ABSTRACTS COLLECTION

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M1

The Role of Activity-Induced DNA Breaks in Neuronal Physiology and Learning Behaviors

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Background: Neuronal activity triggers the rapid expression of immediate early genes that play important roles in experience-driven synaptic changes, learning, and memory. While immediate early genes are primed for rapid induction, the specific impediments to their expression under basal conditions, and the mechanisms that relieve these constraints are still poorly understood. Recently, we reported that activity-dependent stimulation of cultured primary neurons triggers the formation of DNA double strand breaks (DSBs) in the promoters of a subset of immediate early genes, including *Fos*, *Npas4*, and *Egr1*. These activity-induced DSBs are generated by the type II topoisomerase, topoisomerase II β (Top2B), and we showed surprisingly that Top2B-mediated DSBs facilitate the rapid induction of these aforementioned IEGs. Together, these results raise intriguing questions about the mechanisms that regulate the formation of stimulus-induced DSBs at specific genomic loci and whether the formation of these DSBs has a role in neuronal functions, including in the development of adaptive behaviors.

Methods: To assess whether stimulus-induced DSBs are also formed at specific genomic loci in vivo, we subjected two-month old C57BL/6 mice to a contextual fear conditioning (CFC) paradigm, following which we dissected the hippocampi and performed ChIP-seq with antibodies against the DSB marker, γ H2AX. We have obtained a mouse model (Top2b^{f/f} mice) in which the expression of Cre recombinase allows for the conditional deletion of endogenous Top2b. To understand whether DSBs formed in vivo are also a result of Top2B activity, conditional Top2b^{f/f} mice were crossed with CaMKII α -Cre mice, which causes for the deletion of Top2b from excitatory forebrain neurons in adult mice. The resultant Top2bCKO mice were subject to CFC at 8 weeks of age, following which hippocampal lysates were prepared and γ H2AX levels were assessed by western blotting. To understand whether the formation of activity-induced DSBs affects learning behaviors, two month-old male Top2bCKO mice (12 animal per group) were subjected to various behavioral paradigms, including open-field and light-dark tests, object recognition and object location tasks, and contextual and cued fear conditioning tests. Finally, molecular mechanisms that regulate the formation of Top2B-mediated DSBs were investigated through a combination of targeted mass spectrometry,

mutagenesis of Top2B, and imaging-based assays to detect the formation of stimulus-induced DSBs in cultured primary neurons.

Results: Our ChIP-seq studies in vivo recapitulated our previous observations in cultured primary neurons, and indicated that physiological learning behaviors also cause DSB formation within the promoters of neuronal IEGs. Interestingly however, we also observed DSB accumulation at many new loci that were not detected in cultured primary neurons. Gene ontology analysis revealed a significant enrichment of biological processes related to synaptic transmission and synaptic function within loci that incur DSBs in the hippocampus following CFC. The formation of stimulus-induced DSBs in the hippocampus was attenuated in Top2bCKO mice, suggesting that Top2B also generates DSBs in response to physiological neuronal activity in vivo. Furthermore, Top2bCKO mice showed significant defects in both contextual and cued fear-conditioning tasks, indicating defects in long-term memory formation. Finally, our targeted mass spectrometry experiments revealed that the activity of Top2B is modulated through activity-dependent changes in Top2B phosphorylation.

Conclusions: Together, our results suggest that physiological learning behaviors trigger the formation of Top2B-mediated DSBs at specific genomic locations, that the activity of Top2B is modulated to generate DSBs, and that the formation of these DSBs is necessary for the activation of stimulus-dependent gene transcription programs and for the development of adaptive behaviors.

Keywords: Topoisomerase, Early Response Genes, Gene Transcription, DNA Double Strand Breaks

Disclosure: Nothing to disclose.

M2

Verubecestat-Induced Brain Volume Loss Occurs Rapidly and Only in Amyloid-Enriched Brain Regions in EPOCH, a Phase 3 Trial in Mild-To-Moderate Alzheimer's Disease Patients

Abstract not included.

M3

Prenatal Stress Exposure Modulates Resting State Functional Connectivity by Sex in Midlife

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Background: Over the lifespan, many factors contribute to risk and resilience in healthy brain aging, even beginning in fetal development. There is growing evidence that brain development beginning in utero has implications for brain aging, potentially through the disruption of stress-immune pathways, known as prenatal stress models of brain aging. The default mode network (DMN) in the brain, which primarily includes medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), lateral parietal cortex (LP), and hippocampus (HIPP), some areas which are shared with stress circuitry regions, has been found to be important for cognitive aging and vulnerable to early Alzheimer's disease pathology. In aging, intrinsic functional connectivity within the DMN breaks down, with decreased connectivity between anterior and posterior regions as well as within posterior regions of the network. In addition to chronological aging, women undergo reproductive aging, during which they experience a depletion of sex steroid hormones such as estradiol, which we previously demonstrated is directly related to decreased memory performance and reorganization of functional memory circuitries. Here, we aim to integrate previous work on brain aging through a combined investigation of prenatal stress exposures, reproductive aging in women, and the DMN. We tested the impact of preeclampsia (PE) or fetal growth restriction (FGR) on sex differences in the intrinsic functional connectivity of the DMN in early midlife as women transition through menopause.

Methods: Two hundred and twelve middle-aged adults (age range 45–55; 106 women and 106 men) recruited from the New England Family Study (NEFS) cohort underwent clinical assessment, blood collection, and fMRI scanning. NEFS is a unique population-based prenatal cohort born between the years 1959 and 1966. Their mothers were followed through pregnancy and the cohort have been followed since birth for > 50 years, contributing to an extensive dataset comprised of prenatal and developmental information. Subjects were siblings discordant for prenatal stress exposure (PE or FGR), such that one sibling was exposed and the other was not. STRAW-10 criteria and serology were used to determine pre-, peri and post-menopausal staging. Subjects underwent a resting state fMRI scan and data were analyzed using ROI-to-ROI-based functional connectivity analyses. ROIs of the DMN included mPFC, PCC, LP, and HIPP.

Results: We found overall sex differences in resting state functional connectivity within the DMN, specifically in midlife adults exposed to prenatal stress. In the exposed group, women had significantly higher functional connectivity between the mPFC and right and left LP compared to men (mPFC – RLP: $t = 2.46$, $pFDR = 0.04$; mPFC – LLP: $t = 1.94$, $pFDR = 0.047$). Women also had increased functional connectivity between the right and left LP compared to men (RLP – LLP: $t = 2.05$, $pFDR = 0.047$). Examining “prenatally exposed” women across the menopausal transition revealed that only premenopausal women differed significantly from men (mPFC – LLP: $t = 2.97$, $pFDR = 0.009$; RLP – LLP: $t = 2.03$, $pFDR = 0.04$). Functional connectivity in the DMN decreased across the menopausal transition (pre > post: mPFC – LLP: $t = 3.02$, $pFDR = 0.02$) and was directly related to declining levels of estradiol ($r = 0.42$, $p = 0.004$). No sex differences were found between postmenopausal women and men. Overall, no significant differences were observed in the unexposed group by sex or menopausal status.

