despite decades of research there are still no approved pharmacotherapies for treatment of cocaine use disorder. In recent years there has been an alarming increase in cocaine-associated emergency room admissions and overdose deaths. In addition to acute adverse health effects, protracted use of cocaine results in enduring neuroadaptations including a decrease in the levels of phosphorylated adenosine monophosphate activated protein kinase (pAMPK) in the nucleus accumbens core (NAcore). It is believed that these drug-induced changes contribute to a persistent vulnerability to relapse. Consequently, there remains a critical need to identify effective treatments for cocaine relapse prevention that may normalize some these neuroadaptations. It has previously been demonstrated that direct bi-directional manipulation of AMPK levels in the NAcore produces opposing effects on cue-induced reinstatement. Here we investigated the potential efficacy of an FDA approved anti-diabetes drug, metformin, for reducing cue-induced reinstatement both centrally and systemically in a rat model of cocaine self-administration. Metformin was chosen because it increases pAMPK levels and shows efficient blood-brain barrier permeability.

Methods: Adult male and female Sprague-Dawley rats were implanted with chronic indwelling jugular catheters with a subset receiving bilateral intracranial cannulae above the NAcore. Rats were trained to self-administer cocaine on a fixed ratio 1 (FR1) schedule in two hour sessions where an active lever press was reinforced with a cocaine infusion (0.2 mg/infusion) paired with a light and tone cue. After meeting criteria for self-administration (10 days >10 infusions) and extinction (≥2 consecutive days with ≤30% SA infusions) rats were tested for cue-induced reinstatement. For the intracranial experiments, rats were microinjected with either metformin (125 µg/side) or saline in a randomized within-subjects design prior to cue-induced reinstatement sessions ($n = 7$-8/group). After behavior, rats were perfused for histological verification of cannula placement. In a separate cohort of rats, we are testing the effects of chronic systemic metformin (125-250 mg/kg) prior to extinction session on later cue-induced reinstatement.

Results: We observed that female rats took a significantly higher number of cocaine infusions compared to their male counterparts (T-test with Welch's correction: $T(7.3)=2.8$, $p = 0.03$) and therefore had much higher cumulative cocaine intake (mg/kg). Female rats displayed much greater variability in active lever pressing compared to male rats although the group difference did not rise to the level of significance (mean ± SE, 81.66 ± 25.09 vs. 32.86 ± 2.96). We did not observe any estrous cycle influence on cocaine seeking during self-administration. We found that intracranial metformin in the NAcore was effective at reducing cue-induced reinstatement in both male (repeated measures two-way ANOVA with main effects of lever [$F(1,14) = 49.99$, $p<0.0001$], condition [$F(2,28)=3.53$, $p = 0.04$], and interaction [$F(2,28)=6.16$, $p = 0.006$]) and female rats (repeated measures two-way ANOVA with main effects of lever [$F(1,12) = 14.05$, $p = 0.003$], condition [$F(2,24)=6.88$, $p = 0.004$], and interaction [$F(2,24)=4.13$, $p = 0.029$]). In the systemic (i.p. injection) experiments we anecdotally observed a difference in sensitivity to the metformin injections between male and female rats. After noting increased lethargy following 250 mg/kg metformin injection in female rats we reduced the dose to 125 mg/kg. These experiments are ongoing.

Conclusions: These findings in rats provide valuable insight towards a possible pharmacological treatment of cocaine use disorder that repurposes an already viable drug. This research lends further support to the hypothesis that dysregulated AMPK signaling may play a role in cocaine use disorder.

Keywords: Metformin, Adenosine Monophosphate Activated Protein Kinase (AMPK), Cocaine Self-Administration and Reinstatement

Disclosure: Nothing to disclose.

W243

Exploring Withdrawal and Negative Affect Following Abstinence in a Sample of Regular Users of Legal Market Cannabis Concentrates

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Background: Increasingly liberal cannabis policies in the U.S. have expanded availability of high-potency cannabis products within the medical and recreational markets. For example, the average potency of legal-market cannabis flower products sold in Colorado is 16-19% delta-9-tetrahydrocannabinol (THC), with strains up to 30% THC commonly available (Orens et al., 2018; Vergara et al., 2017). Additionally, concentrated forms of cannabis containing up to 80-90% THC are gaining popularity in the legal market (Daniulaityte et al., 2015; Daniulaityte et al., 2017; Stogner & Miller, 2015). At present, little is known about the specific risks associated with the consumption of highly potent cannabis concentrates, however correlational studies of high-potency cannabis suggests that higher THC products may increase cannabis-related harms and contribute to increased dependence and withdrawal over time (Loflin & Earleywine, 2014; Volkow et al., 2014). Prior evidence suggests that withdrawal is more severe in women than men, and that more cannabis use (or higher potency cannabis) pre-cessation could lead to stronger withdrawal symptoms (Bonnet & Preuss, 2017). Further, affective dysregulation is a symptom of cannabis withdrawal, and withdrawal may be more severe in those with affective symptomatology (Hasin et al., 2008). The present study examines the effects of short-term abstinence on THC blood levels, withdrawal, depression and anxiety in a sample of regular cannabis concentrate users.

Methods: We recruited $N = 142$ cannabis concentrate users (47% female; mean age=28.94 years, $SD = 9.7$ range=21-69) to participate in one of two Sub-studies: in Sub-study 1 (S1; $n = 95$), subjects were asked to use cannabis as they normally would prior to their baseline session. In Sub-study 2 (S2; $n = 47$), subjects were asked to abstain for 3 days prior to baseline. At baseline, all subjects completed demographic and self-report questionnaires related to cannabis use (e.g., a 30-day Timeline Followback), mood (Beck Depression Inventory [BDI-II] and Beck Anxiety Inventory [BAI]) and withdrawal (Marijuana Withdrawal Scale [MWS]) and underwent a blood draw. Withdrawal and mood symptoms as well as THC blood levels were compared between subjects in S1(no abstinence) and S2 (3-days

attempted abstinence) using independent samples t-tests. Gender was explored as a moderator using ANOVA. To examine relationships among abstinence and affective symptomatology, we also explored correlations between blood THC, withdrawal, depression and anxiety in S2.

Results: Individuals in S1 and S2 did not differ in days of cannabis use during the 26-days prior to 3-day abstinence period (S1 mean = 23.14 days, SD = 5.2, S2 mean = 21.22 days, SD = 7.4). Of n = 47 S2 subjects asked to abstain for 3 days, n = 26 abstained all 3 days, n = 12 abstained for 2 days, n = 3 abstained for 1 day and n = 5 did not abstain for any of the 3-days. Blood THC and its metabolites (THC-COOH and 11-Hydroxy-THC) were significantly lower in S2 compared to S1, consistent with the S2 abstinence instructions, and suggesting that S2 subjects decreased their use during the 3-day period even if they did not completely abstain. There were no gender differences between successful and unsuccessful abstainers. A significant correlation emerged between days of cannabis use during the abstinence period and days of cannabis during the past month overall ($r = .335$, $p = .023$). Differences in MWC scores also emerged, such that S1 reported significantly less withdrawal at baseline ($M=25.53$, $SD = 7.77$) compared to S2 ($M=30.00$, $SD = 9.63$), $t(76.53) = -2.771$, $p = .007$. The groups also differed in their endorsement of particular MWC items; S2 had greater endorsement of shakiness/tremulousness ($p = .004$), sweating ($p = .003$), restlessness ($p = .009$), increased aggression ($p = .045$) and increased anger ($p = .003$). Results of a two-way ANOVA indicate trend-level gender moderation of this effect, such that females reported greater withdrawal than males in both studies (S1 female mean = 27 SD = 1.16, male mean = 24.06 SD = 1.19; S2 female mean = 34.81 SD = 2.01, male mean = 25.71 SD = 1.76), but the difference was greater in S2; $F(1,127) = 3.848$, $p = .052$. In S2, significant positive associations emerged between cannabis use during the 3-day abstinence period and BAI ($r = .435$, $p = .003$) and BDI ($r = .348$, $p = .018$) and trend-level associations emerged with MWS ($r = .266$, $p = .074$).

Conclusions: S1 and S2 used cannabis at equivalent levels prior to the 3-day abstinence period. Thus, the lower levels of THC and its metabolites in the blood of S2 subjects suggest that they attempted to decrease their cannabis use during abstinence days, though only 55% were able to completely abstain. These results suggest that heavier users may have more difficulty abstaining. Further, individuals who were unable to abstain reported more severe negative affect and withdrawal symptoms. Relatedly, differences in withdrawal symptoms between S1 and S2 suggest that abstaining and/or attempting to abstain (e.g., cutting down) from cannabis concentrates is associated with withdrawal symptoms (particularly negative affect and anxiety). Gender moderation results support the hypothesis that cannabis withdrawal is more severe in female compared to male users. Future work should compare withdrawal symptoms in flower cannabis users to concentrate users, to shed light on potential differences in severity of the withdrawal syndrome related to use of cannabis products containing various THC potencies.

Keywords: Cannabis, High Potency THC, Withdrawal

Disclosure: Nothing to disclose.

W244

Long-Lasting Effects of Methocinnamox (MCAM) on Fentanyl Self-Administration in Rhesus Monkeys

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Background: Opioid abuse remains a serious public health challenge despite the availability of effective medications. Naltrexone is the only opioid receptor antagonist currently approved to treat opioid use disorder, and although it is effective in some patients, a short duration of action and surmountability of antagonism limit its utility. Recent studies showed that the pseudoirreversible mu opioid receptor antagonist methocinnamox (MCAM) attenuated the positive-reinforcing and respiratory-depressant effects of heroin in rhesus monkeys, suggesting it could be an effective treatment for opioid abuse and overdose. The current study extends this work by evaluating MCAM for its capacity to attenuate the reinforcing effects of the potent, high-efficacy opioid fentanyl, which has contributed to a recent dramatic increase in mortality associated with opioid abuse.

Methods: Male and female rhesus monkeys (n = 7; 4 females) responded for i.v. infusions of fentanyl or cocaine under a fixed-ratio 30 schedule during daily 90-min sessions. Once responding was stable, naltrexone or MCAM was administered s.c. 15 or 60 min, respectively, prior to a session. Experiments were conducted using a within-subject design. Data were analyzed using repeated-measures analysis of variance with Dunnett's post-hoc comparisons; $p<.05$ was considered statistically significant.

Results: Under baseline conditions, fentanyl produced an inverted U-shaped dose-effect curve with monkeys obtaining 20 to 25 infusions per session when a moderate unit dose (0.00032 mg/kg) was available and 10 to 15 infusions per session when a 10-fold larger unit dose (0.0032 mg/kg) was available. Monkeys obtained more than 20 infusions per session when responding for cocaine (0.032 mg/kg). MCAM (0.1-0.32 mg/kg) and naltrexone (0.0032-0.1 mg/kg) dose-dependently decreased responding for the smaller unit dose of fentanyl. Effects of MCAM lasted for several days or weeks, whereas effects of naltrexone lasted less than 24 hr. For example, a dose of 0.32 mg/kg of MCAM significantly decreased the mean (SEM) number of infusions from 21.3 (0.8) under baseline to 7.3 (4.1) on the day of treatment. Responding for fentanyl remained decreased for up to 12 days following MCAM administration; during that time, the mean number of infusions per session ranged from 2.5 (1.0) to 7.0 (2.6). A dose of 0.01 mg/kg of naltrexone also significantly decreased the number of infusions from 24.3 (1.1) under baseline to 6.3 (1.7) on the day of treatment; however, the number of infusions obtained one day after naltrexone treatment (21.5 [2.6]) was not significantly different from baseline. When the larger unit dose of fentanyl was available for self-administration, effects of MCAM (0.32-3.2 mg/kg) and naltrexone (0.0032-0.1 mg/kg) depended on the dose of antagonist, with smaller doses of MCAM and naltrexone increasing and larger doses decreasing responding, consistent with shifting the peak of the fentanyl dose-effect curve rightward. Neither MCAM nor naltrexone altered responding for cocaine.

Conclusions: MCAM and naltrexone attenuated the reinforcing effects of the potent, high-efficacy opioid fentanyl, but not cocaine. Both MCAM and naltrexone appeared to shift the peak of the fentanyl dose-effect curve rightward; however, effects of MCAM lasted much longer (> 1 week) than those of naltrexone (< 24 hr). These results confirm and extend previous studies, demonstrating that MCAM produces long-lasting and selective effects on opioid self-administration. The extraordinarily long duration of action of MCAM is likely due, at least in part, to pseudoirreversible binding to the mu opioid receptor, suggesting that it would insurmountably block the reinforcing (abuse-related) effects of opioids. Taken together with previous studies, these results indicate that MCAM could be a safe, effective, and long-acting treatment for opioid use disorder and overdose with several advantages over currently available treatments. This work was supported by the National Institutes of Health [R01DA005018, R01DA048417, and R01DA007315] and the Welch Foundation [AQ-0039].

Keywords: Opioid Abuse, Opioid Antagonist Treatment, Drug Self-Administration, Fentanyl, Rhesus

Disclosure: Nothing to disclose.

W245

Cellular Specificity of Matrix Metalloproteinase Activation on Accumbens Medium Spiny Neurons During Heroin Seeking

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Background: Heroin abuse is a leading cause of drug overdose-related deaths in the United States, highlighting a need for further research elucidating effects of maladaptive neuroadaptations following prolonged heroin use. Activation of the tetrapartite synapse in nucleus accumbens core (NAcore), which comprises of pre- and postsynapse, astrocytic processes, and surrounding extracellular matrix (ECM), has been linked to increased relapse vulnerability. Specifically, degradation of the ECM by activated matrix metalloproteinases (MMPs) is involved in extracellular synaptic remodeling both constitutively and transiently. Following chronic heroin self-administration and extinction training, transient increases in MMP-9 activity in NAcore were elicited after 15 mins of cued heroin seeking compared to heroin-extinguished and saline control rats. Although increases in MMP-2,9 fluorescence can be localized to the soma and dendritic processes of medium spiny neurons (MSNs) in accumbens, it is unknown which specific cell types harbor changes in MMP activity under heroin-extinguished and cued reinstatement conditions. We hypothesized that D1-receptor expressing MSNs express increased pericellular localization with MMPs during transient cued heroin seeking, while D2-receptor expressing MSNs express increased localization following extinction.

Methods: We used an AAV cre-dependent mCherry virus to transfect accumbens MSNs in D1 and D2 cre-dependent rats ($n = 4$-5 rats/group) and measured the localization of activated MMP-2,9 after FITC-gelatin microinjection under extinguished and reinstated conditions. Results were analyzed using one-way ANOVA following by Tukey's post hoc tests for multiple comparisons. Both sexes were included in these studies.

Results: For D1 MSNs, we observed increased MMP-2,9 localization with dendritic surfaces in reinstated animals compared to both yoked saline controls and heroin-extinguished animals ($p = 0.0012$). While D2 MSNs showed increased MMP-2,9 localization only in heroin-extinguished animals, but MMP-2,9 localization after 15 min reinstatement was reduced to yoked saline levels ($p < 0.0001$). We also investigated whether the previously mentioned increased MMP activity selectively around D2 MSNs is mediated by extinction training. Following, heroin self-administration and home cage abstinence, MMP localization around D2 MSNs is diminished, indicating an extinction-mediated process ($p < 0.0001$). Next, we used pharmacological MMP-2,9 inhibitors to determine which were contributing to increased localization, specifically around D1 MSNs during reinstatement and D2 MSNs after extinction. Finally, we studied the involvement of tissue inhibitors of metalloproteinases (TIMPs) in NAcore during reinstatement to potentially determine if local MMP inhibition around D2 MSNs is necessary for cued heroin seeking.

Conclusions: These findings reveal how NAcore extracellular matrix signaling underlying constitutive and transient synaptic plasticity relies in part on specific cell-types.

Keywords: Matrix Metalloproteinase-9 (MMP-9), Nucleus Accumbens, Cue Reinstatement, Opioid Addiction, Extracellular Matrix

Disclosure: Nothing to disclose.

W246

Adolescent Nicotine Exposure Dose-Dependently Increases Thalamo-Striatal and Thalamo-Cortical Functional Connectivity and Associated nAChR Binding

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Background: Smoking remains a major health burden as the leading cause of preventable death in the US. Approximately 88% of smokers begin smoking before the age of 18, during adolescence, an evolutionarily conserved period of brain development. Preclinical models of adolescent exposure to nicotine have observed distinct short- and long-term changes as a function of adolescent nicotine exposure in behavior, brain nAChR expression and network organization. However, the relationship between these physical changes in receptors and changes in brain-wide communication remain unclear. Preclinical models of adolescent exposure to nicotine offer the distinct opportunity to explore these linkages. Given the pervasive health burden of smoking as well as the increasing popularity of nicotine vapor administration among adolescents, a better understanding of the development of nicotine addiction from adolescence through adulthood may inform treatment interventions for smokers.

Methods: We exposed groups of adolescent (postnatal day (p33)) and adult (p68) rats to 6 continuous weeks of saline, low (LN; 1.2mg/kg/d) or high dose (HN; 4.8mg/kg/d) nicotine ($N = 10$-13/group), which was continuously and passively administered using subcutaneous osmotic minipumps. At baseline and at 2 week intervals, nicotine dependence was assessed using a precipitated withdrawal test and conditioned place avoidance (CPA). Immediately after CPA testing, rats were lightly anesthetized using a combination of low dose isoflurane (0.25-0.75%) and dexmedetomidine (0.015mg/kg/h). High-resolution anatomical images and resting BOLD functional MRI (fMRI) data were acquired using a Bruker 9.4T/30cm scanner. Immediately after the final scanning session, rats were euthanized and their brains were harvested and processed for autoradiographic quantification of total nicotine acetylcholine receptor (nAChR) binding and two highly expressed nicotinic receptor subtypes, α4ß2 and α7. Herein, we describe the differences observed after 6 weeks of continuous exposure to nicotine.

Slide-mounted cryostat sectioned brains (20μm) were assessed for nAChR binding using nicotine, L-(-)-N-methyl-[3H], [3H]-3-(2(s))-azetidinylmethoxy and [3H]ASEM to quantify total, α4ß2 and α7 nAChR binding, respectively. Autoradiograms were analyzed used Image-J-Fiji. We conducted a whole brain search to identify brain regions of interest (ROIs; $n = 90$) with significantly different binding as a function of adolescent exposure to nicotine. We then used these ROIs as seeds in a whole brain resting-state functional connectivity (rsFC) analysis to determine the relationship between nAChR binding and alterations in rsFC following adolescent exposure to nicotine.

Results: We observed global up-regulation of total and α4ß2 nAChR binding and regional up-regulation of α7 binding in the amygdala and insula as a function of nicotine dose, regardless of age group. Distinct ROIs, comprising thalamic, cortical and subcortical regions, including the dorsal hippocampus, lateral posterior thalamus and posterior thalamic nuclear group, displayed significant changes in nAChR density, dependent on receptor subtype, as a function of adolescent exposure to nicotine. Particularly, thalamic regions displayed significant increases in total nAChR binding exclusively in adolescents exposed to HN. Binding to α4ß2 nAChR paralleled differences in total nAChR binding, although α4ß2 nAChR binding varied in the dorsal hippocampus as a function

of adolescent exposure to nicotine. LN exposed adolescents displayed increased α7 nAChR binding in amygdalar subregions as compared to HN and saline adolescent counterparts.

ROIs with significant differences in total, α4ß2 and/or α7 nAChR binding also displayed significant changes in rsFC as a function of age of exposure to nicotine. Adolescents exposed to HN displayed increases in rsFC whereas adults exposed to HN displayed a decrease in rsFC across these seed ROIs. For example, we observed increased connectivity between lateral posterior thalamus and the nucleus accumbens and ectorhinal cortex in HN exposed adolescents while HN decreased connectivity between these regions for their adult exposed counterparts. These results suggest common thalamo-cortical-striatal loops that are differentially altered with adolescent exposure to nicotine.

Conclusions: Thus, we observed dose-dependent changes in receptor specific nAChR binding, which paralleled changes in rsFC. To our knowledge, this is one of the first studies that merges nAChR binding density to a fMRI-based metric of brain functional connectivity. Identifying these sensitive circuits highlights their potential for use as targets for treatment strategies in smokers, most of whom want to quit and began smoking in adolescence.

Supported by the NIDA-IRP, a grant from the FDA Center for Tobacco Products (NDA 13001-001-00000 to EAS) and a Canadian Institute for Health Research fellowship (RJK).

Keywords: Adolescence, Nicotine, Nicotinic Acetylcholine Receptors, Resting State Functional Connectivity, fMRI

Disclosure: Nothing to disclose.

W247

Oxytocin Reduces Alcohol Drinking and Relapse Behavior Through Signaling in Extended Amygdala in Mice

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Background: The neuropeptide oxytocin (OT) has emerged as a potential therapeutic for alcohol use disorder (AUD) and stress-related psychiatric illnesses. We previously showed that systemic administration of OT reduced binge-like alcohol drinking, operant oral self-administration of alcohol, and stress-induced relapse-like behavior in a dose-related manner in male and female mice. It is not known whether these effects are mediated by OT release and signaling at oxytocin receptors (OTR) in brain or due to peripheral effects of OT. The present series of studies used pharmacological and chemogenetic approaches to address this research question.

Methods: Adult male and female C57BL/6J or OT-IRES-Cre mice were used to study binge-like alcohol drinking using the 'Drinking-in-the-Dark' (DID) model (limited access to 20% alcohol: 2-hr/day for 3 days, then 4-hr on day-4). Separate mice were trained using standard operant procedures to establish stable responding on a fixed ratio (FR4) schedule for 12% (v/v) alcohol (20 ul) during daily 20-min sessions. After 14 days of extinction testing, mice were exposed 15 min to a predator odor (2,3,5-Trimethyl-3-thiazoline; TMT) for reinstatement testing. Mice were injected (IP) with the peripherally-restricted OTR antagonist Atosiban (1 mg/kg) or central OTR antagonist L368,899 (10 mg/kg) 45 min prior to injection of OT (0.5 mg/kg) or vehicle, which was given 30 min prior to DID and reinstatement testing. For chemogenetic studies, OT-IRES-Cre mice were injected with AAV-DIO-hM3Dq-mCherry or control virus to target excitation of OT neurons in the hypothalamus (PVN), or retroAAV-DIO-hM3Dq-mCherry or control virus was injected to target their projections to the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST). CNO (3 mg/kg) was injected (IP) to activate the excitatory DREADD 30 min prior to DID testing.

Results: The centrally active (BBB-penetrant) OTR antagonist L368,899 blocked the ability of OT to reduce binge-like drinking and stress-induced alcohol relapse-like behavior while the peripherally-restricted OTR antagonist Atosiban did not alter these OT effects. Chemogenetic activation of OT neurons in the PVN reduced binge-like drinking, mimicking the effects of systemic OT treatment. CNO had no effects in mice injected with control virus. Additionally, this effect was blocked by prior treatment with L368,899 but not Atosiban. Chemogenetic activation of PVN OT neurons projecting to BNST or CeA reduced binge-like alcohol drinking as well.

Conclusions: Results from these studies indicate that pharmacological antagonism of OTR in the brain, but not in the periphery, blocked the ability of systemically administered OT to reduce binge-like drinking and stress-induced reinstatement of alcohol seeking behavior. Targeted chemogenetic activation of OT neurons in the PVN reduced binge-like alcohol drinking in a similar manner as systemic administration of the neuropeptide. Further, this effect was reversed by pretreatment with the centrally-active OTR antagonist. Finally, targeted activation of PVN OT neurons that project to CeA and BNST reduced alcohol binge-like drinking as well. Collectively, these results suggest that OT effects in reducing alcohol drinking and relapse are mediated by signaling within extended amygdala circuitry and support the therapeutic potential for OT in treating AUD.

Keywords: Oxytocin and Addiction, Alcohol Relapse Treatment, Alcohol Self-Administration

Disclosure: Nothing to disclose.

W248

Identification of the Dorsal Peduncular Area as a Highly Novel Regulator of Opioid Reward

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Background: The US is in the midst of an opioid abuse and overdose epidemic, with over 115 people dying each day from opioid overdose; this has been declared a public health emergency. Oxycodone is one of the most prescribed analgesics, is the first opioid many people experience, and has physiochemical properties that allow it to accumulate in the brain at rates higher than other opioids, perhaps explaining its considerable abuse potential. In 2016, 1% of people over age 18 reported abusing heroin in the previous 12 months, whereas 11% reported abusing prescription painkillers. Approximately 80% of heroin addicts began by first abusing prescription opioids (SAMHSA). Currently, all FDA-approved medications for opioid dependence are partial (e.g. buprenorphine) or full (e.g. methadone) agonists at the μ opioid receptor, and thus serve as replacement therapies. Such approaches may not address the root cause of addiction-related behavioral abnormalities. In the past two decades, a great deal of research using animal models drug addiction have focused on a small number of neurobiological systems, most notably the mesocorticolimbic dopamine system, and the corticostriatal glutamate system. While examination of these circuits has been informative regarding the roles of these systems in motivation and reward, it is very likely that understudied brain systems and circuitries play a critical role in driving addictive behaviors.

Methods: We injected male C57 mice with 5mg/kg oxycodone, and performed whole-brain immunostaining for c-Fos using the iDISCO+ tissue clearing protocol. The ClearMap Python package was then used to perform automated cell detection and

registration to the Allen Brain Atlas. This yielded a large data set including cell counts throughout the entire brain, which revealed 39 regions showing significant alterations in c-Fos expression following acute oxycodone injection. Of these regions, we chose to further study the dorsal peduncular cortex (DP), a highly understudied structure that sits ventral to the infralimbic cortex. In order to investigate a behavioral role for the DP, we used optogenetic stimulation of ChR2 during a real-time place preference paradigm. To molecularly characterize the DP, we used single-nuclei RNA sequencing, and quantitative polymerase chain reaction (qPCR). Electrophysiological characterization of μ-opioid receptor (MOR)-expressing neurons in the DP occurred in MOR-mCherry mice, and both baseline characteristics, as well as responses to MOR agonism, were compared to neighboring non-MOR-expressing neurons. Finally, to characterize direct mono-synaptic projections from the DP, we used Fos-CreERT2 mice to perform targeted recombination in active populations (FosTRAP), to fluorescently 'tagging' the efferent projections of the oxycodone-responsive with an AAV expressing the anterograde tracer Synaptophysin-mRuby. High-throughput analysis of outputs from the DP was then performed using iDISCO+, light-sheet microscopy, and computational analysis.

Results: Optogenetic stimulation of ChR2 in the DP (10Hz, 5ms pulse width) produced a real-time place aversion, and this aversion was blocked by prior administration of oxycodone. qPCR analysis of Oprm1 expression showed ~2.5-fold enrichment in the DP compared to the neighboring infralimbic/prelimbic cortices (PL/IL). Interestingly, single-nuclei RNA sequencing and bioinformatic clustering indicate that Oprm1 expression in DP neurons is highly overlapping with markers of glutamatergic neurotransmission, contradicting the canonical belief that opioids primarily act on GABAergic interneurons in the cerebral cortex. Furthermore, electrophysiological characterization of MOR(+) neurons showed that they have relatively depolarized resting membrane potential compared to neighboring MOR(-) neurons, and were hyperpolarized by DAMGO application. While still preliminary, high-throughput analysis of outputs of opioid-responsive neurons in the DP indicate dense projections to hindbrain regions known to regulate pain, stress, autonomic function, and aversion, including the trigeminal nucleus, parabrachial nucleus, and rostromedial tegmentum.

Conclusions: These data thoroughly characterize the DP, a highly novel and unique opioid-responsive cortical region. The DP shows enriched expression of the μ opioid receptor, and most interestingly, electrophysiological and single-nuclei sequencing data indicate that this receptor is expressed on glutamatergic output neurons. Furthermore, the connectivity profile of the DP makes it a highly likely candidate to be an important regulator of opioid reward, analgesia, and withdrawal. Additional studies are currently underway to further functionally characterize this region.

Keywords: Opioid Addiction, Prescription Opioids, Mu-Opioid Receptors, Neural Circuit and Animal Behavior, Circuitry-Based Approach

Disclosure: Nothing to disclose.

W249

Role of Genotype in the Effect of Adolescent Cannabis Exposure on Heroin Reinforcement

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Background: Genetic studies have shown that drugs of abuse, particularly illicit ones, share some common genetic risk factors and these factors have been posited to be at the basis of the association between cannabis use and abuse of other illicit drugs (Common Liability Theory of Addiction, CLA). In principle, epidemiological studies are unable to provide unequivocal evidence of causality, while animal models might provide such evidence. Recently, in an animal model of genetic vulnerability to drug addiction, the Fischer 344 and Lewis inbred rat strains, we observed that adolescent exposure to Δ9-tetrahydrocannabinol (THC), the active ingredient of cannabis, increases the rewarding and reinforcing properties of heroin at adulthood, selectively in the addiction prone strain (Lewis), and this effect was associated with a potentiation of drug-induced activation of dopamine (DA) transmission in the nucleus accumbens (NAc) shell (Di Chiara et al., Neuropsychopharmacol, 38, W171, 2013; Cadoni et al., Addiction Biol. 20,132-142, 2015; Lecca et al., submitted). In order to investigate the effects of adolescent THC exposure in genetically vulnerable individuals on heroin reinforcement we utilized an experimental approach consisting of a pattern of long access self-administration (SA) of increasing doses of heroin. All experimental procedures were approved by the Ethical Committee of the University of Cagliari (OPBA) and were conducted in accordance with the European Community Council Directive (2010/63/UE L. 276 20/10/2010).

Methods: Male Fischer 344 (F344) and Lewis (LEW) rats of 6 weeks of age (38-42 PND) were administered with increasing doses of THC (2, 4, 8 mg/kg i.p.) or vehicle twice daily for three days. When adults (10 weeks of age, 63-70 PND) rats were implanted with a catheter in the jugular vein and after 10 days of recovery allowed to acquire heroin SA (0.025 mg/kg) according to a Fixed Ratio 1 (FR1) schedule of responding (modus operandi nose-poking) in 1h session, 5 sessions/week. Following acquisition of operant responding by all groups (15 sessions) the length of the session was extended to 4h leaving unchanged the heroin dose. After 7 sessions the dose was increased to 0.05 mg/kg (7 sessions) and then to 0.1 mg/kg for other 7 sessions. Extinction of heroin SA was performed by substitution of heroin with saline, in absence of visual cues, and after 6 sessions operant SA was reinstated by heroin priming injection (0.5 mg/kg s.c.) in presence of cues. Statistical analysis was carried out by Statistica V.8 (StatSoft, Inc. Tulsa, OK, USA). Responding was analyzed by two- or three-way ANOVA with strain (LEW vs F344) and pretreatment (THC vs saline) as between subjects' factor, and sessions as repeated measure. Drug intake (mg/kg) was expressed as daily amount of heroin self-administered during each sessions and as overall amount means. Responding elicited by heroin priming during reinstatement session was analyzed by three-way ANOVA, with strain, pretreatment and cumulative nose-pokes (active vs inactive) as between subjects' factors. Significant main effects or interactions revealed by ANOVA were further analyzed by Tukey's HSD or Duncan's post-hoc tests. Significance was set at $p<0.05$.

Results: Acquisition of heroin SA (0.025 mg/kg) under FR1 in 1h sessions (sessions 1-15) was not different in the 4 experimental groups. Extending the length of the session to 4h, at the same heroin dose, induced a significant increase of nose-poking behavior in all experimental groups but THC pretreatment increased operant responding, as well as heroin intake, selectively in LEW-THC rats as compared to LEW-VEH, while no difference was observed between treatment groups of F344 strain. Increasing the heroin dose (0.05-0.1 mg/kg) did not affect operant responding in LEW-THC rats who kept constant operant responding, thus increasing heroin intake, while LEW-VEH reduced nose-poking behavior in response to the highest heroin dose. The increase of heroin dose to 0.1 mg/kg reduced operant responding and heroin intake in similar manner in F344-VEH and F344-THC groups. Following 6 sessions of extinction, by substituting heroin with saline and removal of visual cues, operant responding was reinstated in all groups but LEW-THC rats showed greater responding compared with the other experimental groups.

Conclusions: The results of the present study extend and further strengthen the notion that adolescent THC exposure increases the rewarding and reinforcing properties of heroin (Di Chiara et al., 2013op.cit.; Cadoni et al., 2015, op.cit.; Lecca et al., submitted), thus facilitating heroin abuse and dependence in genetically vulnerable individuals. The long access pattern of responding highlighted the effect of THC pre-exposure selectively in the addiction prone LEW strain. Thus, in this condition LEW rat escalated their drug intake and THC potentiated this effect particularly at the highest dose tested (0.1 mg/kg). As previously suggested this effect might be based on differential adaptive changes of mesolimbic DA transmission induced by THC adolescent exposure in the two strains (Cadoni et al., 2015). Thus the increased DA transmission responsiveness in the NAc shell might have enhanced the rewarding and incentive value of heroin thus facilitating the development of addiction.

Keywords: THC, Heroin Self-Administration, Adolescence

Disclosure: Nothing to disclose.

W250

Reduced GABA-B Currents in VTA Dopamine Neurons During Prolonged Abstinence After Fentanyl Vapor Self-Administration in Mice

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Background: Opioid addiction is a growing problem in the United States and deaths from opioid overdoses reached epidemic proportions; fentanyl alone accounts for nearly half of these deaths. The intravenous (IV) opioid self-administration (SA) is considered the "gold standard" in the opioid addiction field. However, applying this model in mice is challenging due to the difficulty in maintaining catheter patency, especially in conditions of long access to the drug. We developed a non-invasive fentanyl vapor SA model of opioid addiction in mice that bypasses the limitations of the IV SA model. We also used this model to study neuroadaptations in the ventral tegmental area (VTA) dopamine neurons during protracted abstinence.

Methods: Mice were trained to self-administer fentanyl on a fixed-ratio (FR) 1 schedule of reinforcement by nosepoking a hole in an airtight operant chamber. Nosepoking the active hole activated a custom-built device that delivers vaporized fentanyl (5 mg/ml dissolved in a mixture of vegetable glycerol/propylene glycol) to the chamber accompanied by a light stimulus for 60 sec during which nosepokes were counted but were inconsequential (i.e., time out). Nosepokes in the inactive hole had no programed consequences. A vacuum system cleared the vapor from the chambers in <60 sec. For the electrophysiology experiments, mice self-administered fentanyl vapor on an FR1 schedule for 2-3 weeks after which they went into forced abstinence. Midbrain slices for patch clamp recordings were prepared 2-5 weeks after the last fentanyl exposure, and whole-cell recordings were made from VTA dopamine neurons.

Results: Mice readily learned to self-administer fentanyl vapor over 1-h sessions with high discrimination between active and inactive holes. They titrated their intake when given different fentanyl concentrations, and increased their responses to receive fentanyl at increasing FR ratios. Mice that were allowed long access, but not short access, to fentanyl escalated their fentanyl intake across sessions. The long access mice showed resistance to the suppression produced by an aversive stimulus by more persistence than short access mice in nosepoking for fentanyl adulterated with capsaicin. Further, mice extinguished their fentanyl-seeking response over 3-4 weeks but reinstated drug-seeking in response to a cue that was previously associated with drug deliveries. Electrophysiological recordings from VTA dopamine neurons in brain slices during protracted abstinence from mice that self-administered fentanyl compared to mice that self-administered the vehicle vapor showed that both GABA-B inhibitory postsynaptic currents and outward currents produced by bath application of the GABA-B agonist, baclofen, were reduced. Reduced GABA-B signaling was due to decreased probability of GABA release at GABA-B receptor synapses on dopamine neurons, and reduced efficacy of postsynaptic GABA-B signaling.

Conclusions: We developed and validated a non-invasive mouse model of multiple indices of opioid addiction and identified neuroadaptations in the VTA dopamine neurons. Given the availability of numerous behaviorally selected and transgenic mouse strains, and the myriad of imaging modalities and genetic tools available for mice, the present model has the potential to provide significant advances in our understanding of the neurobiology of opioid addiction.

Keywords: Fentanyl, VTA, GABAB, Vapor

Disclosure: Nothing to disclose.

W251

Evidence for a Role of Central Amygdala in Compulsive Alcohol Self-Administration in Rats

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Background: Continued alcohol use despite adverse consequences, or "compulsive use", is a core feature of alcohol addiction. Understanding the molecular mechanisms sustaining this behavior is a critical challenge for addiction research. Only a subset of users transitions from recreational to compulsive alcohol use. In contrast, in commonly used animal models, nearly all rats learn to self-administer alcohol. This observation points to the possibility that focusing on alcohol self-administration alone may be insufficient to identify key mechanisms of alcohol addiction.

Methods: We first trained male Wistar rats ($n = 64$) to lever press for a 20% alcohol solution. Next, we examined individual variation in resistance to mild footshock punishment (0.2 mA) (a rat model of compulsive alcohol use). We then identified compulsive and non-compulsive rats using k-means clustering. We also included yoked shock-control groups ($N = 18$) for both punishment- resistant and punishment-sensitive rats. We then carried out an extensive Fos (a neuronal activity marker)-mapping to identify cell populations that are active in a manner specifically associated with punishment resistance (and not merely with shock exposure). To identify large scale networks whose activity is associated with punishment resistance rather than individual structures, we conducted a principal component extraction, followed by a factor analysis. To establish the neurochemical phenotypes of activated cells, we used confocal microscopy with double or triple labeling. To probe the mechanisms underlying punishment resistance, we used viral vector and pharmacological manipulations. An shRNA AAV vector targeting PKC-δ was kindly provided by Dr. R. Messing, UT Austin. Its efficacy was confirmed using RNAscope in situ hybridization and real-time PCR.