Conclusions: Results suggest that prenatal stress exposure modulates the impact of sex and reproductive aging on the intrinsic functional connectivity of the brain in early midlife. In the prenatal exposure group, women had significantly higher functional connectivity in the DMN compared to men. Further, only in the exposed group, DMN functional connectivity declined across the menopausal transition in a similar manner observed with chronological aging (anterior – posterior), despite minimal differences in chronological age between groups. Taken together,

these results suggest that menopause may present a critical period of accelerated brain aging in women and that prenatal stress exposure may increase vulnerability to these changes. Uniquely, at a human population-level, findings demonstrated that prenatal stress exposure is significantly associated with sex differences, that implicate reproductive aging, in the intrinsic functional connectivity in the brain.

Keywords: Prenatal Stress, Sex Differences, Menopause, Default Mode Network (DMN), Resting-state fMRI

Disclosure: Nothing to disclose.

M4

Optogenetic Inactivation of Prefrontal Cortex During Intertemporal Choice Reveals Unique Roles for This Structure in Young and Aged Rat Decision Making

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Background: The medial prefrontal cortex (mPFC) is the rodent homologue of human dorsolateral prefrontal cortex and is critical for mediating executive functions such as working memory and cognitive flexibility. These executive functions are important for supporting cost-benefit decision making such as whether to choose an option that yields a small reward delivered immediately versus an option that yields a larger reward delivered at some point in the future (intertemporal choice). Previous work in both rats and humans indicates that older subjects exhibit greater preference than young for large, delayed over small, immediate rewards. These preferences correlate with age-associated impairments on a PFC-dependent task of cognitive flexibility, suggesting PFC dysfunction in aging contributes to altered intertemporal decision making. The current study used an optogenetic approach to more precisely define the temporally-specific contributions of mPFC neural activity to intertemporal decision-making, and to determine if the engagement of PFC in decision making changes in aging.

Methods: Young adult (6 mo.) and aged (24 mo.) Fischer 344 x Brown Norway F1 hybrid rats were surgically implanted with guide cannulae targeting mPFC, through which pAAV-CaMKIIa-eNpHR3.0-mCherry (halorhodopsin) was delivered and optic fibers were implanted. Rats were subsequently trained on an adjusting-delay intertemporal choice task in which preference for small vs. large rewards was evaluated in the presence of ascending delays to large rewards. Upon reaching stable performance, a within-subjects design was used to inactivate mPFC during discrete phases of each choice trial

Results: In young rats, inactivation of the mPFC prior to choices increased young rats' preference for the large, delayed reward (produced less impulsive behavior). In contrast, mPFC inactivation prior to the choice had no effect in aged rats. Instead, inactivation of mPFC in aged rats during the delay interval or during evaluation of the large, delayed reward, produced less impulsive behavior.

Conclusions: The data suggest that differential engagement of mPFC during intertemporal decision making contributes to robust age differences in intertemporal choice behavior. These results contrast previously published data from our lab on the role of the basolateral amygdala in intertemporal choice.

Keywords: Aging, PFC, Executive Function, Optogenetics, Decision Making

Disclosure: Nothing to disclose.

M5

Memory Network Activation Associated With Insulin Resistance in Postmenopausal Women With Obesity**Laura Holsen*, Benjamin Ryder, Sarah Boukezzzi***Harvard Medical School, Boston, Massachusetts, United States*

Background: Postmenopausal women have the highest rate of obesity of any sex- and age-group, and those with obesity are at an increased risk of developing dementia, even after controlling for age, education, and mid-to-late life cardiometabolic comorbidity. Insulin resistance plays a key role in hippocampal-mediated memory functioning, with prior studies reporting inverse relationships between the homeostatic model assessment of insulin resistance (HOMA-IR), memory performance, and hippocampal volume in women at risk for Alzheimer's disease. Additional data support the role of HOMA-IR in mediating the relationship between BMI and brain activation during standard working memory tasks. However, these paradigms have limited sensitivity to detect early phases memory decline. Associative memory tasks, such as the face-name pair encoding paradigm, on the other hand, elicit robust activation of memory networks which is highly sensitive to mild memory impairment. Previous reports indicate that cognitively intact older adults with poor task-related memory performance exhibit dysfunction in the normative reciprocal relationships between activation in medial temporal lobe structures [hippocampus, parahippocampal gyrus (PHG), inferior frontal gyrus (IFG); increased activity] and default mode structures [precuneus, superior parietal lobule (SPL); deactivation] during successful associative encoding. To date, there is a paucity of data focused on relationships between BMI status, insulin resistance, and brain activation in response to associative encoding paradigms. The goal of this preliminary study was to examine differences between obese and healthy-weight postmenopausal women in memory network activation during associative encoding, and relationships with insulin resistance.

Methods: Postmenopausal, non-diabetic women with no history of hormone replacement therapy [10 women with obesity (OB group), 54.1 ± 3.0 years; 33.7 ± 4.0 BMI; 12 age-matched healthy-weight women (HW group), 55.6 ± 2.7 years; 22.4 ± 2.1 BMI] completed a visit including a fasting blood draw and an fMRI scanning session involving an associative memory paradigm, during which they viewed novel and repeated pairings of faces and names while undergoing fMRI scanning on a 3T Siemens Skyra MR scanner, followed by a retention test outside the scanner and a neuropsychological battery (verbal fluency, verbal IQ, working memory). Glucose and insulin were analyzed in duplicate using commercial immunoassay kits. Data analysis: fMRI data were analyzed using SPM12 to examine between-group (HW vs. OB) contrasts: Novel > Repeated; Fixation > Novel + Repeated (to assess deactivation during encoding). Regions of interest (ROIs) were based on prior reports using this paradigm; for Novel > Repeated: hippocampus, PHG, IFG; for Fixation > Novel + Repeated: precuneus, SPL. Given small sample sizes in each group, clusters meeting a threshold of $k > 10$ and $p(\text{uncorrected}) < 0.005$ are reported. Individual subject beta estimates were extracted from selected ROIs using REX. Relationships between beta estimates and HOMA-IR were examined using general linear models in SPSSv19.

Results: Groups did not differ on retention of the face-name pairings [$t(16) = 0.76$, n.s.] or neuropsychological test performance [$t(20) = 0.18$ – 1.85 , n.s.]. OB had significantly higher HOMA-IR (2.57 ± 1.66) compared to HW (0.92 ± 0.42) [$t(18) = 2.90$, $p = 0.018$], and HOMA-IR was positively associated with verbal fluency errors in the OB group ($r = 0.89$, $p = 0.002$), but unrelated in the

HW group ($r = 0.15$, n.s.). For the contrast of Novel > Repeated, relative to the HW group, the OB group exhibited elevated activation in the right PHG [$t(19) = 4.28$, $p(\text{FWE-corr.}) = 0.042$, $p(\text{uncorr.}) < 0.001$], right hippocampus [$t(19) = 3.22$, $p(\text{FWE-corr.}) = 0.23$, $p(\text{uncorr.}) = 0.002$], and left IFG [$t(19) = 3.21$, $p(\text{FWE-corr.}) = 0.15$, $p(\text{uncorr.}) = 0.002$]. For the contrast of Fixation > Novel + Repeated, relative to the OB group, those with HW exhibited greater deactivation in the right precuneus [$t(19) = 3.44$, $p(\text{FWE-corr.}) = 0.42$, $p(\text{uncorr.}) = 0.001$], right SPL [$t(19) = 4.33$, $p(\text{FWE-corr.}) = 0.11$, $p(\text{uncorr.}) < 0.001$], and left SPL [$t(19) = 4.28$, $p(\text{FWE-corr.}) = 0.12$, $p(\text{uncorr.}) < 0.001$]. Activation in the PHG was inversely related to superior parietal lobule activation in the HW group ($r = -0.63$, $p = 0.037$) but positively related in the OB group ($r = 0.64$, $p = 0.045$). Across groups, HOMA-IR was positively associated with activation in the left IFG ($r = 0.054$, $p = 0.05$) and negatively associated with deactivation in the right SPL during Novel + Repeated face-name pairs ($r = -0.50$, $p = 0.028$).

Conclusions: Results indicate hyperactivation of medial temporal lobe and prefrontal structures, in parallel with impaired deactivation of default mode network regions, in postmenopausal women with obesity relative to healthy weight women, despite similar task performance, potentially suggesting compensatory responses in the obese group. Moreover, these patterns of activation were significantly associated with insulin resistance, providing evidence for a link between impaired insulin sensitivity and impaired coordinated recruitment during associative memory encoding. Although preliminary, given the sample size, findings reveal key associations between metabolic dysfunction and memory network impairment in postmenopausal women with obesity, providing insight into potential neurometabolic biomarkers to target in future interventional studies.

Keywords: Memory Encoding and Retrieval, Obesity, Brain Insulin Resistance, Menopause

Disclosure: Nothing to disclose.

M6

Stress and Low-Level Inflammation Interact to Alter Cognition and Synaptosomal Respiration in C57Bl/6 Mice**Gretchen Neigh*, Gladys Shaw, Imogen Targett, Kimaya Council, Susie Turkson***Virginia Commonwealth University, Richmond, Virginia, United States*

Background: Chronic exposure to highly stressful situations is detrimental to the wellbeing of both body and brain. Chronic stress, especially during the unique developmental timeframe of adolescence and early adulthood, can have adverse effects on both behavioral and metabolic outcomes later in life. Chronic exposure to stress dysregulates the hypothalamic-pituitary-adrenal (HPA) axis causing an increase in systemic inflammation resulting in increased risk of neurodegenerative disorders, such as Alzheimer's disease. The current model has shown that chronic stress in rodents alters glucose transporters in the brain, suggesting implications in metabolism and cognitive impairment. This project aims to assess the influence of chronic stress and chronic inflammation on synaptosomal metabolism and related changes in cognitive ability.

Methods: Male ($n = 31$) and female ($n = 32$) C57Bl/6 mice were subject to chronic predation stress (male $n = 15$; female $n = 16$) or daily handling (male $n = 16$; female $n = 16$) for 15 days during adolescence (PND 34–48) and again during adulthood (PND 57–71). Mice were then subject to 8 weeks of chronic lipopolysaccharide (LPS) or saline injections at 7.5×105 EU/kg (PND 76–121) administered every 3 days and equally split between both sex and

stress groups to induce chronic low-level inflammation. Mice were subject to the open field (PND 97) test to assess anxiety-like behavior. Cognition was assessed via Barnes Maze testing. Behavioral data were recorded, and raw videos analyzed through EthoVision 14.0 software. On PND 147-148 brain tissue (one hemisphere and partial second hemisphere) was homogenized and processed for synaptosomal isolation using a discontinuous Percoll gradient. Synaptosomally enriched samples were plated immediately for respiratory analysis using Agilent's SeahorseXFe24 and Cell Mitochondrial Stress test for mitochondrial respiration. All statistical data were analyzed using single or repeated measures 2- or 3-way ANOVA ($\alpha < 0.05$) where appropriate (GraphPad 8.1).

Results: A 2-way ANOVA indicates that mice with a history of stress, regardless of sex, spend less time in the center of the arena ($F(1,27) = 4.473$, $p = 0.0438$). However, when treated with LPS, the effect of stress was not present ($p > 0.05$). During the acquisition phase of the Barnes Maze task, males displayed a trial by stress interaction on latency to locate the goal box ($F(5,405) = 1.809$, $p = 0.0314$). Post hoc analysis showed an interaction between trial and stress such that non-stress males had a shorter latency to the goal box on day 5 of testing ($F(15,435) = 1.812$, $p = 0.0308$). In females, there was an interaction between trial and LPS ($F(15,420) = 1.889$, $p = 0.0226$). Post hoc analysis showed that LPS-treated females took longer to locate the goal box on day 8 of testing ($F(15,450) = 1.900$, $p = 0.0214$). During reversal learning, males showed an interaction between stress and LPS ($F(1,27) = 4.768$, $p = 0.0379$). Post hoc analysis demonstrated a main effect of treatment within the non-stress males ($F(1,14) = 4.980$, $p = 0.0425$) indicating that LPS-treated males exhibited increased latency to enter the goal box compared to saline controls. Females did not display an effect of stress or treatment on latency to locate the new goal box during reversal learning ($p > 0.05$). Synaptosomal respiration was modified by treatment in both males ($F(1,276) = 13.72$, $p = 0.0003$) and females ($F(1,300) = 5.725$, $p = 0.0173$). Both sexes also displayed an interaction between stress and LPS (males: $F(1,276) = 3.959$, $p = 0.0476$; females: $F(1,300) = 33.16$, $p < 0.0001$). Post hoc analysis demonstrated that in males ($F(1,120) = 16.66$, $p < 0.0001$) and females ($F(1,144) = 28.21$, $p < 0.0001$), LPS decreased overall synaptosomal respiration when combined with a history of chronic stress. In non-stress females, LPS increased overall synaptosomal respiration ($F(1,156) = 6.727$, $p = 0.0104$) but did not impact respiration if there was a history of chronic stress.

Conclusions: We show that the combination of chronic stress and inflammation equally impact anxiety-like behaviors in males and females despite differentially influencing learning, cognitive flexibility, and synaptosomal respiration. Chronic inflammation appears to induce marginal learning deficits and impair cognitive flexibility in males while females require the combination of chronic inflammation and stress before deficits are evident. Chronic repeated stress induces long-lasting anxiety-like behavior which is attenuated by chronic inflammation in both male and female mice with a history of stress. These data indicate a unique interaction between sex, stress, and chronic inflammation on behavior – specifically, while stress and inflammation can both cause detrimental effects to behavior, the extent of these changes and their direction are modified by sex. The changes in synaptosomal respiration suggest that sex, stress history, and inflammation again interact to create a unique, individualized metabolic profile. Changes in synaptic metabolism can undermine synaptic function leading to deficits in behavior. Collectively, these data highlight how sex, stress history, and inflammation can interact to shape individualized patterns of behavior possibly driven by altered synaptic metabolism. Alterations in synaptic metabolism may have implications for neuropsychiatric and neurodegenerative disorders.