Results: We found that a stable proportion of ~35% across multiple batches of outbred Wistar rats showed a punishment

resistance (compulsive-like) phenotype. Central amygdala (CeA), together with nucleus accumbens and the periaqueductal gray, loaded significantly on "Network 1" which accounted for 32% of the total variance and was positively correlated with punishment resistance ($r2=0.61$; $p < 0.001$). Particularly, CeA activity showed a significant positive correlation with punishment resistance ($r2=0.69$; $p < 0.001$). The activated cells in CeA were mainly PKC-δ positive GABAergic neurons. We found that downregulation of PKC-δ (AAV vector expressing a shRNA to PKC-δ) in the CeA suppressed punishment resistant self-administration ($F(1,22) = 5.52$; $p<0.05$) without affecting locomotor activity. Moreover, systemic (1, 3 mg/kg) and CeA (70 ng/0.3 µl) injections with the GABA-B agonist baclofen, reduced CeA activity measured as Fos expression, and this was also associated with a selective suppression punishment resistant self-administration ($F=(2,39) = 6.49$; $p < 0.01$; $F=(1,16)=7.40$; $p<0.05$ respectively). CeA baclofen injections had no effect on saccharin self-administration or locomotor activity.

Conclusions: Our findings demonstrate that PKC-δ down-regulation or inhibition of neuronal activity through activation of GABA-B receptors in the CeA are sufficient to reduce compulsive alcohol self-administration, a core feature of alcohol addiction. Whether these findings converge in a unique regulatory mechanism within the CeA microcircuit requires further investigation.

Keywords: Compulsive Models of Drug Use, Central Amygdala, Alcohol Self-Administration

Disclosure: Nothing to disclose.

W252

Evidence of Central Signaling After Systemic Administration of Oxytocin: A PET Study in Nonhuman Primates

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Background: Preclinical studies suggest that central endogenous signaling of the nine amino acid peptide, oxytocin (OT) and its receptor plays a role in the neurobiological mechanisms related to drug and alcohol addiction. There are preliminary preclinical and clinical studies indicating that administration of OT reduces addiction related behaviors such as self-administration, conditioned place preference and withdrawal symptoms. As such, OT may represent a novel treatment for alcohol and drug dependence. However, there are still many unanswered questions that limit further clinical development of this promising treatment. One question is the effect of systemically administered OT on central signaling peripheral administration of OT such as its modulation of dopamine (DA) signaling in mesocorticolimbic pathways in response to drugs of abuse.

Methods: We conducted an [11C] raclopride positron emission tomography (PET) study in nonhuman primates ($N = 6$ male rhesus macaques) to investigate the effect of intravenous OT (80 IU) on [11C] raclopride binding potential in the striatum after an IV methylphenidate challenge.

Results: In the caudate and ventral striatum, there was a significant main effect of methylphenidate ($p < 0.001$), a main effect of OT ($p = 0.01$) and a significant OT x methylphenidate interaction ($p = 0.04$) where OT reduced the raclopride binding potential and attenuated the methylphenidate induced reduction in raclopride binding potential.

Conclusions: These results indicate a possible mechanism underlying the effect of OT to reduce the rewarding effect of psychostimulants as reported in previous preclinical studies. The demonstration of this effect in nonhuman primates has translational relevance particularly as the brain receptor distributions of OT vary considerably between rodents and primate species.

Keywords: Oxytocin and Addiction, Nonhuman Primates, Intravenous Administration

Disclosure: Nothing to disclose.

W253

A Novel Neuropeptide Decreases Opioid Self-Administration

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Background: Neuromedin U (NMU) and its NMU receptor 2 (NMUR2) are expressed in the limbic-corticostriatal circuitry involved in the enhanced motivational attributes of drugs of abuse, which are key factors in sustained opioid use disorder (OUD) and relapse. The NMUR2 is a G protein-coupled receptor which is enriched in a key node of this neurocircuitry, the nucleus accumbens. NMU regulates behavioral responses to psychostimulants and alcohol, but NMU has not been evaluated in the context of any opioid nor has the neural pathway expressing NMUR2 been delineated. Therefore, we assessed the neurocircuitry expressing NMUR2 and evaluated the effects of NMU to alter self-administration of the prescription opioid oxycodone.

Methods: Male Sprague-Dawley rats ($n = 11$/group) were implanted with indwelling jugular catheters and trained to self-administer oxycodone (0.1 mg/kg/inf) to criterion (less than 10% infusion variability for three days) on fixed ratio schedule (FR 5). The effects of systemic pretreatment with NMU (0.3 mg/kg) or vehicle were assessed on both fixed and progressive ratio responding for oxycodone. Data were analyzed using students t-test or repeated measure ANOVA. Rabies virus-based transsynaptic neural tracing was used to identify the neural circuit expressing NMUR2.

Results: NMU decreased oxycodone taking throughout the session ($p<0.05$ for main effect of time, treatment, and interaction), but did not alter latency to respond. NMU also decreased progressive ratio breakpoints ($p<0.05$ for main effect of time, treatment, and interaction), but, again, did not significantly alter latency to respond. Neural tracing demonstrates that NMUR2 is localized to neurons which project from the dorsal raphe nucleus, to the nucleus accumbens, and then on to the ventral pallidum.

Conclusions: NMU decreased the rewarding and motivational effects of oxycodone in a rat model and NMUR2 is localized to a neural circuit consistent with the indirect pathway. The current study suggests that NMU/NMUR2 has promise for future drug discovery efforts for the suppression of relapse in OUD.

Keywords: Opioid Abuse, Neuromedin U, Neuropeptides, Novel Therapeutics, Neuronal Tracing

Disclosure: Nothing to disclose.

W254

Classing It up: Cluster Analysis as an Outcome-Measure Strategy for Ongoing Drug Use During Treatment for Opioid-Use Disorder

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Background: During medication-assisted treatment for opioid-use disorder (OUD), nearly all patients reduce their use of illicit drugs, but most do not stop entirely, at least during the time frame of a typical clinical trial. The modal pattern of use is sporadic and fluctuating; it almost never conforms to simple categories such as "relapse," especially among people who continue to attend treatment. This hampers clear statements about base rates of success and, in turn, stymies quantification of the benefits of new adjunctive treatments. In clinical trials, such effects are often expressed in terms of group-level percentages of drug-negative urine specimens. This gives no information about the proportions of participants who respond in particular ways, and thus no way to calculate clinically useful effect-size indicators such as number needed to treat (NNT). That was the issue we sought to address: the absence of natural categories for pure measures of drug-use frequency during agonist maintenance.

Methods: We conducted thrice-weekly urine drug screens in 307 outpatients being treated with daily methadone or buprenorphine-naloxone for up to 16 weeks in natural-history studies (i.e., no additional randomized intervention) from 2009 through 2018. We designated dropouts (n = 86; 28%) as a separate category a priori. For the remaining 221 participants (72%), we used agglomerative hierarchical clustering to classify urinalysis results, with an optimal-matching algorithm that accounted for sequential patterns rather than just ordinal position. In additional analyses, we determined whether we could identify similar clusters in three of our prior published randomized clinical trials (RCTs), each of which included adjunctive treatments in the context of agonist maintenance for OUD. The addition of these older data sets (collected 1994-1999, 1999-2002, and 2004-2010) also provided a test of the stability of outcome classes across temporally distinct cohorts.

Results: Apart from dropout (28%), there were five patterns of urine results: abstinence or occasional use (30%) frequent opioid use (13%), frequent cocaine use (10%), frequent use of both drugs (11%), and sporadic use of either or both drugs (8%). These clusters were replicable in urine data from our prior RCTs (N = 193-252; 17-20% dropout, 21-23% abstinent or infrequent, 5-6% opioid, 13% cocaine, 22-30% both, 11-18% sporadic; higher membership in the cocaine and both-drugs clusters was due to the focus of the RCTs on cocaine use in OUD treatment). In the RCTs, patterns of cluster membership tended to differ in accordance with the addition of specific adjunctive treatments (e.g., provision of incentives for cocaine-negative urine specimens), though these differences did not always reach statistical significance because the RCTs had not been powered for a person-level categorical outcome.

Conclusions: Even in a treatment-research setting with flexible, individualized dosing of methadone or buprenorphine, only 20-30% of patients will become mostly abstinent from heroin and cocaine in the time frame of a typical clinical trial. An outcome-classification scheme based on clustering can be used for power and sample-size planning for randomized trials of adjunctive treatments intended to shift cluster membership. Reporting results in those terms will facilitate calculation of NNT, an effect-size indicator of interest to the FDA and clinicians. The price of this added clarity and utility may be a need for larger samples (possibly on the order of 100 participants per experimental group) to detect effects that have traditionally been detected in terms of groups' overall percentages of negative urine specimens.

We want to emphasize that the clustering method we used here is based entirely on detected patterns of drug use, without reference to accompanying OUD symptomatology or quality-of-life considerations. Therefore, we should expect to see discrepancies between cluster membership and broader indices of clinical outcome--and those discrepancies should be of considerable interest. A rational, integrated approach to outcome assessment will include a broad array of measures of functioning, but will benefit from initial categorical clarity about patterns of ongoing drug use.

Keywords: Cluster Analysis, Opioid Addiction, Clinical Trial Methodology

Disclosure: Nothing to disclose.

W255

Hormonal Regulation of Risky Decision Making in Male and Female Rats

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Background: Many psychiatric diseases characterized by altered risk taking are differentially represented in males and females. Progress towards understanding the relationship between psychiatric disorders and risk taking is constrained, however, by our limited knowledge regarding sex differences in risk taking. As a first step toward addressing this issue, we showed previously that females are more risk averse than males in a rat model of risky decision making. We hypothesized that these sex differences are due to differences in hormonal modulation of risk taking.

Methods: In Experiment 1, female rats were trained in a risky decision making task in which rats chose between a small, "safe" food reward and a large, "risky" food reward accompanied by varying probabilities of mild footshock. Subsequently, half of the females were ovariectomized (OVX) and were then re-tested. After choice behavior stabilized, estradiol (E) was subchronically administered (0, 10, 20 μg/0.1 mL) while rats were tested in the decision-making task. Finally, to determine whether effects of OVX and/or E administration were due to changes in motivation for food or shock sensitivity, females were tested under a progressive ratio (PR) schedule of reinforcement followed by a shock sensitivity threshold assay. In Experiment 2, male rats were trained in the same decision-making task, after which half of the males were orchiectomized (ORX) and then re-tested. After choice behavioral stabilized, testosterone (T) was subchronically administered (0, 0.75, 2.25, 7.5 μg/0.1 mL) while rats were tested in the task. All male rats were also tested in the decision-making task while receiving a subchronic regimen of E administration (0, 20 μg/0.1 mL). Finally, as in Experiment 1, males were tested under a PR schedule of reinforcement followed by a shock sensitivity threshold assay.

Results: In Experiment 1, there was a significant increase in choice of the large, risky reward (increased risk taking or risky choice) in OVX females relative to their sham counterparts and their pre-surgery baseline. This effect was not due to differences in motivation for food or to changes in sensitivity to footshock. The highest dose of E decreased risk taking in OVX females, thus rescuing the OVX-induced increase in risky choice, but did not affect PR performance. In Experiment 2, there was a significant decrease in risk taking in ORX rats relative to their sham counterparts and their pre-surgery baseline. Surprisingly, subchronic T had no effect on risk taking in either ORX or sham males. Similar to females, however, subchronic E administration decreased risk taking in both ORX and sham males without affecting PR performance.

Conclusions: These data show that circulating levels of E are necessary for the characteristic risk aversion in females, as OVX increased risk taking and E replacement attenuated this effect. Risk taking in males is also regulated by gonadal hormones as ORX decreased risk taking. Surprisingly, T replacement did not mitigate this change in choice performance, although E administration did cause decreases in risk taking in males, irrespective of gonadal

status. Collectively, these data demonstrate a robust modulatory role for gonadal hormones in risk-based decision making and, in particular, reveal a role for E in suppression of risk taking in both males and females.

Keywords: Decision Making, Gonadal Hormones, Sex Differences

Disclosure: Nothing to disclose.

W256

Effect of Level of Drug Use on the Relationships Between Craving, Stress, and Cue Exposure

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Background: Nearly all people who enter agonist treatment for opioid use disorder (OUD) reduce their drug use, but many do not stop entirely. Ongoing use might result from stress or from drug cue exposure. Our lab has been using ecological momentary assessment (EMA) to collect data on the dynamics of those risk factors in relation to specific drug-use events during treatment.

Methods: In this natural history study, 307 participants [78% male; 64% African American; 34% white] being treated with an opioid agonist medication (methadone or buprenorphine-naloxone) made EMA entries at randomly prompted (RP) times and when they used opioids or cocaine. Urine was tested thrice weekly. Sequences of drug-test results were classified by hierarchical clustering to classify patients into three groups (clusters) with high, sporadic, and low (predominantly no) use. In multilevel models, we analyzed cluster differences in momentary craving, momentary stress, momentary mood, and reasons for ongoing drug use. We then analyzed interactions between time, cluster, momentary cue exposure, and momentary stress as predictors of momentary craving during the periods that led up to a drug-use event. Effects were decomposed to distinguish within-person associations from between-person differences.

Results: When the EMA variables from electronic diary entries were decomposed to isolate their within-subject and between-subject components, participants' overall levels of the of stress, negative mood and craving all had moderate to strong correlations with each other (p's<.05, rPearson range = .46 to .80); within subjects, participants tended to experience more than their usual level of stress during the same times that they experienced more than their usual level of negative mood. The high-use, sporadic-use and low-use clusters averaged 91%, 58% and 11% drug-positive urines, respectively. Patients in the high-use cluster reported significantly higher craving (F2, 221 = 6.0, p = .003; reffect = .19) and lower positive mood (F2, 221 = 4.56, p = .01; reffect = .16) in RPs compared to those in the low-use cluster, but no differences in stress or negative mood. Patients in the sporadic-use cluster endorsed "being asked to use" as a reason for their use more frequently than those in either the high or low use clusters (F14, 175 = 6.4, p = .002; reffect range= .24 to .37). There were 108 participants (80 high-use and 28 low/sporadic-Use) who had drug use events that met the criteria for an interaction analysis focusing on craving during periods in which no use was reported for at least 24 hours before a use event. There were a few moderately sized effects reflecting between-person differences, such as interactions between average level of stress and cue exposure as predictors of craving (reffect range= .24 to .25). The strongest effects (reffect range= .19 to .76) occurred at the within-person level—i.e., they involved changes in stress and cue exposure relative to the person's own average. There was a multi-way interaction of cluster, within-subject stress, within-subject cue-exposure and time: when momentary levels of stress and cues were near the person's average level, craving was minimal and flat over time in all clusters, but the strongest craving was seen when people in the high-use cluster were exposed to high momentary levels of cues and stress and they would not be using drugs for the next several days.

Conclusions: Adopting the heuristic that stress and cues trigger craving allowed us to ask whether the relationships between these variables might differ between people who have different patterns of drug use. We found that the effects of stress and cues on craving varied depending on how frequently a person typically uses and how long it will be until they will use again. Such information helps us better understand these complex factors and could potentially be useful in personalizing treatment.

Keywords: Ecological Momentary Assessment, Opioid Use Disorder, Emotional Stress, Craving, Opioid Agonist Treatment

Disclosure: Nothing to disclose.

W257

High Prevalence of Chronic Pain Among Individuals With Opioid Use Disorder but no Pain Diagnosis: A National Pilot Survey in Norway

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Background: Chronic pain in opioid dependent individuals with no primary pain diagnosis is not well understood, and may contribute to maintain the opioid dependence in a high number of these individuals. Our aim was to investigate the prevalence of chronic pain and clinical pain characteristics of an opioid dependent population in Norway with no primary pain diagnosis.

Methods: A total of 569 patients referred to treatment for opioid dependence (DSM-V) were assessed for chronic pain conditions in a multi-center cross-sectional survey. All participants filled out a brief questionnaire including location, onset and characteristics of the pain condition. NRS-11 was used to measure the pain intensity.

Results: Fifty-five per cent of the patients (n = 306) reported current chronic pain lasting for at least 3 months. The mean age was 42 years (median: 42, range: 19-65) and 32% were women. The age distribution was similar for both genders. The prevalence of chronic pain was higher among women (61%) than men (52%) (p = 0.032%) and associated with higher age (p<.001). There was a higher prevalence of chronic pain among methadone patients compared to those receiving buprenorphine (-naloxone) or not receiving any opioid agonist treatment (p<.001). In the chronic pain group mild, moderate and severe pain intensity was reported by about one third each.

The severe pain group had a higher number of pain locations, was more likely to endorse the pain descriptors "cramping" and "cruel" and was less likely to report that pain medication had an effect on their pain, compared to the mild pain group.

Conclusions: The high prevalence of chronic pain found in our survey underscores the importance of pain recognition and management in the treatment of opioid dependent patients. Chronic pain may contribute to maintain opioid dependence and interact negatively with recovery and treatment outcomes for this group of patients.

Keywords: Opioid Addiction, Chronic Pain, Disease Prevalence

Disclosure: Nothing to disclose.

W258

Transcranial Direct Current Stimulation Applied to the Left Dorsolateral Prefrontal Cortex in Smokers Modifies Cognitive Circuits Implicated in the Nicotine Withdrawal Syndrome

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Background: Symptoms of nicotine withdrawal remain a major impediment for smokers trying to quit; most quit attempts fail within the first week of abstinence. The Nicotine Withdrawal Syndrome (NWS) is characterized by both cognitive (reduced attention, working memory, "WM") and affective (irritability, anxiety) disturbances. Studies employing functional magnetic resonance imaging (fMRI) have identified reductions in strength of the Executive Control Network (ECN) and related nodes - and increases in strength of the Default Mode Network (DMN) and related nodes - as associated with NWS cognitive deficits, while hyperactivity of the amygdala and related circuits have been associated with NWS affective dysfunction. Transcranial Direct Current Stimulation (tDCS) has the potential to modify these neuronal circuits by producing a subthreshold conductive current through the scalp and into the brain. Two potential targets for tDCS as a smoking cessation aid are the dorsolateral prefrontal cortex (dlPFC), a node of the ECN, and the ventromedial prefrontal cortex (vmPFC), a node of the DMN. We hypothesized that functional activity in cognitive control networks, and downstream amygdala circuits, would be modified by acute application of tDCS to the left (L) dlPFC and right (R) vmPFC.