Keywords: Lifetime Stress, Inflammation, Cognition, Anxiety, Mitochondrial Function

Disclosure: Nothing to disclose.

M7

Sources of Heterogeneity of Bipolar Disorder: Multidomain Discrimination of Bipolar-I From Bipolar-II Subtypes in the NIMH Family Study of Affective and Anxiety Spectrum Disorders

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Background: Heterogeneity of Bipolar Disorder (BD) has been a major challenge to our understanding of etiologic factors and treatment. Identification of biomarkers as potential endophenotypes for BD in the context of a family study may assist in examining sources of heterogeneity. This study examines whether Bipolar-I disorder (BD-I) and Bipolar-II disorder (BD-II) have qualitative versus quantitative differences on clinical and biologic measures of its underlying domains. We then examine whether the biomarkers that discriminate between the subgroups are familial.

Methods: Data for these analyses are based on a clinically-enriched community sample of 578 probands and their 1,035 relatives who were recruited from the greater Washington, DC metropolitan area; data were collected using standard family study methodology through a best-estimate procedure based on direct semi-structured interviews or family history reports. The sample consists of 121 probands with a lifetime history of BD-I, 70 probands with a lifetime history of BD-II, 188 probands with a lifetime history of Major depressive disorder (MDD) and 113 controls. There are 174 relatives with BD-I and 93 relatives with BD-II. Probands and relatives underwent a series of comprehensive measures of potential endophenotypes for mood disorders, including: sleep and circadian rhythms via both interviews, actigraphy and electronic diaries; temperamental measures; cognitive assessments; autonomic function; psychophysiological assessments; olfactory testing. Analyses compared probands and relatives with BD-I and BD-II on these domains.

Results: Probands and relatives with BD-II differ from those with BD-I on several biological and clinical features: greater variability of sad and anxious mood on electronic diaries and night-time objectively-assessed motor activity; greater accuracy and speed in executive function assessments; greater autonomic reactivity to challenge in terms of lower blood pressure and lower epinephrine and dopamine; greater rates of the atypical subtype of depression; more comorbidity with migraine; lower rates of comorbid anxiety disorders and rates of suicide attempts. These findings are all significant at the level of $p < .05$. No differences between BD-I and BD-II emerged for temperamental factors, reactivity to startle, olfactory function, evening chronotype, reactivity to life events and most neuro-cognitive domains.

Conclusions: These differences between BD-I and BD-II may reflect heterogeneous etiologic factors between subtypes. Domains in which there was similarity between BD-I and BD-II are likely manifestations of common underlying diatheses. Classification modeling of these findings will be presented to derive higher order factors both in common and unique to these two subtypes of BD. Findings have important implications for understanding etiologic pathways and potentially different pharmacologic treatments for these two subtypes of BD.

Keywords: Bipolar Disorder, Bipolar I & II disorder, Comorbidity, Endophenotype, Biomarkers

Disclosure: Nothing to disclose.

M8

Single-Nucleus RNA Sequencing of Medial Prefrontal Cortex and Amygdala During Fear Conditioning and Extinction**Robert Fenster*, Kenneth McCullough, Shahin Mohammadi, Kerry Ressler***McLean Hospital, Harvard Medical School, Belmont, Massachusetts, United States*

Background: Post-Traumatic Stress Disorder (PTSD) is a debilitating psychiatric disorder with profound social burden and few effective treatments. Fear extinction deficits are thought to contribute to PTSD pathogenesis. Research from animal models and from human neuroimaging studies implicate medial prefrontal cortex (mPFC) and amygdala, among other structures, as playing a crucial role in fear extinction memory formation. The molecular fingerprints of cell-types that are necessary and sufficient for fear extinction processes in these regions are incompletely understood but could lead to the identification of novel drug targets for PTSD.

Methods: We used single nuclear sequencing (InDrops) to sequence over 100,000 nuclei from both mPFC and amygdala from three behavioral groups of mice, home cage, fear conditioning, and fear extinction ($n = 8$ groups of $n = 2-3$ male mice). We identified clusters of cells in both regions that exhibit immediate early gene (IEG) expression two hours after either fear conditioning or fear extinction when compared with home cage. We have also utilized the novel ACTION algorithm to identify cellular markers that co-vary with IEG expression. We have used fluorescent in situ hybridization (FISH) and immunohistochemical techniques to confirm markers for cell clusters found with single nuclear sequencing. Retrograde viruses expressing Rpl10a-eGFP were also used to identify molecular signatures of projection neuron populations from mPFC to amygdala using translating ribosomal affinity purification (TRAP).

Results: We have identified over 20 distinct cell-type populations within the mPFC, and over 16 cell-types within the amygdala, including populations of glutamatergic neurons, GABAergic interneurons, astrocytes, microglia, and endothelial cells. Many of these clusters express known cell-type specific markers. We have also identified populations of neurons with previously undescribed molecular signatures. Several neuronal clusters exhibit IEG expression (including Fos, Junb, Npas4, Egr1, Egr4, and Nr4a1) following fear conditioning or extinction. In the mPFC, one of the clusters with strongest IEG expression after fear extinction upregulates the plasticity master-regulator BDNF and also Ptgs2, a potential pharmacologic target for PTSD. Ptgs2 is enriched in mPFC neurons that project to the amygdala (fold change 2.7 IP fraction versus input, FDR=0.002). Pharmacological inhibition of Ptgs2 with rofecoxib, a Ptgs2-inhibitor, suggests that it is necessary for fear extinction (Two-Way ANOVA, Vehicle vs. Rofecoxib, $p = 0.007$, $n = 13$ Vehicle, $n = 14$ rofecoxib, administered i.p.).

Conclusions: We provide data to begin construction of a comprehensive map of cell-types within the mouse mPFC and amygdala. We have identified both known and uncharacterized cell-types. Some of these cell-types possess transcriptional signatures suggestive of activity during fear extinction. Follow-up studies will assess the functional role of these cell-types in the process of fear extinction formation and consolidation.

Keywords: Medial Prefrontal Cortex, Single-cell RNA Sequencing, Fear Extinction, Fear Conditioning, Amygdala

Disclosure: Nothing to disclose.

M9

High-Frequency rTMS to the Right dlPFC Increases Anxiety Potentiated Startle in Healthy Volunteers

Abstract not included.

M10

Neural Reinstatement Promotes Context-Dependent Extinction Memory Retrieval**Joseph Dunsmoor*, Augustin Hennings, Mason McClay, Jarrod Lewis-Peacock***University of Texas at Austin, Austin, Texas, United States*

Background: Fear extinction provides a model for exposure-based therapy. It is well known that extinction is context-specific, and fears return during periods of threat ambiguity outside the extinction context in a form of relapse known as renewal. Multivoxel pattern analysis (MVPA) of functional MRI data allows experimenters to decode context reinstatement during memory retrieval. But whether reinstatement of an extinction context during a period of threat ambiguity is associated with prevention of fear relapse is unknown. We hypothesized that neural reactivation of the extinction context, 24-hours after extinction, would predict successful retrieval of extinction memories in healthy controls, but not patients with PTSD.

Methods: We used MVPA of functional MRI data in humans to decode a multivariate signature selective to encoding and 24-hour retrieval of extinction memories. Subjects were 24 healthy adults and 24 adults with PTSD symptoms. Subjects first learned that conditioned stimuli predicted an electric shock, but that these stimuli were safe in a specific context. We trained a pattern classifier to decode the extinction context at a 24-hour test of fear relapse, when subjects viewed the conditioned stimuli under threat ambiguity in a different context.

Results: We report three key findings. First, the degree to which patterns of activity in visual cortex matched those from the extinction context predicted activity in the ventromedial prefrontal cortex (vmPFC) and hippocampus, regions critical for the contextual modulation of safety memory retrieval. In contrast, context reactivation was a poor predictor of safety memory retrieval in subjects with PTSD symptoms, a disorder characterized by severe contextual processing deficits. Second, the magnitude of spontaneously retrieved safety context disambiguated feelings of safety versus feeling of danger in healthy adults, but not in PTSD. Finally, item-level neural similarity patterns between safety encoding and memory retrieval were enhanced in the vmPFC in healthy adults as compared to PTSD.

Conclusions: These results indicate that neural reinstatement and retrieved context may help resolve memory competition between opposing sources of emotional information. Understanding how the human brain separately encodes and retrieves memories of fear and safety has broad implications for the study of healthy emotional memory and the neuropathophysiology of affective disorders. Quantifying mental context reactivation could be used as an innovative measure to evaluate successful treatment in disorders marked by excessive fear and anxiety.

Keywords: Fear Conditioning, Fear Extinction, Fear Renewal, Anxiety & PTSD, MVPA

Disclosure: Nothing to disclose.

M11

Neural Activation and Connectivity During Attention Shifting and Emotional Appraisal Predicts Treatment Response in PTSD

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Background: Posttraumatic stress disorder (PTSD) has been associated with difficulties modulating emotion. Deficits in emotion modulation are associated with increased activation in brain regions associated with emotion processing (insula, amygdala), decreased activation in emotion modulation regions (regions in prefrontal cortex), and differences in connectivity within and between these emotion processing and modulation regions. The purpose of this study was to examine neural mechanisms underlying emotion processing and modulation as predictors of PTSD treatment response.

Methods: Fifty-eight military veterans with PTSD were assigned to three evidence-based treatment groups: Prolonged exposure plus pill placebo (PE + PLB), sertraline plus enhanced medication management (SERT + EMM), and PE plus sertraline (PE + SERT). Symptom assessment and fMRI scanning occurred before and after treatment. During fMRI scanning, the Shifted Attention Emotion Appraisal Task (SEAT) probed brain function during implicit emotional processing, attention modulation of emotion, and emotion modulation by appraisal. An additional 27 combat control (CC) participants were studied at pre-treatment as a baseline comparison.

Results: During emotion modulation by appraisal, brain activation at pre-treatment predicted change in PTSD symptoms from pre- to post-treatment ($R^2 = .28$, $F(7, 42) = 2.33$, $p = .040$). Specifically, activation in insula ($\beta = 2.03$, $p = .049$), dlPFC ($\beta = -.414$, $p = .012$), and vmPFC ($\beta = 2.33$, $p = .025$) before treatment was associated with symptom change from pre- to post-treatment. Greater connectivity between left amygdala and superior parietal cortex during appraisal at pre-treatment predicted greater reductions in symptoms from pre- to post-treatment ($p < .05$, FWE corrected). Greater connectivity between right dorsolateral prefrontal cortex and superior parietal cortex during attention modulation at pre-treatment predicted reduction in PTSD symptoms from pre- to post-treatment (trend-level; $p = .052$, FWE corrected). Increased connectivity between these regions from pre- to post-treatment was associated with greater reduction in symptoms ($ps < .05$, FWE corrected). There were no main effects of, or interactions with, treatment group. Differences in neural function during emotion processing and modulation were not associated with treatment drop out.

Conclusions: This study is one of the first to examine task-based activation and connectivity in a PTSD treatment trial, with evidence to suggest that the function of regions involved in emotion processing and modulation are important predictors of treatment response.

Keywords: PTSD, Sertraline, Exposure Therapy, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

M12

Amygdala Activation During Threat Learning Predicts Subsequent Implicit Approach-Avoidance Behavior

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Background: Fear and avoidance are two key characteristics of anxiety disorders. Differential threat conditioning is often used to examine how individuals learn fear. In differential threat conditioning studies, an aversive stimulus (i.e., unconditioned stimulus, UCS) is paired with a neutral stimulus (i.e., conditioned stimulus, CS+), but not a second (i.e., CS-). As a result of learning, threat responses (e.g., amygdala activation) are generally elevated to the CS+ relative to the CS-; however, meta-analytic findings demonstrate individual differences in the amygdala pattern of activation (e.g., Fullana, et al., 2016). Additionally, work is needed to understand the relationship between threat learning and avoidance behavior. Here, we aimed to characterize how variability in threat learning evidenced by amygdala activation influences behavioral and neural differences in automatic approach and avoidance of newly learned threat (CS+) and non-threat (CS-).

Methods: Thirty adults (16 females, 20.3 ± 2.6 , ethnicity: 12 Hispanic, race: 6 Asian, 1 Mixed) underwent threat conditioning paradigm followed by an approach-avoidance task inside the MRI scanner. In the threat conditioning paradigm, the CS+ and CS- were two neutral female facial expressions and the UCS was a brief, aversive scream. In the approach avoidance task, generalization stimuli, i.e., 11 morphed images varying from CS+ to CS- in 10% increments, were presented. Individuals distinguished between two background colors via button press. Each button pressed either enlarged the image size to mimic approach behavior or decreased the image size to mimic avoidance behavior. Imaging data for both paradigms were modeled at the individual level. To capture variability in threat learning, bilateral amygdala activation was extracted from the threat conditioning paradigm using an anatomical mask. The sample was divided into two groups, learners and non-learners, based on whether amygdala activation was greater in response to CS+ relative to CS- or not. Behavioral data and neural data were examined using a trial type (approach, avoid), condition (CS+, CS-) and threat learning group (learners, non-learners) repeated measures ANOVA. Statistical data were assessed using $\alpha = 0.05$ for behavioral data and $p < 0.005$ and cluster size 60, which effectively corrects for multiple comparisons at an alpha level of 0.05.