Methods: 15 smokers (in 12-hours nicotine abstinence, crossed between nicotine and placebo patch) and 28 matched nonsmokers served in a randomized, sham-controlled, double-blind, crossover design with 3 conditions of 25 min, 2mA tDCS: anodal L-dlPFC + cathodal R-vmPFC ("An-dlPFC"); polarity reversed ("Cat-dlPFC"); and sham. Tasks probed relevant cognitive constructs (error monitoring: parametric Flanker task; WM: N-back task; emotional reactivity: matching faces task), and brain activity measured with simultaneous fMRI (3T Siemens Prisma; NIDA-IRP, Baltimore, MD; 2017-2019). We generated two statistical models to test tDCS effects on two aspects of smoking addiction: Trait, the between-subjects factor of nonsmokers vs. sated smokers; and State, the within-smokers factor of nicotine withdrawal vs. nicotine sated. Behavioral outcomes included task accuracy, response time, and d-prime sensitivity. Images were preprocessed with BIDS-app fmriprep, 1st level and group processed in AFNI, with statistical testing completed in R. We measured BOLD signal to detect tDCS effects on a priori regions of interest (ROIs) for each task, Bonferroni-corrected for number of ROIs: N-back, 9 ECN-related ROIs; Flanker, 3 salience processing ROIs; Emotion, 6 amygdala sub-regions. We further conducted an exploratory, familywise error corrected (FWE, $\alpha < 0.01$, p-voxelwise $= 0.001$), whole-brain search for effects of tDCS on each task.

Results: Behavior: Sated smokers were more accurate than Nonsmokers ($p = 0.03$), and had a lower criterion to detect signal ($p = 0.03$), on the N-back task. Within smokers, nicotine deprivation induced slower response times ($p < 0.05$, N-back, Flanker, and Matching), reduced accuracy ($p < 0.05$, N-back and Matching), higher signal criterion and response omission rate (N-back, $p < 0.05$). We did not observe tDCS effects on task behavior. ROI analysis: An-dlPFC tDCS increased right anterior cingulate cortex (ACC) activity in the smoker group to a greater degree than the nonsmoker group ($p = 0.02$) across all difficulty levels of Flanker. Whole-brain analysis: We observed that An-dlPFC tDCS strengthened the deactivation of 14 DMN associated regions (including hippocampal and parahippocampal gyri, mid-cingulate gyrus, precuneus, and temporal regions) across all subjects on the N-back Trait model. In the State model, An-dlPFC tDCS reduced activity in the same DMN-related regions, however the effect was more prominent in the nicotine sated vs. withdrawal state.

Conclusions: Single session acute tDCS enhanced the deactivation of DMN nodes during a WM task, and enhanced ACC activity during an error monitoring task. While all subjects were sensitive to tDCS effects on DMN, smokers were more sensitive to tDCS effects on ACC. Further, smokers were more sensitive to tDCS effects on DMN in the nicotine-sated state, in which subjects were generally more attentive to task and therefore potentially more receptive to an adjuvant modifier, than during withdrawal, when subjects demonstrated reduced task engagement (slower responses, reduced accuracies) and attention (more omissions). Thus, the cognitive circuit dysregulation associated with NWS, and often alleviated by nicotine replacement therapy, may be further modifiable by "adjuvant" anodal, excitatory tDCS applied to L-dlPFC. Use of tDCS as a complement to standard therapy has been successfully tested in depression, in which combined tDCS + antidepressant medication improved outcomes more than either alone. The present data support the possible use of tDCS as a complementary therapy to other, standard treatments for nicotine addiction, especially the dysregulated cognitive processes seen during the NWS. This work was supported by the NIDA-IRP.

Keywords: tDCS, Brain Stimulation, Nicotine Dependence, Cognitive Control, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

W259

Sex-Dependent Effects of Adolescent Social Isolation on Glutamatergic Transmission in the Nucleus Accumbens: Input-Specific Alterations

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Background: Adolescent social stress puts individuals at increased risk for multiple psychiatric diseases including substance use disorders. The nucleus accumbens (NAc) plays a central role in the development and expression of addictive behaviors and is influenced by stress exposure. However, little is known about how adolescent social isolation alters signaling in the NAc to make an individual more vulnerable to addiction.

Methods: The current studies utilized an adolescent isolation stress model that elicits an increase motivation for cocaine in adulthood to examine the effects of stress on NAc physiology in both male and female mice. In additional to examining presynaptic and postsynaptic transmission using traditional whole-cell electrophysiological techniques, we used ex vivo optogenetics to interrogate the effect of adolescent social isolation on three inputs to the NAc: projections from the prefrontal cortex (PFC), ventral hippocampus (vHIPP) and basolateral amygdala (BLA).

Results: Adolescent social isolation leads to a decrease in paired pulse ratio in the NAc of both male and female mice ($p = .01$, $n = 6$-11/group) but the mechanisms underlying this difference are sex-dependent, with females exhibiting a decrease in the size of the readily releasable pool and males exhibiting alterations in release probability. When examining specific inputs, both males and females exhibit a decrease in PPR following adolescent social isolation at vHIPP to NAc projections ($p<.05$, $n =$ 10-13/group) while females also exhibit this decrease at PFC to NAc projections ($p<.05$, $n = 9$-13/group). Post-synaptically, the effects of adolescent social isolation seem more similar across the

sexes, with both exhibiting an increase in asEPSCs at BLA to NAc synapses ($p<.05$, $n = 6$-8/group).

Conclusions: Taken together, our research shows that adolescent social isolation leads to input-specific alterations in both presynaptic and postsynaptic glutamate transmission and these changes differ in males and females. As many of the endpoints examined also exhibit baseline sex differences, the importance of these findings will also be discussed.

Keywords: Adolescent Stress, Nucleus Accumbens, Sex Differences, Circuit Optogenetics

Disclosure: Nothing to disclose.

W260

Neural Mechanisms Underlying Effects of Propranolol on Drug Cue Reactivity

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Background: A characteristic feature of addiction is intense urges/cravings that emerge in specific contexts or in response to discrete drug-related stimuli. This effect is believed to emerge due to cues promoting the retrieval of memories associating the stimuli with previous episodes of use and may contribute to ongoing drug use and relapse among those have abstained. A growing body of literature implicates the noradrenergic system in retrieval of emotionally-laden memories. A well-established body of pre-clinical literature has revealed that administration of beta-adrenergic antagonists can disrupt the memory retrieval and reconsolidation process, reducing the impact of these stimuli on drug-taking behavior. Local infusion studies indicate noradrenergic activity within the hippocampus may be particularly critical for mediating this effect. Early clinical research findings are mixed, but suggest some potential therapeutic effects on drug cue-induced craving. However, no studies in humans to date have examined the neural mechanisms that underlie any potential therapeutic effects. Accordingly, our ability to deduce potential causes of mixed effects in humans is quite limited. The proposed study examined the effects of propranolol hydrochloride (a centrally-acting beta-adrenergic antagonist) on neural responses to smoking and non-smoking stimuli.

Methods: Male and female daily cigarette smokers ($n = 42$) not actively engaged in a cessation attempt were recruited. During an initial screening visit, a semi-structured interview was used to identify their most common smoking and non-smoking environments (i.e. places they frequently smoke during the week and places they never or very rarely smoke). Using an approach validated in our previous research participants were trained to photograph each of these environments using a study-provided camera. Upon camera return, staff reviewed images for content and quality. Participants were subsequently scheduled for an MRI visit set to occur after 24 hours of smoking abstinence. Two hours prior to the scan, participants were administered a single dose of propranolol hydrochloride (40 mg; immediate release) or placebo. Both researchers and participants were blind to condition. During the scan, participants viewed images of their personal smoking and non-smoking environments, as well as images of standard smoking and non-smoking environments and proximal smoking and non-smoking cues. A series of 4 images from the same location were presented across 16 seconds for each block. Immediately following each block, participants rated their urge to smoke while viewing these images. Behavioral analyses examined the interaction between drug condition and image type for urge ratings. Following pre-processing, preliminary MRI analyses examined this interaction using an uncorrected (whole-brain) threshold of $Z = 2.3$.

Results: A significant Drug x Cue Type ($F = 11.6$, $p < .001$) indicated participants receiving propranolol had reduced smoking cue-induced craving relative to those who received placebo. This effect was most robust for proximal cues ($p = .028$), effects for standard distal and personal distal cues were in a consistent direction but did not reach significance ($p = .117$ and $p = .155$, respectively). MRI analyses indicated propranolol also reduced activation to smoking cues relative to neutral cues in the left hippocampus and the parahippocampal gyrus. As with findings for cue-induced craving, these effects were also most pronounced for proximal cues. In addition, propranolol reduced reactivity to personal smoking environments (relative to standard smoking environments) in the precentral gyrus.

Conclusions: Findings confirmed hypotheses that propranolol may assist in reducing drug cue reactivity in ongoing cigarette smokers across both neural and behavioral indices. Propranolol (versus placebo) reduced self-reported smoking urge in response to smoking stimuli. Critically, it also reduced activation in response to smoking-related stimuli in the left hippocampus and parahippocampal gyrus—regions that are central to memory reconsolidation and retrieval. This pattern of findings is highly consistent with findings seen in pre-clinical models of drug use, suggesting that the mechanisms underlying these effects may indeed be consistent across species. This provides an important framework for translational research targeting the noradrenergic system and memory processes both in addiction and other related disorders (e.g. PTSD, anxiety). Additional research should examine these effects in the context of a larger-scale trial with clinical endpoints and explore potential moderators. Ideally, nuanced exploration of mechanistic effects outside the laboratory (e.g. assessment of context-specific cravings using ecological-momentary assessment) should also be included.

Keywords: Smoking Cessation, Propranolol, Cue Reactivity, Memory Encoding and Retrieval, Translational Neuroscience

Disclosure: Nothing to disclose.

W261

The Role of Insula Function in Emotion Regulation and Risk for Alcohol Use Disorder

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Background: The ability to change an emotional response to a circumstance by thinking about it differently is called emotion regulation, and stronger ability to regulate emotions has been associated with greater resilience to psychopathology. There is some evidence that individuals with an alcohol use disorder suppress negative emotions more often than healthy individuals, whereas healthy individuals are more likely to change thinking patterns to relieve negative emotions. The insula and amygdala have been shown to contribute to ability to regulate emotions. However, it is unclear if deficits in ability to regulate emotions predisposes an individual to alcohol use disorder, and what role neural activation plays.

Methods: This study recruited young adults between the ages of 18 and 22 years who had no previous history of alcohol or substance use problems. Eleven individuals have completed the study to date. Emotion regulation was assessed by the Emotion Regulation Questionnaire, and by a task completed in a functional MRI scan. The task involved viewing a series of neutral or highly negative images from the International Affective Picture System.

During some images, participants were instructed to "look" or "decrease". For "look", participants viewed the image without trying to change their thoughts or feelings. For "decrease", participants were instructed to decrease their negative feelings by changing the way they think about the image. Participants rated how negatively they felt on a 1-5 scale following each image. Participants also completed the Alcohol Use Disorder Identification Test (AUDIT) and the Customary Drinking and Drug Use Record to assess substance use.

Results: Participants who scored higher in ability to regulate emotion on the Emotion Regulation Questionnaire also reported feeling less negatively during the "decrease" condition relative to the "look" condition ($r = 0.73$). Participants who reported feeling less negatively during the "decrease" relative to "look" condition also reported higher AUDIT scores ($r = 0.42$). There was no relationship between marijuana use and emotion regulation ($r<0.1$). A linear mixed effects model of neural activation during the three conditions revealed a main effect of task in the left anterior insula, where activation was higher during the "decrease" condition, intermediate during the "look" condition, and lower during the neutral condition. Individuals with higher insula activation during the "decrease" condition reported lower ability to regulation emotions on the Emotion Regulation Questionnaire ($r = -.52$).

Conclusions: The relationship between task and questionnaire data suggests that the fMRI task shows face and construct validity in the current sample. Ability to regulate emotions may relate to alcohol use. Insula activation during the task also relates to ability to regulate emotion.

Keywords: Emotional Regulation, Adolescent Alcohol, Insula, Functional MRI (fMRI), Cannabis

Disclosure: Nothing to disclose.

W262

Frontal Theta/Beta Power Ratio Associates With Cue-Induced Craving and Impulsivity in Abstinent Methamphetamine Dependents

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Background: Craving and impulse action act as two major causes for relapse. Incubation of craving has been demonstrated in human subjects with cocaine and methamphetamine abuse. Yet the dynamic cortical network changes and neuropsychological functions (e.g. impulsivity) during abstinence have not been fully elucidated.

Methods: We recruited 72 male methamphetamine dependents (MAs) and 34 male control subjects (HCs) to record 128-channel resting-state electroencephalogram, with measurement of cue-induced craving, stop-signal task and neuropsychological states.

Results: The results showed decreasing trend for cued-craving and frontal theta/beta power ratio (TBR) with prolonged abstinence, while impulse control and beta power remained at similar level. Increased beta power was found across all abstinent MAs relative to HCs; while Frontal TBR significantly correlated to low craving and reduced impulsivity in MA subjects.

Conclusions: These results suggest frontal TBR may serve as an effective neurophysiological indicator to delineate the rehabilitation status and may predict relapse for methamphetamine dependents.

Keywords: Addiction, Methamphetamine, Abstinence, EEG

Disclosure: Nothing to disclose.

W263

"And the Winners are….": Both the Amygdalar Brain Response, and the (Implicit) Positive Affective Bias, to Cocaine Cues can Predict Future Drug Use Outcomes -- While (Explicit) Ratings of the Cues' Hedonics and "Crave-Ability" Do Not

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Background: The sharp recent rise in stimulant users, in new users, in overdose deaths with cocaine detected, and in governmental seizures of stimulants has led to identification of stimulants as a National Emerging Threat (NET, 2018). The insidious upturn in the national cocaine epidemic developed in the shadow of the opioid crisis, but the two public health problems are now lethally inter-twined: e.g., cocaine is present in nearly half the opioid overdose deaths in Philadelphia, with fentanyl present in more than 85% of these overdose deaths. In this context, the lack of an FDA-approved medication for stimulants takes on additional urgency. Toward this goal, our laboratory has focused on the brain substrates of relapse, pushing toward targets that have a clear link to drug use outcome, and that may be sensitive to candidate anti-relapse medications. We have identified a 'cue-vulnerable' phenotype, showing that some cocaine patients have a heightened brain response to drug cues in nodes of the brain's motivational circuitry, that this brain response can be linked to poor drug use outcomes, and that it is medication-sensitive. Though neuroimaging offers critical mechanistic information about the brain targets for medication, we are always on the look-out for inexpensive 'proxy' measures, whether tasks or ratings, that may correlate with the brain and/or the clinical outcome. If validated, these 'proxies' could help speed the screening of candidate anti-relapse medications, across a wide variety of settings. Our guiding hypothesis for this work is that implicit, task-based measures of the affective/feeling state for drug (cocaine) cues may show a stronger link to brain and clinical outcomes than explicit self-report measures, as explicit responses may be undermined both by social demand effects and by our patients' difficulty in labeling/scaling internal states.

Methods: Using BOLD fMRI (3T), we scanned stabilized male cocaine inpatients during passive ("Just watch") exposure to a quasi-random alternation of 6 sec (Cocaine and Neutral) videos. Following the scan day, patients participated in an affective priming task (testing the ability of visual images to facilitate the rapid identification of positive vs. negative nouns), yielding an (implicit) affective bias score for brief (500 msec) cocaine cues ($n = 15$, CPDD 2018). Additionally, they ($n = 23$, ongoing) also (explicitly) rated a subgroup of the same cues for pleasantness (1-9), and for their ability to trigger craving (1-9) in a cocaine user. The implicit task scores and explicit ratings were then examined for correlation (SPM 12) with the limbic brain response during the first half of the video task, and with future cocaine use (% cocaine urines positive or missing) across the 12 outpatient treatment weeks following hospital discharge.

Results: The brain response to cocaine cues in the amygdala (r. amygdala, 5 mm sphere centered on (x,y,z): 20,-5,-22; CPDD 2018) showed significant positive correlations with future cocaine use ($r = 0.69$; $p<0.004$). Consistent with our hypothesis, the (implicit) affective bias scores for cocaine showed a strong relationship both

with the brain response to cocaine cues ($r = 0.71$; $p<0.002$) and with future cocaine use ($r = 0.66$; $p<0.007$). In contrast, the explicit subjective ratings (of cocaine cue hedonics; estimated ability to trigger craving) showed no suprathreshold voxels (no significant correlations) with the amygdala (or any other limbic regions of interest), and were also uncorrelated with cocaine outcomes ($r = 0.029$ and 0.000 respectively; both $p>0.2$).

Conclusions: Cue-triggered activity in the amygdala, a brain region conferring motivational valence to incoming stimuli, accounted for nearly half of the variance in future cocaine relapse, highlighting the potential utility of the brain cue response in medication development. Encouragingly, cocaine patients' responses in the implicit bias task (revealing 'positive' feelings toward cocaine cues) showed a strong relationship to the same amygdala brain region, and to clinical cocaine outcome – potentially offering a low-cost 'proxy' for use in identifying patients at greatest relapse risk, and for screening candidate medications. Explicit subjective ratings (whether for hedonic valence or 'crave-ability' of the cocaine cues) were uncorrelated with brain and clinical outcome, highlighting the potential limitation of self-report measures in outcome prediction and in medication development.

Keywords: Brain Imaging, Functional MRI (fMRI), Cue Reactivity, Relapse, Implicit Priming

Disclosure: Nothing to disclose.

W264

Circadian Regulation of Incentive Motivation Influences Cocaine Demand: Role of Cholinergic Interneuron Modulation of Dopamine Signals in the Striatum

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Background: The ability of an organism to learn associations between stimuli in the environment and specific outcomes as well possessing sufficient motivation to respond to outcome-predicting cues (i.e., approach or avoid) is essential for guiding behaviors towards health and survival. Rapid dopamine signals in the nucleus accumbens (NAc) encode cue-outcome associations and provide a signal for incentive motivation. Indeed, the magnitude of dopamine release in the NAc tracks motivation towards reward-associated cues in the environment and to rewards themselves. Moreover, bursting and pausing of cholinergic interneurons (CIN) in the NAc is known to influence the magnitude of dopamine release to reward-associated cues and therefore plays a critical role in motivated behavior.

Advances in technology which allow for either measuring or manipulating cell specific populations during rodent behavior has illuminated key neural substrates for learning and motivation. However, much of these studies are designed around single points in time in order to control for potential circadian or time-of-day oscillations in neuronal function. This leaves open for investigation questions pertaining to whether the activity and function of these neural substrates shift dynamically throughout time-of-day and lead to circadian rhythms in motivation for natural rewards and drugs of abuse. For an accurate representation of how the brain mediates learning and motivation, the question is not only how the brain controls what stimuli associate and motivate action toward drugs and rewards, but how the brain controls when stimuli motivate action.