Results: Amygdala percent signal change values for CS+ relative to CS- were significantly different between learners ($n = 17$, 0.155 ± 0.12) and non-learners ($n = 13$, -0.095 ± 0.11 , $t(28) = 5.9$, $p < 0.001$). Behaviorally, a near significant trend in the trial $\times$ condition $\times$ group interaction $F(1,28) = 4.1$, $p = 0.053$ was detected. Group differences were observed in the speed of approaching CS+ relative to CS- ($t(28) = 2.8$, $p < 0.01$). Learners tended to be slower than non-learners to approach the CS+ ($t(28) = 2.0$, $p = 0.053$). Moreover, learners were slower to approach CS+ than CS- ($p = 0.004$). Additionally, although group differences were only at trend level ($p = 0.083$), learners were faster to avoid the CS+ than the CS- ($p = 0.021$). Non-learners did not exhibit any significant CS+ vs. CS- differences (all $p > 0.54$). Examining similar contrasts in the neural data, compared to non-learners, learners exhibited greater activation in a region including the nucleus accumbens ($-16, -1, -9$, $k = 96$) and posterior cingulate ($1, -66, 44$, $k = 403$) when approaching the CS+ relative to the CS-. The group difference in the posterior cingulate can be explained by learners exhibiting less activation than non-learners when approaching the CS- ($-1, -41, 41$, $k = 114$).

Conclusions: Subgroups based on differences in bilateral amygdala activation during threat learning exhibited differential approach and avoidance behavior in response to learned threat (CS+ vs. CS-). Based on bilateral amygdala activation, individuals who successfully learned the CS+–threat association

exhibited slower approach and faster avoidance to the threat cue. Neural data indicated that learning that the CS+ was threatening yield in group differences in nucleus accumbens and poster cingulate activation when approaching threat relative to non-threatening stimuli. These preliminary data suggest that the amygdala activation during threat learning may be an important factor in subsequent approach and avoidance behavior, which offers clinical implications for exposure-based treatments.

Keywords: Fear Conditioning, Approach/Avoidance, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

M13

Gender-Specific Responses to Stress and Treatment in the Chronic Social Defeat Stress Model for Depression

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Background: There is a higher prevalence of depression in women than in men and there are strong sexual dimorphism in symptom manifestation and in response to antidepressant treatment. Previously, we have shown that combination treatment to simultaneously target stress-induced peripheral inflammation and synaptic maladaptation can promote resilience in the chronic social defeat stress (CSDS) model for depression in male mice. In this study, we tested its efficacy in a newly developed female mouse model of CSDS.

Methods: We used the CSDS paradigm to test the efficacy of the combination treatment. A battery of behavioral testings including the social avoidance test, elevated plus maze test were used to evaluate the efficacy of lead compounds on depression-like phenotypes. Multiplex ELISA was used to assess the peripheral immune responses. RNA-seq was conducted in the brain regions that are pathophysiologically associated with depression including the nucleus accumbens (NAc) and the prefrontal cortex (PFC) to assess for transcriptome changes in response to stress and treatment.

Results: We found that the combination treatment significantly attenuated depression-like behavior in female mice. Similar to male mice, CSDS also induced peripheral inflammation in female mice as reflected by increased IL-6 production following the defeat. We also identified gender-specific cytokine/chemokine changes in female mice in response to stress and to the treatment. Transcriptome profiling of the NAc identified 791 genes that were significantly upregulated by stress and treatment decreased about 15% of these genes to non-stressed control level. 822 genes were significantly downregulated by stress and 13% of these genes were normalized to non-stressed level by the treatment. In the NAc of male mice, we found 446 genes that were significantly upregulated and 431 genes were significantly downregulated by stress. Among these stress-induced differentially expressed genes, 20% were reverted by the treatment to non-stressed level. Interestingly, we found that only 2.8% of the genes regulated by stress in females overlapped with the same genes in male mice. We are currently conducting gene ontology of these regulated transcripts to identify top networks that are dysregulated by stress

in both male and female mice. Similar analysis is carried out in the PFC.

Conclusions: Our study demonstrated that the combination therapy is also effective in attenuating depression-like phenotype in female mice. We found that though stress induces peripheral inflammation and treatment reduced the inflammation in both male and female mice, there is gender-specific cytokine/chemokine cascade and time course differences in response to stress. Transcriptome profiling of NAc showed different gene regulation in response to stress in male and female and in response to treatment. Our results point to distinction in the biological process following stress and provide novel insights into transcriptional mechanisms underlying stress-related depression between male and females.

Keywords: Depression Inflammation Cytokine, Transcriptome, Network-Analysis, Prefrontal Circuit, Nucleus Accumbens

Disclosure: Nothing to disclose.

M14

Open Board

M15

Traumatic Distress Symptom Clusters in Complicated Grief: Response to Treatment

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Background: Complicated grief (CG) is hypothesized to include both attachment and traumatic distress symptoms, and a preliminary diagnosis has been placed in the trauma and stressor related DSM-5 category (APA, 2013). Posttraumatic stress disorder (PTSD) and CG often present comorbidly, and both result from a major stressor (Simon et al., 2007; Marques et al., 2013; Lenferink et al., 2018). Preliminary data suggest posttraumatic stress symptoms (PTSS) may be present across patients with CG, and not vary by whether the loss is violent or accidental in nature such as required for PTSD diagnoses (Simon et al., 2013; Kersting et al., 2011). Much less is known about how PTSS changes with CG targeted treatment, whether this change is impacted by the nature of the death, or whether it may be necessary to target PTSS separately from grief to improve functional outcomes.

Methods: Participants were 395 individuals (mean age $\pm$ SD = 53.0 $\pm$ 14.5 years; 78.0% women) with a primary diagnosis of CG based on structured clinical interviews and an Inventory of Complicated Grief (ICG) score $\geq$ 30. Data were derived from the previously published 20-week multi-center RCT of complicated grief therapy plus pill placebo (CGT + PLA), CGT plus citalopram (CGT + CIT), citalopram (CIT), or placebo (PLA) (Shear et al., 2016). DSM-IV PTSS were assessed using the 17-item self-report Davidson Trauma Scale (DTS). DTS total score of 40 was proposed by the developers of the scale as a cut-off for a diagnosis of PTSD, and has been frequently used as a threshold in previous studies (Davidson et al., 1997; Kastello et al., 2016; Khitab et al., 2013). Our primary analysis examined the adjusted mean difference from baseline in the DTS total and subscale

scores (i.e., intrusion, avoidance-numbing, hyperarousal) over three follow-up periods (week 12, 16, and 20) by treatment arm using longitudinal mixed effects regression with participant specific random intercepts. In follow-up analyses, we investigated whether cause of death (violent vs. non-violent) moderated the relationship between treatments and DTS total score by introducing interaction terms between cause of death and treatment arms in the mixed effects regression model.