Methods: Male sprague-dawley or long-evans rats were used for each experiment ($N = 8$-12 per group) and maintained on a 12:12 light/dark cycle. We investigated the circadian nature and diurnal variation in both baseline and nicotine-induced facilitation of incentive motivational value towards reward-associated cues using a pavlovian conditioned approach (PCA) task at several time points across a 24 hours (zeitgeber time (ZT)1 (one hour into light), ZT6, ZT13 (one hour into dark), and ZT18). We then built on this understanding of the circadian control of cue responding to examine circadian rhythms in the degree to which a discriminative stimulus could invigorate motivation for cocaine self-administration using an behavioral economic demand approach (i.e., within-session threshold procedure). This threshold procedure generates data that can be modeled using an equation, $\log(Q)=\log(Q0)+k \times (e\text{-alpha} \times Q0 \times C - 1)$ to establish economic demand curves, subjective value, and motivation for cocaine.

After or during behavioral tasks, we assessed rapid NAc dopamine signaling and modulation of dopamine signals by CINs at the 4 time-points tested during the behavioral tasks using both in vivo and ex vivo voltammetry combined with optogenetics.

Each outcome measure was assessed within task using mixed-model analysis of variance (ANOVA) and relationships between behavioral and neurochemical measures were correlated when sampled from the same animal.

Results: For PCA, rats increased light cue (CS+) contacts (sign-tracking) that reliably predicted reward (sugar pellet) delivery as their cycle progressed to midway through their dark cycle (ZT18), while rats in their light cycle (ZT6) learn to engage the reward directly with head entries into the food port (goal-tracking). No animal was sleeping during the task; rather, the behavioral repertoire and learning shifts in a manner that corresponds to shifts in phasic dopamine signaling in the NAc.

Indeed, there was greater dopamine release to in the NAc following in vivo electrical stimulation of the ventral tegmental area in rats midway through their dark cycle. Moreover, the magnitude of spontaneous dopamine bursts in awake, freely-moving animals is increased and more robust during the dark cycle compared to light cycle. Acetylcholine modulation of dopamine release, as tested with selective activation of CINs in the NAc or application nicotine (full agonist) in slices, increased dopamine release magnitude only during the dark cycle.

For cocaine threshold, there were substantial time-of-day differences in the measures of motivation (maximal price paid (Pmax), cocaine value/demand elasticity (alpha), but not consumption (Q0). Rats exhibit increased Pmax and decreased alpha (inelastic demand) for cocaine starting midway through their dark cycle (ZT18 to ZT1) compared to light cycle (ZT6) and beginning of their dark cycle. In fact, ZT6 rats stopped lever pressing almost entirely for the last 3 doses of cocaine.

Conclusions: We show that rats exhibit robust time-of-day variation in 1) the incentive motivational impact of cue-reward associations in the PCA task, 2) subjective value of cocaine / demand elasticity in the threshold tasks, 3) dopamine signaling, and 4) CIN modulation of dopamine signals. Our data suggest the conclusion that there are times within the day that an individual will exhibit increased sensitivity to reward-associated cues that can lead to increased probability of drug seeking and conditioned behaviors, which are mediated by time-of-day variations in the CIN modulation of rapid DA signaling. The interaction between CIN and dopamine release is mediated by nAChRs located directly on DA terminals. We further conclude that this variation is governed by a circadian rhythm and can be entrained by environmental cues such as light / dark cycle.

Keywords: Dopamine, Acetylcholine, Cocaine Self-Administration, Behavioral Economics, Pavlovian Conditioning

Disclosure: Nothing to disclose.

W265

Sex-Specific Associations Between Polygenic Risk Scores for Major Depressive Disorder and Pretreatment Alcohol Consumption in Alcoholic Males and Females

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Background: Clinical and epidemiological studies indicate presence of sex-related differences in alcohol consumption and susceptibility for alcohol use disorders (AUD) in men and women. Although biological mechanisms underlying these differences remain poorly understood, it is reasonable to hypothesize that genetic variation may contribute to those differences. We have previously demonstrated that a life-time history of major depressive disorder (MDD) impacted pretreatment alcohol consumption in alcoholic males and females in a sex-specific manner. Specifically, lifetime MDD history was associated with less drinking days in alcohol dependent men, while current MDD was associated with higher alcohol use per day in alcohol dependent women. In this study, we aimed to investigate the impact of genetic load for MDD (measured by polygenic risk scores, PRS) on alcohol consumption in men and women entering AUD treatment while considering clinical diagnosis of MDD as a potential covariate.

Methods: Total number of drinks and number of drinking days in the 90 days before enrolment was assessed by Time Line Follow Back (TLFB) in 287 men and 156 women aged 18-80 meeting DSM-IV-TR criteria for alcohol dependence. They were also assessed for psychiatric comorbidities including life-time MDD using Psychiatric Research Interview for Substance and Mood Disorders (PRISM). Based on summary statistics from the Psychiatric Genomics Consortium genome-wide association study of over 130,000 MDD cases of European ancestry, the PRS for MDD was estimated for a subset of European-ancestry subjects in our sample (262 men and 151 women) using PRSice2. Linear models were used to regress the normalized alcohol consumption measures onto MDD PRS with or without adjustment for life-time MDD diagnosis as a covariate.

Results: In alcohol dependent men, genetic risk for MDD was associated with lower total number of drinks ($p = 0.002$). The numbers of drinking days, maximum drinks per day, and drinks per day were also lower in subjects with higher MDD PRS, but with weaker statistical evidence for the associations ($p = 0.052$, $p = 0.072$, and $p = 0.169$, respectively). On the contrary, in alcohol dependent women, MDD PRS was marginally associated with higher total number of drinks ($p = 0.076$) and number of drinking days ($p = 0.077$). These results did not substantially change after adjustment for clinical diagnosis of lifetime MDD.

Conclusions: Our findings indicate that genetic load for MDD has a sex-specific impact on pre-treatment alcohol consumption; specifically, higher genetic load for MDD is associated with lower alcohol consumption in alcohol dependent men, but with higher alcohol consumption in alcohol dependent women. These effects are similar to the association of consumption with lifetime clinical diagnosis of MDD, but independent of this effect, Future research is needed to replicate these findings and investigate the biological mechanisms underlying these associations.

Keywords: Alcohol Dependence, Major Depressive Disorder (MDD), Polygenic Risk Score, Alcohol Consumption

Disclosure: Nothing to disclose.

W266

Depletion of the Gut Microbiome Reduces Opioid Seeking and Alters Opioid-Induced Gene Expression in the Nucleus Accumbens

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Background: The resident population of bacteria in the gastrointestinal tract, collectively termed the gut microbiome, have been shown to have profound effects on brain and behavior. Studies in models of depression, autism spectrum disorder, Parkinson's disease and other neuropsychiatric conditions have all showed demonstrable effects of gut microbiome manipulations on brain function. While the mechanisms of this gut-brain interaction remain to be fully elucidated, the microbiome and its metabolites affect neuronal architecture, microglial responsiveness, function of the blood brain barrier, and many other aspects of brain function. Recently, our group was one of the first to demonstrate that manipulations of the microbiome can affect the rewarding properties of the psychostimulant drug cocaine. Here, we performed a similar series of studies in a model of opioid use disorder to determine differential effects across classes of drugs of abuse.

Methods: To knock down the gut microbiome, C57/BL6J mice were given a cocktail of non-absorbable broad-spectrum antibiotics (Neomycin 2mg/ml, Bacitracin 0.5mg/ml, Vancomycin 0.4mg/ml) via their drinking water for two weeks prior to any experimental manipulation. For behavioral experiments mice were trained on conditioned place preference across a range of doses (2.5 – 15mg/kg), and for self-administration animals were trained to lever press for infusions of fentanyl on an FR1 schedule for 3H daily sessions. Drug seeking behavior was elicited via presentation of the drug-paired cue in the operant chamber following ten days of abstinence. RNA and ATAC-sequencing experiments were performed on the nucleus accumbens of mice treated with high dose (20mg/kg) morphine for seven days followed by 24h of withdrawal. For RNA-sequencing analysis reads were aligned to the mm10 genome using the Bowtie2 alignment tool followed by differential gene expression analysis with DESeq2. For ATAC-seq analysis fragments were aligned to the genome, nucleosome free reads were filtered using esATAC, peak calls were performed using F-Seq and peaks were annotated using ChIPseeker. For all sequencing experiments gene ontology analysis and predicted transcription factor binding were performed using g:profiler and TRANSFAC. For all behavioral and molecular biology studies experiments were performed with an N of 6-10 animals per group, which is powered to detect a moderate to large effect size between groups. Behavior was analyzed via a two-way ANOVA with post-hoc tests as appropriate. All sequencing studies utilized pairwise comparisons with FDR-corrected p-values.

Results: On conditioned place preference tests, antibiotic-treated animals showed decreased morphine preference compared to controls across all doses, with significant differences being seen at the higher 10mg/kg and 15mg/kg doses ($p < 0.01$, Holm-Sidak post-hoc). Similarly, on the self-administration task animals showed a decreased acquisition of fentanyl self-administration and decreased levels of administration of acquisition (RM two-way ANOVA main effect of treatment $p < 0.0001$). RNA-sequencing analysis revealed that while morphine alone resulted in the significant alterations of several hundred genes, the transcriptional response was much more pronounced in microbiome depleted animals – with several thousand genes being significantly regulated. Pathway analysis of significantly

regulated genes demonstrated significant enrichment of genes involved in transcription and epigenetic regulation. Confirmatory qPCR analysis confirms regulation of numerous histone deacetylases. Given the widespread changes in gene expression and the effects on epigenetic regulation machinery, we next performed ATAC-sequencing to examine changes in chromatin accessibility across the genome. Interestingly, while there were no changes in microbiome depleted saline injected animals on RNA-sequencing, this group demonstrated the most robust alterations in chromatin accessibility particularly in gene promoters.

Conclusions: Taken together, these data suggest that the gut microbiome is a critical homeostatic regulator of both brain and behavior in response to repeated opioids. These studies lay the early foundation for possible translational research strategies to reduce harmful opioid use behaviors.

Keywords: Microbiome, Opioid Use Disorder, Transcription

Disclosure: Nothing to disclose.

W267

Nicotine Dependence (Trait) and Acute Nicotinic Stimulation (State) Modulate Attention but Not Cognitive Control in Smokers: Converging Evidence From Go-Nogo and Flanker fMRI Tasks

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Background: Nicotine withdrawal includes cognitive, affective and reward processing deficits. The cognitive deficits have been hypothesized to play an important role in relapse to smoking but the exact nature of these deficits and their relation to nicotine-related neurotransmitter systems remains unclear. This study focused on cognitive control and error monitoring in smokers and non-smokers while manipulating state with a nicotinic agonist, a partial agonist and withdrawal (smokers), assessing both behavior and fMRI task activation, to elucidate differences between smokers and non-smokers as well as characteristics of nicotine withdrawal and response to nicotinic treatments.

Methods: 24 smokers and 20 non-smokers participated in a double-blind, placebo controlled cross-over study in which they performed a Go-Nogo task and a Flanker task during BOLD fMRI scanning under placebo, nicotine patch, varenicline and varenicline plus patch conditions. Smokers were 12 hours abstinent for every scan. Varenicline or placebo was administered for two weeks prior to two scans with placebo or nicotine patch. Prior to the pill phase, subjects also underwent two scans with nicotine or placebo patch which are not reported here. Mixed-model ANOVAs (GROUP (smoker vs. non-smoker) X NICOTINE (nicotine vs. placebo) X VARENICLINE (varenicline vs. placebo) were performed on inhibitory failures and errors of omission for the Go-Nogo task and on the error rate and missed trial rate separately for both congruent and incongruent trials in the Flanker task. Using the BOLD data, the same ANOVAs were applied to the contrast of successful inhibitions vs baseline and unsuccessful vs. successful inhibitions for the Go-Nogo task and to correct incongruent vs. congruent trials and incongruent correct vs. incorrect trials for the Flanker task.

Results: Behavior: In the Go-Nogo task, there were no main nor interactive effects of GROUP, NICOTINE, or VARENICLINE on ability to inhibit a prepotent response, however, omission errors (missed responses on Go trials) showed significant NICOTINE ($F(1,123) = 33.72$, $p < 0.001$), NICOTINE*GROUP ($F(1,123) = 6.22$, $p = 0.014$) and NICOTINE*VARENICLINE ($F(1,123) = 11.55$, $p < 0.001$) effects driven by abstinent smokers committing more omissions with the expected agonist/partial agonist interaction between nicotine and varenicline. Nicotine also slightly reduced omissions in non-smokers. Similarly, there were no main effects nor interactions in the incongruent condition of the Flanker task but the congruent condition showed a main effect of NICOTINE ($F(1,123) = 5.68$, $p = 0.018$), with smokers showing particularly strong improvement on nicotine ($F(1,69) = 9.49$, $p = 0.003$). Both groups missed fewer trials in the nicotine patch condition ($F(1,287) = 31.20$, $p < 0.001$), an effect that was mitigated by varenicline (NICOTINE*VARENICLINE: $F(1,287) = 5.95$, $p = 0.015$). While the GROUP effect was non-significant, smokers showed a strong reduction in missed trials on nicotine ($F(1,69) = 19.10$, $p < 0.001$), as hypothesized. Non-smokers also had a significant reduction in missed trial ($F(1,54) = 5.84$, $p = 0.019$).

Imaging: Successful inhibitions in the Go-Nogo task showed a main effect of GROUP such that smokers had less activation in right anterior insula and right putamen. There was also a main effect of NICOTINE such that pre-SMA/dACC was less activated under the nicotine condition. There were no ANOVA results for incorrect vs. correct Nogo trials. The Flanker task yielded no significant ANOVA results for either contrast.

Conclusions: Our behavioral results indicate that cognitive deficits in abstinent smokers are observed in less demanding situations and that these deficits are mitigated by nicotinic stimulation. Smokers were able to muster the resources to complete the more demanding task conditions but faltered when demands were low, indicating a failure to maintain attention. Nicotine also improved attention in non-smokers but to a lesser degree. Nicotine reduced activation during successful inhibitions in the pre-SMA/dACC but this effect was present in both smokers and non-smokers indicating it is unlikely to reflect alleviation of withdrawal but may be an effect on processing efficiency. Smokers showed reduced inhibition-related activity in right anterior insula and right putamen as a trait difference, unaffected by nicotinic manipulations. Reduced inhibition-related activation in right anterior insula, a region implicated in allocation of attentional resources, may point to inadequate activation for appropriate attentional allocation in less demanding situations, although there is no evidence of such a deficit in the current data. Future studies may be designed to examine this question. Our results indicate that smoking cessation medication may help smokers avoid attentional lapses, especially in non-demanding tasks, and support use of cognitive behavioral approaches to help smokers enhance awareness of the possibility of lapses in seemingly less challenging situations. This research was supported in part by the Intramural Research Program of the NIH, NIDA.

Keywords: Functional MRI (fMRI), Varenicline, Nicotine Withdrawal, Inhibitory Control, Attention

Disclosure: Nothing to disclose.

W268

Incentive Salience During Early Abstinence is Associated With Amygdala Resting State Functional Connectivity in Individuals With Alcohol Use Disorder That Later Relapse

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Background: Incentive salience is a distinctive phenotype thought to characterize those with alcohol use disorder (AUD) (Kwako et al. 2019). Incentive salience is defined as motivation for rewards (or 'wanting' a reward) after an individual's physiological urges are triggered by previously learned associations related to a

reward cue (Berridge and Robinson 2016). There is evidence that incentive salience is mediated by activation of mesocorticolimbic dopamine system. More specifically, the amygdala within the mesocorticolimbic dopamine system exerts positive and negative emotional behavioral dispositions to rewards (Alcaro, Huber, and Panksepp 2007; G. F. Koob 1999; George F. Koob 2003). To determine whether the degree of the association between incentive salience and amygdala functional organization is related to treatment outcome, we compared correlations between amygdala resting state functional connectivity (rsFC) and incentive salience scores between individuals with AUD that subsequently relapsed versus those that remained abstinent.

Methods: We present data from 37 individuals with AUD (Age: M=43.0, SD = 10.2; Gender: 12 females, 32%) recruited from an addiction treatment program. Eyes-closed resting state fMRI data were collected from all individuals at ~2 weeks of abstinence. Rs-fMRI data were preprocessed/denoised using the Human Connectome Project pre-processing pipeline, including motion correction, dewarping, MNI registration and denoising. Incentive salience or motivation for rewards was measured with the Obsessive-Compulsive Drinking Scale (OCDS). We calculated resting state connectivity matrices between amygdala (left and right) and (i) cortical parcellations previously defined by intrinsic functional networks by Yeo et al. (2011) and (ii) subcortical Harvard-Oxford parcellations (Desikan et al. 2006). We first explored whether the degree of left or right amygdala rsFC was different between those that relapsed or remained abstinent 4-months later (independent samples t-tests). We then examined the associations (Pearson's) between amygdala rsFC and OCDS scores for AUD participants that subsequently relapsed to alcohol use and those that remained abstinent, separately.

Results: After a 4-month follow-up, 18 individuals with AUD relapsed to alcohol use (Age: M=40.9, SD = 10.5; Gender: 8 females, 47%), and 19 remained abstinent (Age: M=45.0, SD = 9.8; Gender: 4 females, 21%; no significant age difference t(35)=1.22, p = 0.23 or gender difference Chi-squared (1)=2.3, p = 0.13). Fisher z tests comparing correlation coefficients between groups (AUD that relapsed vs. AUD that remained abstinent) showed significant differences in correlations of OCDS scores and left amygdala rsFC with (i) right anterior cingulate cortex (z = −2.366, p = 0.009), (ii) parietal regions including right and left posterior cingulate cortex (right: z = −2.865, p = 0.002; left: z = −2.806, p = 0.003), right and left inferior parietal lobule (right: z = −2.445, p = 0.007; left: z = −1.964, p = 0.025), (iii) right thalamus (z = −1.762, p = 0.039), (iv) left cerebellum (z = −2.204, p = 0.014) and (v) right pallidum (z = −2.16, p = 0.015). No significant correlation differences between right amygdala rsFC and OCDS scores were observed. T-tests showed no significant differences in the strength of left or right amygdala rsFC between AUD that later relapsed vs AUD that remained abstinent (p>0.05). T-tests also showed no significant differences in OCDS scores between groups (t(34)-0.088, p = 930).

Conclusions: Findings from this preliminary study suggest that there are characteristic differences in the association of OCDS scores and left amygdala rsFC networks at ~2 weeks of abstinence between AUD that later relapse vs AUD that remain abstinent. Only AUD that subsequently relapsed showed significant negative correlations between these variables suggesting that higher incentive salience (i.e. higher obsessive drinking thoughts or urges) is associated with lower rsFC between left amygdala and a distributed cortico-thalamic-striatal network (or those with low incentive salience had higher amygdala rsFC). Interestingly, there were no significant group differences when only examining amygdala rsFC strength or OCDS scores. Further studies with larger sample sizes examining additional incentive salience or neurofunctional domains (Kwako et al. 2019) and additional regions within the mesocorticolimbic dopamine system need to be conducted to confirm robustness of current findings.

Keywords: Alcohol Use Disorder, Amygdala-Based Networks, Incentive Salience, Resting-State fMRI, Relapse Biomarkers

Disclosure: Nothing to disclose.

W269

Resting State Functional Connectivity and Parenting Stress in Postpartum Women Receiving Buprenorphine Treatment for Opioid Use Disorder

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Background: Every day, more than 130 people in the U.S. die after overdosing on opioids. The number of pregnant women with OUD quadrupled from 1999 to 2014. Thus about 100,000 women are affected by OUD per year and may receive buprenorphine replacement treatment (BT) to prevent withdrawal. Despite animal models that document opioid-induced deficits in maternal behavior, little is known about human maternal brain and behavior under conditions of OUD and BT – known to involve increased parenting stress. We conducted a longitudinal functional magnetic resonance imaging (fMRI) pilot study in humans to examine effects of BT on maternal resting-state functional connectivity (RSFC), focusing on two brain circuits and parental stress. RSFC in the default mode network (DMN) is a key brain circuit for stimulus-independent, internally focused thought that has been associated with cognitive function and altered in OUD. Maternal behaviors are known to be governed by evolutionary conserved Maternal Behavior Neurocircuit (MBN) to regulate maternal caring and defensive behaviors, with RSFC between periaqueductal gray (PAG) and hypothalamus (HYP) indicating normally reciprocal regulation required for sensitive parental care.

Methods: We studied 32 mothers who completed fMRI scans at 1 month (T1) and 4 months postpartum (T2), including seven mothers receiving buprenorphine for OUD and 25 non-OUD mothers as a comparison group (CG). The participants underwent a 6-minute resting-state fMRI scan at each time point. We measured parenting-related stress using the Parenting Stress Index (PSI) at both timepoints. We explored RSFC in the DMN using a seed in the precuneus; and the maternal behavior neurocircuit by measuring PAG-HYP RSFC.

Results: While there was no group difference in PSI at either time point, the groups differed in RSFC in DMN and MBN. At T1, BT mothers, as compared to CG, showed greater precuneus-dependent RSFC with the right inferior frontal gyrus, bilateral lentiform nuclei, and bilateral visual cortex, and lesser precuneus-dependent RSFC with the dorsal medial prefrontal cortex; the parenting stress was associated with precuneus-dependent RSFC with posterior cingulate cortex, superior frontal gyrus, right posterior insula, and dorsal anterior cingulate cortex; in BT, but not CG, mothers, the precuneus-dependent RSFC with dorsomedial prefrontal cortex was positively associated with parenting stress, while the precuneus-dependent RSFC with right ventrolateral prefrontal cortex and midbrain was negatively associated with parenting stress. Within MBN, PAG-hypothalamus RSFC in the PAG was stress dependent in the BT group but not the HC group at T1. At T2, BT mothers, as compared to CG, showed greater precuneus-dependent RSFC with the dorsal precuneus and left superior frontal gyrus; the parenting stress was associated with precuneus-dependent RSFC with the midbrain and left orbital frontal cortex. From T1 to T2, BT mothers, as compared to CG, showed greater increases in the precuneus-dependent RSFC with the left temporal pole and right superior parietal lobule and greater decreases in the precuneus-dependent RSFC with the right

inferior frontal gyrus, right lentiform nucleus, bilateral caudate nuclei, right precentral gyrus, right middle temporal gyrus, and left posterior insula; the changes of parenting stress from T1 to T2, controlling for the baseline, was associated with the changes in precuneus-dependent RSFC with middle cingulate cortex.

Conclusions: Parental stress has been linked with insensitive parenting that may contribute to adverse child socioemotional outcomes and is increased for mothers with OUD and BT. Our data suggest that parental stress, among early postpartum mothers with OUD and BT vs. controls, affects connectivity in the DMN and MBN – critical brain circuits for general cognition and specific maternal behavior respectively. Our results on parenting stress effects also fit with the literature on reduced DMN connectivity for opioid replacement treated OUD patients conferring increased risk of relapse. This exploratory study supports potential endophenotypes and biological mechanisms for investigating both the therapeutic benefits and risks of OUD and buprenorphine for maternal care behavior and infant outcome.

Keywords: Perinatal Stress, Functional Connectivity, Opioid Use Disorder, Buprenorphine, Maternal Behavior

Disclosure: Nothing to disclose.

W270

PCSK9 and the Liver-Brain Axis: A Novel Therapeutic Target for Alcohol Use Disorder

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Background: Alcohol Use Disorder (AUD) is a chronic disorder that negatively impacts personal and public health; however, the underlying pathophysiology is poorly understood but robust evidence suggests genetic and environmental components. We recently identified proprotein convertase subtilisin/kexin 9 (PCSK9) as a novel target that is epigenetically regulated by alcohol intake. While a major role of PCSK9 in hepatic function and lipid regulation has been clearly established, other pleiotropic effects remain poorly understood. Given the well-established deleterious effects of alcohol on the liver, we hypothesized that PCSK9 inhibition would attenuate alcohol-induced inflammation in an animal model. Moreover, since existing research suggests a positive association between PCSK9 expression in the brain and psychopathology, with increased levels of PCSK9 in the cerebrospinal fluid (CSF) of individuals with neurodegenerative diseases, we tested if chronic alcohol use would increase PCSK9 expression in CSF of individuals with AUD.

Methods: PCSK9 inhibitor (alirocumab, 50 mg/kg) or vehicle was administered weekly for 6 weeks to rats receiving a 12% alcohol liquid diet or an isocaloric control diet ($n = 32$). For molecular characterization and histopathology analysis, rat serum and liver samples were obtained at the end of the alcohol exposure. For the quantification of PCSK9 levels in human CSF, samples from subjects with AUD admitted to an inpatient rehabilitation program ($n = 42$) and controls ($n = 25$) were utilized. CSF samples in AUD were assessed at two-time points, at day 5 and day 21 after admission. Furthermore, human plasma samples were collected and measured from the individuals with AUD.

Results: In chronically alcohol-fed rats, inhibition of PCSK9 improved alcohol-induced hepatic triglyceride accumulation ($p < 0.0001$), hepatocellular injury ($p = 0.0133$ for AST), and hepatic inflammation ($p < 0.0001$ for TNFa, IL-1β, and MCP-1). Clinically, PCSK9 in human CSF was significantly increased in the AUD group at day 5 and day 21 compared to the controls ($p < 0.0001$). Plasma PCSK9 levels were correlated positively with CSF PCSK9 levels in the subjects with AUD ($p = 0.0493$).

Conclusions: We demonstrate that chronic anti-PCSK9 treatment using the monoclonal antibody alirocumab attenuates alcohol-induced steatohepatitis in a rat model of alcohol dependence. Given the large unmet clinical need for effective and novel treatments for AUD and alcohol liver disease, anti-PCSK9 treatment with a monoclonal antibody that spares liver metabolism is a viable new therapeutic possibility. Furthermore, we show that PCSK9 is increased in CSF in individuals with AUD, suggesting a potential role of PCSK9 in AUD beyond the liver including the brain.

Keywords: Alcohol Use Disorder, Proprotein Convertase Subtilisin/Kexin 9, Liver-Brain Axis, Alcohol-Induced Inflammation, Cerebrospinal Fluid

Disclosure: Nothing to disclose.

W271

Zinc Modulates Cocaine Mediated DAT Function and Abuse Vulnerability

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Background: Cocaine binds to the dopamine (DA) transporter (DAT) which inhibits the removal of DA from the synapse and results in an accumulation of synaptic DA concentrations. This process underlies the "euphoria" that is typically associated with cocaine use and is central to the development of cocaine abuse and addiction. The trace element zinc ($Zn2+$) interacts with numerous proteins throughout the body and is essential for many biological processes, yet its role in the brain is understudied. $Zn2+$ can be found in two forms, bound (structural) and labile (free) which is tightly regulated by a family of 24 transporters that move $Zn2+$ around the cell. This unbound free $Zn2+$ can be co-packaged into glutamate-containing vesicles by the transporter ZnT3 (Slc30a3) and co-released with glutamate from presynaptic neuron terminals. Using in vitro cellular assays, it has been shown that $Zn2+$ decreases [3H]dopamine uptake by DAT and increases [3H]-WIN 35,428 (a cocaine analog) binding to DAT. Thus, our lab has focused on the interaction between DAT and $Zn2+$ as a function of cocaine abuse vulnerability.

Methods: Animals consisted of male C57Bl/6J mice on a special diet with low $Zn2+$ (5ppm, deficient) or a genetic knockout of ZnT3 which prevented these mice from packaging $Zn2+$ into glutamate vesicles and subsequent $Zn2+$ release. To determine if cocaine alters $Zn2+$ levels in the striatum, mice were chronically treated with cocaine, and brain tissue was collected and processed to quantitatively assess trace metal content in the striatum. $Zn2+$ was chemically removed from the striatum with TPEN to assess striatal $Zn2+$ availability on cocaine psychomotor sensitization. In vivo voltammetry was utilized to assess dopamine release and clearance in ZnT3 wild-type, knockout, and C57 mice on a deficient diet. To assess behavior, we used cocaine related tasks such as: locomotor sensitization, conditioned place preference, and self-administration. The metabolic activity of ZnT3 wild-type and knockout mice was assessed prior to and after chronic cocaine exposure using the radiotracer [18F]FDG with positron emission tomography (PET). Finally, human caudate putamen samples from chronic cocaine users were assessed for $Zn2+$ levels and correlated to benzoylecgonine (a long-lasting cocaine metabolite) to determine any relationship between cocaine use and $Zn2+$ levels.

Results: Without manipulating cocaine exposure, both ZnT3 knockout and C57 mice on a deficient Zn2+ diet had significantly lower levels of Zn2+ in the striatum, allowing for a suitable experimental model to decrease striatal Zn2+. Cocaine exposure significantly increased striatal Zn2+ levels compared to saline exposed C57 mice. Compared to wild-type, ZnT3 knockout mice show significantly lower dopamine release and greater dopamine clearance when exposed to increasing doses of cocaine (0, 5, 10, 20 mg/kg). Dietary Zn2+ deficiency significantly decreased dopamine release but had no effect on clearance. Directly removing Zn2+ by chemical chelation via TPEN in the dorsal striatum led to a significant decrease in sensitization to cocaine. Using our genetic and dietary models, we found that expression of cocaine sensitization and place preference were reduced in both ZnT3 knockout and dietary Zn2+ deficient mice. Metabolically, knockout mice have significantly less [18F]FDG uptake prior to and after chronic exposure to cocaine in striatal and cortical regions. The TXRF analysis of human tissue found a significant negative correlation between striatal Zn2+ concentration and plasma benzoylecgonine levels.

Conclusions: The relationship between Zn2+ and the drug abuse potential of cocaine was examined in a comprehensive and translational manner to reveal key novel insights into the mechanism of action of cocaine by showing that Zn2+ modulates cocaine potency, reinforcement, and abuse vulnerability via direct modulation of cocaine-mediated DAT inhibition. Our findings have important implications for environmental and biological factors relating to Zn2+ availability and suggest that Zn2+ manipulations may be important for the treatment of cocaine use disorders.

Keywords: Cocaine, Addiction, Zinc

Disclosure: Nothing to disclose.

W272

Genetics of Alcohol Use Disorder Treatment Outcomes: Genome-Wide Pharmacogenomics Analyses

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Background: Randomized controlled trials suggest that naltrexone prevents excessive alcohol consumption, while acamprosate is effective in supporting abstinence. While there is evidence of efficacy for both medications, and both are FDA approved for treating alcohol use disorder (AUD), many patients do not benefit from these treatments. Thus, identification of genetic variants that influence response to these medications may help determine their mechanisms of action, and eventually help with treatment outcome prediction leading to improved individualized treatment selection. No genome-wide association studies (GWASs) of AUD treatment outcomes have been published. Here, we performed a series of GWAS using data from three of the largest studies of acamprosate and naltrexone completed to date: The US COMBINE study and the German PREDICT study, both of which investigated response to acamprosate and naltrexone, and the Mayo Clinic Center for Individualized Treatment of Alcoholism (CITA) study, which investigated only acamprosate treatment outcomes.

Methods: The primary analyses included patients treated with acamprosate, naltrexone, or placebo to identify predictors of treatment outcomes regardless of pharmacological intervention. Drug-stratified analyses were then run to identify treatment-specific (i.e. pharmacogenomic) predictors of acamprosate and naltrexone response. In these analyses, the primary treatment outcome measures were: (1) relapse to any drinking during the first three months of treatment and (2) relapse to heavy drinking (≥5 drinks for men, ≥4 drinks for women in one day) within the same time period. All GWAS were performed on the three datasets (COMBINE N = 505, PREDICT N = 266, CITA N = 436) separately, followed by fixed-effects meta-analysis. In each dataset, SNP associations with relapse and heavy relapse were assessed using logistic regression models adjusted for genetic principal components, if needed, to control for population stratification; all analyses were run with and without relevant clinical covariates, such as baseline consumption measures, which may represent mediating factors. Gene-level tests and gene-set analyses were performed using MAGMA implemented in FUMA, and leave-one-out polygenic risk score (PRS) analyses were used to test for a reproducible polygenic predictor of treatment outcomes between datasets.

Results: No individual SNPs were associated with relapse or heavy-drinking relapse at a genome-wide significance level ($p<5E-08$) in the meta-analyses when analyzing data across all drug groups, regardless of whether the analysis was adjusted for clinical covariates or not. Similarly, in the separate meta-analyses restricted to acamprosate-treated and naltrexone-treated subjects, no SNPs were significantly associated with relapse or heavy-drinking relapse. Gene-level meta-analyses in the full dataset and the acamprosate-treated subset also identified no significant associations; however, in the gene-level analyses of heavy-drinking relapse in naltrexone-treated subjects without clinical covariate adjustment the GNPTAB gene ($p = 3.6E-07$) achieved the significance threshold for gene-level tests. In leave-one-out PRS analyses, a relapse PRS derived from PREDICT+CITA GWAS results was marginally associated with higher risk of relapse in COMBINE data (max $R2=0.023$, $p = 0.0045$). Similarly, a heavy-drinking relapse PRS based on PREDICT+CITA GWAS results showed marginal evidence of association with higher risk of relapse in the COMBINE data (max $R2=0.012$, $p = 0.027$). Relapse and heavy-drinking relapse in PREDICT and CITA cohorts was not predicted by corresponding PRSs based on a meta-analyses of the other two studies.

Conclusions: Our GWAS did not identify individual variants associated with treatment outcomes in AUD at a genome-wide significance level. One gene was significantly associated with heavy relapse after correction for the number of genes in the genome – this gene, GNPTAB, encodes the N-acetylglucosamine-1-phosphate transferase subunits alpha and beta. Although this association should be interpreted cautiously given the multiple GWAS that were performed here, it warrants further investigation. PRS analyses suggest a possible polygenic signal associated with relapse and heavy-drinking relapse. Further analyses of these data are ongoing, including investigation of candidate genes, such as OPRM1, and medication-specific neurotransmitter/receptor gene pathways.

Funding: R21 AA25214 (Biernacka/Karpyak)

Keywords: Pharmacogenomics, Alcohol Use Disorder, Acamprosate, Naltrexone, Genome-Wide Association Studies

Disclosure: Nothing to disclose.

W273

Cannabis 'Side Effect' Syndromes Seen Within 90 Days After 1st Use: Refined Estimates Based on a Novel 'Gamma Prime' Statistic

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Background: Among newly incident cannabis users in the United States (US) observed within ~90 days after 1st cannabis use, some 1.2% to 2.2% are affected by a 'mild' or more serious DSM-IV-like cannabis dependence syndrome (CDS). Studying pairs of CDS indicators and other cannabis 'side effect' problem-experiences (SEPE), we aim to identify 'target product profiles' (TPP) of importance in pathological processes leading toward CDS. These TPP should become important in the CDS medications development context, as well as cannabis product safety pharmacovigilance, and in early case identification efforts.

By conventional definition in epidemiology as applied in CDS research, any TPP-candidate syndrome requires greater than chance co-occurrence of at least two CDS indicators or other SEPE, as might be measured via familiar odds ratio (OR) estimates from generalized linear models with logit link functions. In this new work, we describe development of a novel Bayesian 'gamma prime' estimator (γ') as a statistically more discriminating and powerful approach in TPP research on syndromes such as CDS.

Methods: Each year, 2004-2014, United States (US) populations under study consisted of non-institutionalized civilian residents age 12-years-and-older, male and female, sampled for National Surveys on Drug Use and Health. Computerized self-interviews identified 3710 newly incident cannabis users (~1-90 days after 1st use) and assessed CDS indicators and side effects (SEPE). Unweighted and analysis-weighted year-specific and aggregate SEPE and SEPE-SEPE pair incidence proportions were estimated, from which OR and novel γ' were derived.

Results: We show traditional odds ratio and novel γ' estimates for the observed 128 SEPE-SEPE pairs (8 potential pairs were unobserved). This contrast helps illustrate a generally greater discrimination and statistical power of the γ' estimator – i.e., a more refined estimate. As an example, our estimate for rapid-onset co-occurrence of 'cannabis causing a serious problem at home or work or school' and 'continuing to use cannabis despite physical problems,' yields an OR estimate of 5.1 (95% CI = 2.3, 12.6). The corresponding gamma prime estimate is 0.56 (CI = 0.31, 0.79).

Conclusions: Studying epidemiological samples of US cannabis initiates very soon after 1st use, we now can draw attention to a number of TPP-candidates of potential interest in cannabis syndrome medications research and development. Some (but not all) of the SEPE-SEPE pairs indicate that a cannabis syndrome of potential clinical significance has formed (e.g., 'mild' DSM-5-like CDS).

These findings can help guide cannabis syndrome investigations now underway as part of the NIH Adolescent Brain and Cognitive Development (ABCD) research initiative. These results also should be useful when drawing up clinical practice guidelines for fast effective syndrome screens of primary care patients seen soon after cannabis onset, akin to clinical screening guides designed for adolescent-onset and adult drinkers.

Finally, the Bayesian 'gamma prime' estimator may prove to be of methodological importance in TPP research on early syndrome formation. This novel estimator seems to be more discriminating and statistically powerful than familiar odds ratios in the medications development research context when early syndrome formation is of interest.

Keywords: Cannabis, Epidemiology, Biostatistics, Screening, Medications

Disclosure: Nothing to disclose.

W274

Alcohol Exposure Affects Brain Energetic Cost and Power

Abstract not included.