Results: In the full sample, the mean DTS total score at baseline was 63.2 ± 27.2 , and 77.7% ($n = 307$) had $DTS \geq 40$. There was a general decreasing trend of mean DTS total scores over the 20-week period with a mean adjusted reduction of 27.4 points ($d = 0.6$) from baseline to week 12 ($p < 0.001$), and a reduction of 30.7 points ($d = 0.7$) from baseline to week 20 ($p < 0.001$). There was no significant difference in change in DTS total score at week 12 by treatment group. However, at weeks 16 and 20, CGT + PLA and CGT + CIT were each associated with a significant DTS reduction compared to placebo alone, while CIT was not. For CGT + PLA vs. PLA, there was 8.8 point ($d = 0.14$) greater reduction in DTS total score from baseline to week 16 ($p = 0.01$), and 12.5 point ($d = 0.19$) greater reduction from baseline to week 20 ($p < 0.001$). For CGT + CIT vs. PLA, there was a 10.0 point ($d = 0.15$) greater reduction in adjusted DTS total score from baseline to week 16 ($p < 0.001$), and 10.7 point ($d = 0.16$) greater decrease from baseline to week 20 ($p < 0.001$). Similar trends were observed for DTS subscales – CGT + PLA and CGT + CIT demonstrated consistent reduction compared to PLA. In the model with interaction terms between treatments and cause of death, the decrease in DTS score for CGT + CIT compared to PLA was 9.5 points ($d = 0.12$) greater for those who had violent death compared to those who did not experience violent death ($p = 0.04$). For CGT + CIT vs. CGT + PLA, however, the reduction in DTS total score was 4.2 points ($d = 0.04$) greater in those who experienced violent death compared to those who did not, but the difference was not statistically significant ($p = 0.53$).

Conclusions: Adults with primary CG assigned to CGT with or without medication demonstrated a significantly larger reduction in PTSS compared to pill placebo, whereas citalopram alone did not. These data parallel findings from the primary study findings for grief (Shear et al., 2016), and demonstrate that CGT may be an effective intervention for PTSS in those with CG. A high level of PTSS were present in this primary CG sample, and PTSS were comparable at baseline for those with violent and non-violent losses. For those who lost someone to violent death, while these data found initial support for greater PTSS reduction for combination therapy with CGT and citalopram compared to placebo, we did not find evidence for a significant benefit of combined therapy over CGT alone for CG due to violent loss. More research is needed to fully understand the role of traumatic distress and its optimal treatment in CG.

Keywords: Complicated Grief, Post Traumatic Stress Disorder, Treatment

Disclosure: Department of Defense, Grant, NIH, Grant, PCORI, Grant, Axovant Sciences, Consultant, Springworks, Consultant, PraxisTherapeutics, Consultant, Aptinyx, Consultant, Genomind, Consultant, G1 Therapeutics, Stock / Equity (Spouse), Wolters Kluwer, Royalties.

M16

Change in Neural Response During Emotion Regulation is Associated With Symptom Reduction in Cognitive Behavioral Therapy for Anxiety Disorders

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Background: Anxiety disorders are common and debilitating conditions that can be treated with cognitive behavioral therapy (CBT). Increased understanding of the neurobiological correlates of CBT may inform treatment improvements and personalization. Prior neuroimaging studies point to treatment-related changes in anterior cingulate, insula, and other prefrontal regions during emotional processing, yet to date the impact of CBT on neural substrates of “top down” emotion regulation remains understudied. We sought to extend earlier work by examining the relationship between symptom changes assessed over the course of CBT treatment sessions and pre- to post-treatment neural change during an emotion regulation task.

Methods: A sample of 30 participants with panic disorder or generalized anxiety disorder completed a reappraisal-based emotion regulation task while undergoing fMRI. During the task, participants viewed negative images and were cued to either reduce emotions using cognitive reappraisal or to maintain emotions. This task was completed before and after completing CBT as part of a larger clinical trial (ClinicalTrials.gov Identifier: NCT00947570). Group analysis was conducted using AFNI's R-based 3dLME program with subjects as a random factor to examine brain regions that changed pre- to post-treatment on the contrast of Reappraise-Maintain. Linear mixed effects models were used to examine if change in neural activation related to improvement trajectory over treatment and/or end-point anxiety severity. Change in neural activation was operationalized as percent signal change extracted from the ROI identified in the 3dLME analysis, i.e., the difference in percent signal change for the Reappraise-Maintain contrast from pre- to post-treatment.

Results: Reduced activation in a region spanning the parahippocampal gyrus and fusiform gyrus was observed from pre- to post-treatment during periods of reducing versus maintaining emotion. Reduction in activation in this region was associated with change in symptoms over the course of treatment, $B = .53$, $SE = 0.25$, $p = .04$, and post-treatment responder status, $B = 4.81$, $SE = 1.58$, $p = .004$.

Conclusions: Results suggest that, from pre- to post-CBT, participants demonstrated downregulation of neural responses during effortful cognitive emotion regulation. Effects were not observed in frontoparietal systems as would be hypothesized based on prior literature, suggesting that treatment-related change could occur outside of top-down control and limbic regions that are central to most models of neural functioning in anxiety disorders. Continued work is needed to better understand how CBT affects cognitive control and memory processes that are hypothesized to support reappraisal as a strategy for emotion regulation.

Keywords: Anxiety, Cognitive Behavior Therapy, Brain Imaging, fMRI

Disclosure: Nothing to disclose.

M17

Altered Infralimbic Plasticity and Diminished Fear Extinction During Adolescence

Abstract not included.

M18

Role of the Suprachiasmatic Nucleus in the Regulation of Anxiety and Depression-Like Behaviors in Mice

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Background: Circadian rhythm disruptions, including a dampening or shifting of rhythms, commonly occur in individuals suffering from depression and/or anxiety disorders. In animal models circadian rhythm disruptions, such as light cycle manipulations, can cause depression and anxiety-like behaviors. However, it is unclear whether light cycle manipulations induce anxiety and depression-like behaviors through a mechanism that is dependent on the suprachiasmatic nucleus (SCN), the master pacemaker in the brain. Currently, there is evidence to both support and refute that the SCN regulates anxiety and/or depression-like behaviors in animal models. Thus, here our goal was to directly determine if chronically dampening or shifting the neural firing rhythms of the SCN increases anxiety and depression-like behaviors by using optogenetics.

Methods: To obtain channelrhodopsin-2 expression in the SCN, we crossed a mouse line where Cre recombinase expression is under the control of the vesicular GABA transporter (Vgat-ires-Cre) with a mouse line expressing Cre-dependent channelrhodopsin-2 (Ai32;ChR2(H134R)-EYFP). Optic fibers were implanted above the SCN of male heterozygous Vgat-ires-Cre;Ai32 mice. Mice were then individually housed in PiezoSleep (Signal Solutions) boxes to measure homecage activity rhythms, a behavioral output of the SCN. To determine the effects of SCN-mediated dampening of rhythms, we unpredictably stimulated (1 h, 10 ms pulse width, 8 Hz) the SCN during the dark phase, when SCN neural activity is low, for nine consecutive days (N = 10-11/group). To determine the effects of SCN-mediated shifting of rhythms, free-running mice received optogenetic stimulation (1 h, 10 ms pulse width, 8 Hz) of the SCN every three days for a total of six stimulations (N = 14-16/group). Here we stimulated the SCN at a time late in the active phase (CT21) of the mice that would be expected to advance rhythms. Control mice received sham stimulations. We then assessed anxiety and depression-like behaviors using a battery of tests (open field, elevated plus maze, light/dark box, forced swim test) while continuing the SCN stimulation paradigms.