W275

An Investigation Into the Role of Metabolic Biomarkers in the Anticipation of Reward in Treatment Seeking Individuals With Alcohol Use Disorder

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Background: Recent studies have highlighted the importance of the gut-liver-brain axis in alcohol use disorder (AUD) and alcohol seeking behaviors. Aspects of the gut-liver-brain axis, such as hormones, have been widely characterized as playing a role in feeding behavior, energy homeostasis, and more recently shown to modulate central reward and stress pathways related to alcohol seeking behaviors. However, the role of other features of the gut-liver-brain axis in reward and stress related pathways and alcohol seeking behaviors remains unknown.

Efficient and normal metabolism is required for energy homeostasis and the production of essential hormones in this system. As such, dis-regulation of metabolism or changes in metabolic factors underlying the gut-liver-brain axis may result in changes in central reward pathways and alcohol related behaviors. Studies focusing on feeding behavior and obesity have revealed changes in circulating gut-liver hormones and triglycerides levels are associated with reductions in neural response to palatable food cues in brain regions regulating reward and feeding behaviors. Moreover, in another study investigating the role of blood glucose levels (HbA1c,) and food reward related brain activation in diabetics, found diabetics with improved HbA1c (after gastric bypass procedure) showed substantially higher activations in brain regions associated with reward compared to diabetics with no improvements to HbA1c. Taken together, these findings suggest that the regulation of metabolism plays a role in feeding and reward related pathways and may also modulate the gut-liver-brain axis and its role in these pathways.

To better understand the role of metabolism in the gut-liver-brain axis and reward and stress related pathways, we investigated the relationship between metabolic factors (cholesterol, triglyceride, and BMI) and reward anticipation in detoxified alcoholics. Using the Monetary Incentive Delay (MID) we assessed neural responses to the anticipation of rewards in three conditions: high reward gain, high reward loss and low reward gain and loss

Methods: Multiple mediation analyses were used to model the association between alcohol use (measured by AUDIT dependence score) and reward anticipation in seventy-three detoxified alcoholics. Triglycerides, Hgb_A1C, and BMI were included as mediators in all models with AUDIT Dependence score predicting reward anticipation.

Results: Alcohol use predicted neural activation in the left nucleus accumbens in anticipation of losing a high value reward, this association was mediated by Hgb_A1C levels ($p = .023$). A1C levels also appeared to be mediating the same association between alcohol use and left ventral striatum activity however it did not meet significance ($p = .05$). Triglycerides also significantly mediated the association between alcohol use and left amygdala activity in anticipation of losing a high value reward ($p = .033$).

Conclusions: These preliminary findings highlight the potential role of metabolism in reward and motivation pathways. Research on feeding related behavior and obesity suggests metabolic factors may play a role in responsiveness to food related cues and modulate feeding behavior and response to food related reward. Similar metabolic factors may also play a role in responsiveness to addiction related reward and may contribute to the role of the gut-liver-brain axis alcohol seeking behavior.

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

W276

Epigenetic Regulation of GABA-A Receptor Subunits and Neurosteroid Biosynthesis in Subjects With Alcohol Use Disorder (AUD)

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Background: Alcohol use disorder (AUD) is a chronic relapsing disorder affecting 6% of the US adult population. There have only been a few studies investigating the mechanisms whereby alcohol affects GABAA receptor (GABAAR) subunit composition and GABAergic positive allosteric modulation elicited by neurosteroids.

The GABAAR is a pentameric protein complex formed by subunits, whose combination accounts for the diverse pharmacological properties of GABAAR. Synaptic GABAAR mainly containing alpha1,2,3 along with beta and gamma subunit variants, mediates phasic currents, while extrasynaptic GABAAR containing alpha4,5,6 and delta are responsible for a persistent tonic inhibition. The extrasynaptic GABAAR-mediated inhibition is enhanced by allopregnanolone (Allo) and its equipotent GABAergic isomer, pregnanolone (PA). The biosynthesis of these neurosteroids is initiated by cholesterol translocation into the inner mitochondrial membrane by the 18 kDa translocator protein (TSPO). Cholesterol is then converted by P450scc into pregnenolone, which is further metabolized into progesterone by 3beta-hydroxysteroid dehydrogenase (3β-HSD). 5alpha-reductase type 1 (5alpha-R1) and 3alpha-HSD then convert progesterone into Allo. Previous work from our group showed reduced mRNA and protein levels of delta subunit in the cerebellum of AUD subjects suggesting an impaired sensitivity of GABAAR to neurosteroids. However, the long-term effects of alcohol exposure on neurosteroid biosynthesis and its consequences on GABAergic neurotransmission in the cerebellum of individuals with AUD remain underinvestigated.

Methods: In a cohort of postmortem brains from 25 pairs of controls and AUD subjects (New South Wales Brain Tissue Resource Centre, University of Sydney, Australia), we determined the mRNA levels of GABAAR subunits and neurosteroid biosynthetic enzymes by RT-qPCR. DNA methylation levels were assessed by methylated DNA immunoprecipitation (MeDIP) using the MagMeDIP kit (Diagenode). Protein levels were determined by Western blot and neurosteroid concentrations were quantified by gas chromatography-mass spectrometry (GC-MS).

Results: As previously observed for the GABAAR delta subunit, GABAAR alpha2 mRNA and protein expression were reduced. These changes were associated with increased DNA methylation levels at the alpha2 promoter region in the cerebellum of AUD subjects. These data suggest that both the primarily synaptic (alpha2) and the extrasynaptic (delta) GABAAR subunit subtypes are affected in AUD. In addition, neurosteroid biosynthesis was altered in the cerebellum with reduced mRNA expression of TSPO, 5alpha-R1 and 3alpha-HSD. Increased DNA methylation levels were observed at the promoter region of 3alpha-HSD. As expected, this resulted in marked reduced levels of Allo and PA in the cerebellum.

Conclusions: Whether changes in GABAAR subunit expression result from a direct effect of alcohol or whether this is secondary to alcohol affecting neurosteroid biosynthesis remains to be further investigated. Given the key role of Allo and PA in the fine-tuning of GABAAR-mediated inhibition, our data suggest that alcohol-induced impairments in GABAergic neurotransmission might be profoundly impacted by reduced neurosteroidogenesis. Thus, neurosteroid-based agents might be promising as a novel treatment approach for AUD.

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Keywords: Alcohol and Substance Use Disorders, Neurosteroids, GABA-A Receptors, Epigenetics

Disclosure: Nothing to disclose.

W277

Subjective and Objective Longitudinal Assessments of Incubation of Cue-Induced Drug Craving in Cocaine-Addicted Individuals

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Background: Cue-induced craving is a major contributor to relapse in treatment-seeking addicted individuals. Animal studies have shown that cue-induced drug-seeking increases (or incubates) during the initial phase of abstinence, presumably reflecting a period of heightened relapse vulnerability. However, these findings have not been robustly translated, as most human studies show a steady decline in craving with increasing abstinence duration. In a previous cross-sectional study, we employed EEG-derived late positive potential (LPP) as a biomarker for cue-induced craving, providing evidence for incubation of cue-induced craving in cocaine addiction. Here, we used a within-subjects longitudinal design spanning 5 follow-ups acquired every 3 months over 12 months of abstinence in individuals with cocaine use disorders (iCUD) in an effort to robustly translate animal findings to human addiction.

Methods: Thirteen mostly treatment-seeking iCUD completed three assessments: at baseline (7-40 days), 3 months (55-96 days) and 6-months (110-185 days) after abstinence. At each visit, participants completed the Cocaine Craving Questionnaire to report their unprovoked subjective craving. In addition, EEG data were recorded to assess cue-reactivity as participants passively viewed 30 cocaine and 30 neutral pictures. For each picture, participants rated the intensity of cocaine 'wanting' (i.e., subjective cue-induced drug craving). The LPP elicited by cocaine-related relative to neutral pictures was extracted.

Results: As expected, subjective unprovoked craving decreased linearly [$F(1,12)=19.42$, $p=.001$] with abstinence. Interestingly, subjective cue-induced cocaine wanting ratings showed an inverted u-shaped quadratic effect [$F(1,19)=19.46$, $p=.011$] such that it was higher at 3 months relative to both baseline and 6-months follow-up. A similar pattern, albeit at a trend level, was also observed for the LPP amplitudes [$F(1,12)=2.86$, $p=.12$]. Analyses with longer time points (9 and 12 months) and more subjects are underway and novel results will be included in the conference presentation.

Conclusions: To our knowledge the current study is the first to use both subjective and objective indices to show longitudinal evidence of incubation and subsequent decline in cue-induced craving in iCUD. These results underscore the need for incorporating measures of cue-induced craving in addition to baseline measures, which are commonly used in clinical settings. Ultimately, we aim to identify precise time-courses of personalized

craving trajectories that could be optimally targeted for individualized treatment deployment for preventing relapse.

Keywords: Incubation of Drug Craving, Cocaine Addiction, EEG/ERP Electrophysiology, Drug Relapse

Disclosure: Nothing to disclose.

W278

Risky Behaviors: Are They Traits or Developmental States?

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Background: A number of behaviors in immature populations are associated with elevated risk for substance use. Increased novel reactivity, preferences for sweet solutions, impulsive choice, novel object recognition, or novelty preferences are the most common and are generally conserved across species. A number of studies, including ours, have examined these risk behaviors at a single age before assessing whether they can predict drug-taking behavior. For example, strong object recognition on the novel object recognition (NOR) task at postnatal day (P) 25 is associated with less cocaine self-administration (Jordan and Andersen, 2018). Here, we examined a number of behaviors in a longitudinal and cross-sectional design in male and female rats to better understand the trajectory of change across age.

Methods: Sprague-Dawley, male and female rats were used for these studies. Only one rat/sex/litter was used for the longitudinal study and any littermates were distributed across the conditions for the cross-sectional study. All subjects were housed under standard conditions and tested on P24 (juvenile), P55 (late adolescence), and P90 (adulthood).

Sucrose preferences: A two bottle free choice was used to measure preferences to a 1% sucrose solution versus water for 2 hours. A total of $N = 20$ subjects were used.

Novel object recognition: Rats are habituated to the testing arena for a 6 min trial, followed by two 6 min trials where two identical objects are placed in the arena; each trial is separated by a three min break. For the test trial, one of the objects is replaced with a novel object and the time spent with the novel and the habituated objects are recorded. The discrimination index: time with novel object/(time with novel and habituated objects) is the dependent measure of novel object recognition. A total of $n = 15$ males and 14 males were used for this study.

Novelty preferences: Following three days of habituation to one side of the testing chamber, rats are placed into a two-sided chamber in a free-choice paradigm for novelty preferences. Great care was taken to assure that the novel side was different at each age of testing, by varying floor texture, scent, and wall decoration. A total of $n = 21$ males and 19 female rats were used for this study.

Results: Sucrose preferences: Preferences for a 1% sucrose solution nearly doubled between P24 and P90 male and female rats (Student's t-test, $P<0.01$). Juvenile male rats started drinking less sucrose than females, but surpassed the amount consumed by females by P90.

Novel object recognition: Sex differences in the discrimination index were not apparent at P24 ($F1, 27=3.7$, $P=0.07$), nor at P55 ($P=0.6$). No age differences were present between P24 and 55 in a longitudinal analysis ($P=0.72$), suggesting that NOR performance is a stable trait.

Novelty preferences: High novelty-seeking rats are associated with greater vulnerability to drug abuse. To determine the trajectory for both a high and a low group of novelty preferring subjects, we subdivided rats into high (>650 sec) or low (<550 sec) groups based on their preference for the novel side. Two different trajectories of development were observed (high/low x Age [within subject variable]: $F1, 9 = 8.5$, $P< 0.05$) whereby high novelty-seeking rats were significantly different at P24, and then decreased preferences; however, preferences were still elevated at P55 and P90 relative to the low group. Similar age- and sex-related trends were observed for males and females in the longitudinal cohort and the cross-sectional cohort. The high novelty-seeking rats represent ~25% of the population.

Conclusions: Early identification of individuals at risk for substance abuse could lead to early intervention that would significantly impact of addiction rates. Age-related changes in sucrose drinking were observed, but the overall group was homogenous and therefore sucrose preferences are unlikely to provide unique identification of risk. The stability of NOR performance across age supports its use as an early index of substance use. The observation that females have higher discrimination indices than males supports suggests that it may be a more sensitive measure for at-risk boys.

High-novelty seeking confers elevated risk to use substances both clinically (in pediatric populations) and pre-clinically (mainly in adult populations). Our observation that these subjects have a unique trajectory and maintain high novelty-seeking into adulthood suggests that this trait can be used for early identification purposes. By identifying behaviors that parallel the clinical phenotype, new targets of intervention could be developed that are appropriate for the younger age group. Novel therapeutics in developing systems will most likely capitalize the age-related changes in the development.

Keywords: Developmental Trajectory, Risk Factors, Novelty Seeking

Disclosure: PESI, Consultant (Spouse), Springer, Board Member.

W279

Extended Cortical Circuit Mechanisms for Large-Scale Cognitive Networks

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Background: Optimal cognitive performance requires the robust activation of large-scale cortical networks. Abnormalities in these cognitive networks appear in many pathologies characterized by cognitive dysfunction including addiction, depression, autism, and schizophrenia. Our inability to treat cognitive dysfunction is reflected by our poor understanding of the neural circuit mechanisms allowing for cognitive network emergence. The claustrum is a subcortical nucleus that is functionally connected (as assessed by fMRI) with cortical regions that are part of both task-positive and task-negative cognitive networks. Here, we test the hypothesis that the claustrum is a central hub for connecting cortical cognitive network components.

Methods: To test this, we used both viral anatomical tract tracing and channelrhodopsin-assisted circuit mapping (CRACM) to probe the existence of long-range, cortico-claustral-cortical circuits in mice ex vivo.

Results: We found that projections arising from prefrontal cortices command posterior parietal regions through the claustrum. We also found that prefrontal cortex drives claustrum neurons projecting back to the contralateral prefrontal cortical area of origin.

Conclusions: These data give rise to a model in which prefrontal cortices command fronto-parietal synchrony through claustrum. As such, this extended cortical circuitry represents a possible neural circuit explanation for fronto-parietal network support.

Keywords: Cognition, Claustrum, Frontoparietal
Disclosure: Nothing to disclose.

W280

PharmacoMRI to Investigate the Functional Selectivity of 5-HT1A Receptor Biased Agonists

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Background: The emerging concept of "functional selectivity" or "biased agonism" denotes the phenomenon whereby agonists can preferentially direct receptor signalling to specific intracellular responses, thus potentially avoiding side effects and improving therapeutic effects. In neuropharmacology, this opens the possibility of identifying drugs that differentially target receptors in distinct brain regions.

Methods: The aim of this study was to investigate biased agonism by using non-invasive functional imaging in vivo. The cerebral blood oxygen level dependent (BOLD) signal changes induced by increasing doses of two serotonin 5-HT1A receptor biased agonists, NLX-112 and NLX-101, were mapped in anaesthetized rats by pharmacological magnetic resonance imaging (phMRI, 7T). Although both compounds display high affinity, selectivity and agonist efficacy for 5-HT1A receptors, NLX-101 is known to preferentially activate post-synaptic receptors, whereas NLX-112 targets both pre- and post-synaptic receptors.

Results: NLX-112 and NLX-101 induced different positive and negative hemodynamic changes patterns at equal doses, in agreement with previous findings. NLX-112 produced higher BOLD changes than NLX-101 in certain brain regions, whereas the drugs had comparable effects in other regions. Importantly, NLX-101 had no effect attributable to pre-synaptic receptors at 0.16 mg/kg, contrary to NLX-112. NLX-112 also induced more widespread changes of functional connectivity.

Conclusions: The present phMRI study demonstrates that two closely-related agonists display notable differences in their hemodynamic "fingerprints". These data support the concept of biased agonism at 5-HT1A receptors and raise the prospect of identifying novel therapeutics which exhibit improved targeting of brain regions implicated in neurological and neuropsychiatric disorders.

Keywords: 5-HT1A Receptors, Biased Agonism, 7T fMRI, Functional Selectivity, Serotonin
Disclosure: Nothing to disclose.

W281

Open Board

W282

Prefrontal Circuitry in Control of Limbic Thalamus Requires Juvenile Social Experience to Establish Adult Sociability

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Background: Loneliness is increasingly being recognized as a serious threat to mental health. Social isolation during the juvenile critical window is particularly detrimental to the maturation of medial prefrontal cortex (mPFC) and establishment of appropriate levels of adult sociability. However, the neural circuit mechanisms underlying these phenomena are poorly understood. Here, we aimed to identify specific mPFC circuits in control of social behavior whose maturation is profoundly affected by juvenile social experience in mouse.

Methods: We integrated techniques to measure (fiber photometry imaging, patch-clamp electrophysiology) and manipulate (optogenetics/chemogenetics) the activities of selective circuits during social behavior (3chamber test, reciprocal interaction) in mice undergo juvenile social isolation (p21-p35).

Results: We find that transient juvenile social isolation leads to a failure to activate adult mPFC neurons projecting to the limbic thalamus ($p = 0.007$, $N = 19$-23 mice, 2way ANOVA), which relays signals to various components of the classical reward circuitry. Chemogenetic or optogenetic suppression of mPFC projection to limbic thalamius is sufficient to induce social behavior deficits ($p = 0.006$, $N = 10$ mice for chemogenetics, $p < 0.001$, $N = 12$ mice for optogenetics, paired t-test) without affecting motor activity, anxiety-related behaviors, or preference toward rewarding food. These results can be mechanistically explained by our findings that juvenile social isolation leads to reduced intrinsic excitability of mPFC neurons projecting to limbic thalamus ($p < 0.001$, t-test, $N =$ 19-23 cells) and an aberrantly increased inhibitory drive from a subclass of deep layer somatostatin-expressing low-threshold spiking (LTS-SST) interneurons ($p = 0.039$, t-test). When these interneurons are aberrantly activated with chemogenetics, sociability is reduced ($p = 0.034$, $N = 10$ mice, paired t-test). Importantly, sociability deficits caused by juvenile social isolation can be rescued by chemogenetic or optogenetic activation of mPFC neurons projecting to limbic thalamus ($p = 0.007$, $N = 12$ mice for chemogenetics, $p = 0.041$, $N = 13$ mice for optogenetics, paired t-test).

Conclusions: Our study identify a novel pair of specific mPFC excitatory and inhibitory circuits in control of social behavior whose maturation is profoundly affected by juvenile social experience. As these circuits are sensitive to experience-dependent modulation, they are attractive circuit targets for the amelioration of social deficits shared across of range of disorders. Ultimately, our study may inspire interventions that improve social functioning in neurodevelopmental and psychiatric disorders by specifically targeting prefrontal top-down circuits to impact subcortical hubs, such as the limbic thalamus.

Keywords: Social Behavior, Medial Prefrontal Cortex, Thalamus, Juvenile Critical Period, Inhibitory Neurons
Disclosure: Nothing to disclose.

W283

Brain Network Connectivity Abnormalities Predict Dimensional Symptoms Across Mood and Anxiety Disorders

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Background: Patients with "anxious misery" disorders, including major depression, post-traumatic stress disorder, social anxiety disorder, generalized anxiety disorder and persistent depressive disorder exhibit heterogeneous symptom profiles, characterized

by the RDoC negative valence constructs of "loss" and response to sustained threat. Studies using resting-state functional magnetic resonance imaging (rsfMRI) have described large scale brain network differences from controls in a variety of DSM diagnoses but have not examined symptom correlations across disorders.

Methods: Participants (n = 100) were recruited with a score > 10 on the PHQ-9, completed a battery of clinician administered and self-report measures and were scanned using the Human Connectome Project (HCP) multiband rsfMRI protocols. Clinical data consisting of 138 individual items from eight symptom scales (Hamilton depression rating scale (HAMD), Montogomery-Asberg depression rating scale (MADRS), Montogomery-Asberg symptom questionnaire (MASQ,), Snaith-Hamilton Pleasure scale (SHAPS), insomnia symptom index (ISI), anxiety symptom index (ASI), ruminative symptoms scale (RTS) and childhood trauma questionnaire (CTQ)) underwent data reduction using symptom network analyses, resulting in nine symptom clusters: anhedonia (from SHAPS), depression, anxiety, sleep problems, vegetative symptoms, rumination, positive affect (from MASQ-ad), history of sexual abuse or physical neglect, h/o emotional/physical abuse or emotional neglect. Extraction of within and between network connectivity of the 9 Power networks produced 55 brain connectivity features. These brain network features were correlated using multidimensional canonical correlation analyses (CCA) with the nine symptom clusters.

Results: A significant correlation with the first pair of CCA modes was observed: canonical correlation r = 0.90, p = 0.02 (FDR-corrected). The first network CCA mode was significantly associated ($p < 0.05$, FDR corrected) with seven of the 55 original network variables, including within network connectivity in three networks: the cingulo-opercular network (CON), salience network (SAN), and somatomotor network (SMN), and four between network connectivities: CON-frontoparietal network (FPN); CON-SMN; SMN-auditory network (AUD); and CON-SAN. There were also specific correlations ($p < 0.05$, FDR-corrected) of the anxiety, sleep problems, rumination and general depression clinical clusters with the first CCA mode.

Conclusions: Data-driven network modeling of rsfMRI suggests that within and between network FC in particular networks is associated with specific symptom clusters. These symptom cluster correlations were present across mood and anxiety psychiatric disorders, supporting the RDoC framework. Future analyses will test these relationships in a validation test set.

Keywords: Resting State Functional Connectivity, RDoC, Canonical Correlation Analysis

Disclosure: Nothing to disclose.

W284

Perinatal Omega-3 Fatty Acid Deficiency Alters Brain Network Connectivity and Response to Psychostimulant Treatment in Adult Rats: A 7 Tesla phMRI Analysis of Brain Network Organization and Connectivity

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Background: The pathoetiological mechanisms contributing to functional connectivity abnormalities observed in individuals with mood and psychotic disorders remain poorly understood. Youth and adults with these disorders exhibit robust blood deficits in omega-3 polyunsaturated fatty acids (n-3 PUFA), including docosahexaenoic acid (DHA; 22:6n-3). Emerging preclinical evidence suggests that n-3 PUFAs promote synaptogenesis and synaptic plasticity, as well as synaptic pruning, in the developing brain. A neuroimaging study found that developmental n-3 PUFA insufficiency reduced resting-state functional connectivity among prefrontal and temporal cortical networks in adult non-human primates. Furthermore, psychostimulants (e.g., amphetamines) are commonly used in youth for the treatment of symptoms of inattention and hyperactivity and have been shown to induce structural and functional connectivity changes in frontostriatal networks. Graph-based network analysis is a technique used to interrogate topological properties of functional brain networks. Here we used graph-based network as well as functional connectivity analyses to investigate the effects of perinatal n-3 PUFA deficiency and/or chronic psychostimulant treatment during adolescence on regional brain network connectivity in adult rats.

Methods: Female Long-Evans rats received either a control diet (CON) containing alpha-linolenic acid (ALA; 18:3n-3) or an ALA-deficient diet (DEF) 30 days prior to mating through lactation and gestation. On postnatal day 21 (P21), male offspring were weaned onto the same diets as their dams. During the adolescent period (P40-P80), rats received 5 injections per week of D-amphetamine (AMPH, 1mg/ml/kg, s.c) or saline (SAL, 1ml/kg, s.c) for 6 weeks (30 injections total). On P90, gradient-echo echo planar imaging data (TE 8.5 ms, TR 1200 ms, 4 segments, 500 repetitions, matrix 160 x 92, field-of-view 32 mm x 32 mm, total scan time 40 minutes) were acquired on a Bruker Biospec 7T horizontal MRI prior to and for 30 minutes following an AMPH (7.5mg/kg i.v.) challenge in CON-SAL (n = 17), CON-AMPH (n = 16), DEF-SAL (n = 13), and DEF-AMPH (n = 11) rats. ROI-to-ROI, Graph Theory, and network-based statistics (NBS) were used to assess connectivity differences between groups before and after the acute AMPH challenge. Postmortem erythrocyte and forebrain membrane fatty acid levels were determined by gas chromatography.

Results: Erythrocyte and forebrain DHA levels were significantly lower in DEF rats compared with CON rats, and there was no effect of prior chronic AMPH treatment. The ROI-to-ROI analysis did not reveal any differences between groups prior to the acute AMPH challenge. Following the AMPH challenge, all DEF rats (SAL +AMPH-pretreated rats, n = 24) displayed increased connectivity between the cingulate and medial prefrontal cortices, lateral hypothalamus and thalamic nuclei relative to all CON rats (n = 33, $p<0.05$, FDR corrected). The Graph Theory analysis also indicated no significant individual group differences prior to the AMPH challenge. Following the AMPH challenge, the Normalized Clustering Efficiency (λ) and the Small World Index (σ) were both reduced in DEF-SAL rats compared with CON-SAL rats (λ p = 0.00023; σ p = 0.00032). Additionally, prior chronic AMPH treatment resulted in reduced Normalized Clustering Efficiency in CON-AMPH rats compared with CON-SAL rats (p = 0.00062). Prior to the acute AMPH challenge, the NBS analysis found reduced connectivity in all DEF rats relative to all CON rats within a network that included the thalamus, mesencephalic motor region, and the internal capsule (T-threshold: 3.00 - 3.62). Following the AMPH challenge, DEF-AMPH rats exhibited increased connectivity in a network lateralized to the right amygdala, mesencephalic motor region, zona incerta, and striatum compared with CON-AMPH rats (T-threshold: 3.75 - 4.11).

Conclusions: Together these findings provide novel evidence that developmental n-3 PUFA deficiency and chronic AMPH exposure alter functional connectivity in brain networks. The observation that developmental n-3 PUFA deficiency in combination with chronic AMPH exposure produce hyper-connectivity in corticolimbic and striatal networks may have implications for emotional and behavioral responses to psychostimulant treatment. Furthermore, n-3 PUFA deficiency resulted in disruptions to overall brain network structure and organization. These preclinical findings encourage future research into the role of n-3 PUFAs in corticolimbic circuit maturation in clinical populations.

Keywords: Functional Neuroimaging, Preclinical, Omega-3 Fatty Acids, Amphetamine, Brain Development

Disclosure: Nothing to disclose.

W285

Evoked and Resting State Gamma Mechanics to Test NMDA Receptor Engagement of Kynurenine Pathway Modulator AV-101 in Healthy Veterans

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Background: Suicide rate is increasing and is higher amongst U.S. military veterans than non-veterans. Medically severe suicide attempts have been associated with changes in kynurenine pathway (KP) functioning, notably an increase in pro-inflammatory metabolite quinolinic acid (QA) and a reduction in protective metabolite kynurenic acid (KYN). KYN is an N-methyl-D-Aspartate receptor (NMDAR) antagonist, whereas QA is an NMDAR agonist, possibly linking KP changes to excessive activation of NMDARs. AV-101 (4-chlorokynurenine) is an oral prodrug that is metabolized by astrocytes to 7-chlorokynurenic acid (7-Cl-KYNA), a potent and selective NMDAR glycine site antagonist. In this phase-1b study, we examined effects of AV-101 on oscillatory gamma mechanics, measures sensitive to changes in NMDAR functioning, captured during rest and with the auditory steady state response (ASSR) to 40Hz click trains. We assessed resting state alpha power to measure vigilance. We hypothesized increases in resting gamma power, representing cortical disinhibition, ASSR gamma amplitude, representing synchronicity of inhibitory neuron firing, and ASSR gamma inter-trial phase coherence (ITPC), representing timing of event-related interneuron firing, to AV-101 compared to placebo.

Methods: Seven healthy veterans of Operation Enduring Freedom, Operation Iraqi Freedom or Operation New Dawn (6M, 1F; age mean ± SD = 31.4 ± 5.1) received a single dose of AV-101 (720 mg or 1,440 mg) or placebo in a double-blind, randomized, cross-over controlled trial. EEG was collected before, and every hour for 5 hours after medication intake. Outcomes are across 64 EEG leads. ASSR was measured during a task consisting of 40Hz click trains interlaced with click-trains of different frequency to ensure task focus. Gamma amplitude was estimated using Morlet wavelets with a width of 10 cycles. The spectrum was baseline corrected by subtracting the mean amplitude between 500 ms and 100 ms before stimulus onset to emphasize stimulus evoked gamma. We measured the average amplitude over the period of 200 ms to 900 ms between 35Hz and 45Hz. Gamma ITPC was estimated by using Morlet wavelets with a width of 10 cycles to measure the phase angle consistency across trials. We measured the average ITPC over the period of 200 ms to 900 ms between 35 Hz and 45 Hz. Resting state EEG was measured during two minutes of eyes open and eyes closed. EEG was de-trended, low-pass filtered at 100 Hz, and divided into 4-second segments. Power spectrum densities (PSD) were computed using Welch's method of windowed averaging (1000 ms window, no overlap). All PSDs were normalized by the sum total power across all frequencies (i.e., relative PSD). Mean power estimates were calculated for each of the frequency bands, alpha (8-13 Hz) and gamma (31-54 Hz) on the relative PSD. Statistical analyses employed generalized estimating equations (GEE) using the factors drug (three levels: placebo, low dose, high dose) and time (6 levels: pre-drug baseline, and 1, 2, 3, 4 and 5 hr post-drug) to estimate the parameters of a generalized linear model to explain gamma power, amplitude, and ITPC.

Results: ASSR gamma amplitude: There was a main effect of drug ($X2 = 19.69$, $p<.001$), but no effect of time. Beta coefficient for the AV-101 conditions were significantly different from the placebo condition. Pairwise comparisons revealed that gamma amplitude for the low dose (difference from placebo = 1.15 microvolts) and for the high dose (difference from placebo = 0.51 microvolts) were significantly greater than for placebo ($p\leq.006$). Gamma amplitude did not differentiate between doses.

ASSR gamma ITPC: There was a main effect of drug ($x2 = 15.3$, $p<.001$), but no effect of time. Gamma ITPC for the high dose was significantly different from placebo ($x2 = 11.6$, $p = .001$), whereas the low dose was not. Pairwise comparisons revealed that gamma ITPC for the high dose was greater compared to low dose (mean difference = 0.041) and placebo (mean difference = 0.034) ($p\leq.007$).

Resting gamma power: There was a main effect of drug ($X2 = 16.96$, $p < .001$) and of time ($X2 = 38.35$, $p<.01$). Beta coefficient for the AV-101 conditions were significantly different from the placebo condition. Pairwise comparisons revealed higher gamma power in both AV-101 conditions compared to placebo, without a difference between doses. Pairwise comparisons revealed differences in 4 hr vs 3 hr and 2 hr vs 1 hr measurements (Bonferroni corrected $p < 0.05$).

Resting alpha power: There was a main effect of time ($X2 = 23.56$, $p < .01$) but no effect of drug. Pairwise comparisons indicated significant differences in 2 hr vs 1 hr measurements (Bonferroni corrected $p < 0.05$).

Safety: There were no serious adverse events. Both doses of drug were well-tolerated and not associated with dissociative adverse events.

Conclusions: In healthy veterans, both a low and high dose of AV-101 are associated with increased gamma power, indicating enhanced cortical disinhibition, and increased gamma amplitude, indicating enhanced interneuron synchronicity. High dose AV-101 is associated with increased gamma ITPC, indicating stronger event-related interneuron firing. These findings are consistent with NMDAR blockade by AV-101. More broadly, these measures could be used as electrophysiological biomarkers of NMDAR engagement. Future studies could examine the effect of AV-101 high dose on suicide risk which has been associated with KP dysregulation and increased activation of NMDARs.

Keywords: Kynurenine Pathway, NMDA Glycine-Site Receptor, ASSR, Resting State, EEG/ERP Electrophysiology

Disclosure: Nothing to disclose.

W286

Neuropsychopharmacology (NPP) Special Projects: Update on Gender Balance in NPP Function and Examination of Relationships Between Social Media and Citation Counts

Abstract not included.

W287

Neuroactive Steroid Levels are Associated With Cortical Thickness

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Background: Neuroactive steroids are endogenous molecules with regenerative, neuroprotective, and anti-inflammatory actions. A number of neurosteroids also demonstrate activity at GABA-A

and/or NMDA receptors. Both cortical thickness and neuroactive steroid levels decline with age and are decreased in several neuropsychiatric disorders. However, few neuroimaging studies have been conducted to date that examine potential associations between brain structure or function and neuroactive steroids. We thus investigated possible relationships between serum neuroactive steroid levels and in vivo measures of cortical thickness on MRI.

Methods: Peripheral serum samples from 115 U.S. military veterans (Iraq/Afghanistan-era) were quantified for neuroactive steroid levels by mass spectrometry (allopregnanolone, pregnenolone, androsterone, pregnanolone). DHEA, DHEAS, and progesterone serum levels were quantified by radioimmunoassay ($n = 143$). A total of 143 veterans underwent high-resolution structural MRI, followed by parcellation of the cortical surface into 148 anatomically-defined regions. Regression modeling was applied to test the association between neuroactive steroid levels and hemispheric total gray matter volume, as well as region-specific cortical thickness. False discovery rate (FDR) correction was used to control for Type 1 error from multiple testing.

Results: Levels of the neuroactive steroids allopregnanolone and pregnenolone were positively correlated with gray matter thickness in multiple regions, primarily in cingulate, parietal, and occipital association cortices ($r = 0.20$ to 0.47; $n = 115$; $p<0.05$; FDR-corrected); this included 59 cortical regions for allopregnanolone, 46 cortical regions for pregnenolone, and 6 cortical regions for androsterone (FDR-corrected).

Conclusions: Positive associations between serum neuroactive steroid levels and gray matter cortical thickness were found in multiple brain regions (FDR-corrected). To our knowledge, this investigation is the first to describe an association between cortical thickness and neuroactive steroid levels in adults. If results are confirmed in independent cohorts, quantifying neuroactive steroid levels and examining cortical thickness may have clinical utility - potentially identifying participants who are most likely to benefit from a neuroactive steroid intervention and providing a target engagement endpoint for therapeutic response.

Keywords: Neuroactive Steroid, Cortical Thickness, MRI, Neuroregeneration, Neuroprotection

Disclosure: Pending patent applications; no patents issued; no licensing in place; VA 208 waiver in place

W288

The Voltage-Gated Calcium Channel, CaV1.3, in Cognitive and Affective Behaviors

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Background: Genetic variation in L-type voltage-gated calcium channels, including CaV1.3, is associated with increased risk for psychiatric disorders including bipolar disorder and schizophrenia. Additionally, rare mutations in CaV1.3 have been linked to epilepsy, developmental delay, and autism. Deletion of CaV1.3 in mice is associated with impaired consolidation of contextual fear conditioning. Some studies have also observed affective behavior deficits in CaV1.3-deficient mice, but other studies have not found affective phenotypes, perhaps due to differences in genetic backgrounds, sex ratios, or task protocols. CaV1.3 is important for slow after-hyperpolarization in hippocampal and amygdala neurons, which prevents excessive firing in response to sustained excitatory input, and CaV1.3-deficient amygdala neurons exhibit hyperexcitability and impaired LTP. CaV1.3 is also expressed in the cerebellum, but its functional role there is not well-understood. Given its importance in shaping neuronal activity in the hippocampus and amygdala, we hypothesized that loss of CaV1.3 would cause abnormalities in motor learning as well as affective and cognitive behaviors.

Methods: Wild-type (WT, $n = 31$), haploinsufficient (Hap, $n = 32$), and knockout (KO, $n = 27$) mice were maintained on a congenic C57BL/6NTac genetic background and were subjected to behavioral tasks including rotarod, ErasmusLadder, forced swim test, and tail suspension test. Data were analyzed with sexes combined and with sexes separated to assess for sex as a biological variable. Studies were analyzed by one-way ANOVA, two-way ANOVA, or generalized linear mixed model, where appropriate.

Results: Loss of CaV1.3 (KO) was associated with impaired motor learning in the rotarod task ($p<0.05$), as well as impaired associative learning in the ErasmusLadder task ($p < 0.01$), despite intact locomotor function on both tasks. Hap mice were not different from WT mice on either rotarod or ErasmusLadder when sexes were combined. When examined by sex, the rotarod phenotypes were driven by motor learning impairments in males (both Hap and KO, $p<0.05$ and $p < 0.01$, respectively), whereas the ErasmusLadder associative learning phenotypes were present in both sexes only in the KO condition, consistent with previously reported impairments in CaV1.3-deficient mice in consolidation of contextual fear conditioning. Although KO mice learned the motor aspects of the ErasmusLadder task, they learned more slowly. They also failed to learn start cues, which requires intact associative learning. In affective tasks, female KO mice demonstrated increased latency to float in the forced swim test ($p<0.05$) and increased escape behavior in the tail suspension test ($p<0.05$), while male KO mice performed no differently from WT.

Conclusions: These preliminary studies provide new evidence that CaV1.3 is important for the function of neural circuits involved in motor learning, and concur with previous data showing its involvement in affect and associative learning. There may also be sex-specific differences in affective behaviors in KO mice. Our data differ slightly from previous studies of CaV1.3 in motor learning, which could be attributable to differences in task protocols and/or genetic background. These results highlight the importance of CaV1.3 in a variety of behaviors, which may help explain why variation in CaV1.3 expression and function has pleiotropic effects in humans.

Keywords: Voltage-Gated Calcium Channel, Mouse Behavior, Motor Learning

Disclosure: Nothing to disclose.