Results: Unpredictable stimulation of the SCN during the dark phase dampened the amplitude of homecage activity rhythms. Stimulating the SCN late in the active phase (CT21) shortened the period of and dampened homecage activity rhythms. In mice that received optogenetic stimulation of the SCN at unpredictable times during the dark phase or late in the active phase, correlations were observed between the amplitude of homecage activity rhythms and anxiety-like behavior; indicating that dampened rhythms were correlated with increased anxiety-like behavior in stimulated mice. We did not observe correlations between homecage activity amplitude and anxiety-like behavior in sham stimulated mice. In addition, we did not observe consistent correlations between homecage activity amplitude and depression-like behavior in the forced swim test.

Conclusions: Overall, our findings suggest that SCN neural activity rhythms directly regulate anxiety-like behavior. Specifically, our findings thus far indicate that dampening SCN neural activity rhythms increases anxiety-like behavior. Follow-up studies will focus on whether enhancing SCN neural activity rhythms has anxiolytic effects.

Keywords: Circadian Rhythms, Anxiety, Suprachiasmatic Nucleus

Disclosure: Nothing to disclose.

M19

L-Dopa and Consolidation of Fear Extinction Learning Among Women With Posttraumatic Stress Disorder

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Background: Posttraumatic stress disorder (PTSD) is associated with marked impairment. Exposure-based therapy is among the best supported interventions for PTSD, yet remission rates are typically only 50-60%. Exposure therapy is hypothesized to work via the mechanisms of fear extinction learning, and considerable efforts have been made to identify ways of boosting extinction learning towards the goal of improving exposure therapy efficacy. The role of dopaminergic signaling during the post-fear extinction consolidation window has not been investigated in PTSD, despite a growing body of data implicating dopamine as a critical mechanism underlying fear extinction learning, consolidation, and subsequent recall.

Methods: Adult women diagnosed with PTSD completed a contextual fear acquisition and extinction task during fMRI and then immediately received either placebo (n = 34), 100/25 mg L-DOPA/carbidopa (n = 28), or 200/50 mg L-DOPA/carbidopa (n = 29). Participants completed a resting-state scan before the task and again 45 min following drug ingestion to characterize effects of L-DOPA on extinction memory neural reactivation patterns during consolidation. 24hrs later, participants returned for tests of context renewal, extinction recall, and reinstatement during fMRI with concurrent skin conductance responding (SCR) assessment.

Results: Both active drug groups demonstrated increased reactivation of extinction encodings in the amygdala during the post-task resting-state scan compared to placebo. For SCR data, both drug groups exhibited decreased Day 2 reinstatement across all stimuli compared to placebo, and there was some evidence for decreased context renewal to the fear stimulus in the 100mg group. For imaging data, both drug groups demonstrated decreased Day 2 reinstatement across stimuli in a bilateral insula network compared to placebo. There was no evidence in SCR or neural activity that L-DOPA improved extinction recall. Reactivation of extinction encodings in the amygdala during consolidation on Day 1 predicted Day 2 activation of the insula network.

Conclusions: Across both SCR, neural network, and voxelwise indices of fear recall, the most robust association with L-DOPA was decreased fear responding following reinstatement. The reduced reinstatement was not specific to either CS or context, suggesting both reduced fear reactivation to the CS+ and reduced fear generalization to the CS-. With respect to potential acute mechanisms by which L-DOPA boosts consolidation of extinction learning, the data demonstrated increased neural reactivation of amygdala patterns engaged in response to stimulus offsets (i.e., prediction error teaching signals) during extinction in both drug groups. The specificity of reactivations to the extinction, rather than acquisition, offset patterns is important, as it rules out an alternative explanation of these data: that L-DOPA impairs consolidation of acquisition memories rather than boosting consolidation of extinction memories. We also observed corroborating functional relationships with degree of amygdala extinction reactivation patterns, such that greater reactivations were associated with less AI / IFG network overall activation and less AI responding specifically to the CS+ in the acquisition context (i.e., decreased context renewal).

These results from a multi-site trial suggest the potential for targeting dopaminergic signaling during the post-extinction consolidation window as a means of boosting clinical outcomes for exposure therapy in PTSD. Future research is needed to corroborate these findings and pinpoint the specific role of dopaminergic consolidation processes on reducing fear reinstatement rather than improving fear extinction recall.

Keywords: PTSD, Dopamine, Fear Extinction

Disclosure: Nothing to disclose.

M20

Neural Correlates of Anger Expression in Patients With PTSD

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Background: Anger and aggression are common and debilitating symptoms of post-traumatic stress disorder (PTSD). Although fMRI studies have identified regions underlying anger experience and expression, how these circuits interact with trauma remains unclear. Here we performed the first study examining neural correlates of anger in patients with PTSD.

Methods: Resting-state fMRI was recorded from 162 trauma-exposed veterans (age 32.9 ± 7.7 , 143 male), including 97 without mental illness and 65 with PTSD. The trait anger measure STAXI-2 was used to classify participants into high (>75th percentile, population norm) or low (<25th percentile) anger groups. Global functional connectivity values were calculated for 133 cortical and subcortical regions and entered into a generalized linear model comparing healthy controls, high-anger PTSD patients, and low-anger PTSD patients, while controlling for age, gender, education, IQ, PTSD severity, depression severity, and site of fMRI acquisition. Regions with significant global connectivity differences after FDR correction were subsequently analyzed for group differences in pairwise functional connectivity. A subset of participants ($n = 44$ controls and 36 patients with PTSD) also underwent combined transcranial magnetic stimulation and electroencephalography (TMS-EEG), a tool to probe cortical excitability by recording EEG potentials evoked by single pulses of TMS.

Results: PTSD patients varied widely in anger scores but overall reported significantly higher anger levels than trauma-exposed controls ($p < 0.001$). Anger was associated with global connectivity in two regions: the left dorsolateral prefrontal cortex (DLPFC, salience network) and the right orbitofrontal cortex (OFC, limbic network) (both $p < 0.05$, FDR-corrected). In both regions, PTSD patients with low anger scores showed stronger global connectivity than high-anger patients, who resembled controls. Subsequent analysis of pairwise connectivity revealed a significant association between anger levels and fronto-striatal connectivity. Specifically, high OFC-striatum connectivity was associated with high anger, while high DLPFC-striatum connectivity was associated with low anger. TMS-EEG revealed that excitability in the same DLPFC node was significantly associated with a participant's anger score, with high-anger patients exhibiting weaker evoked potentials in the stimulated region ($p < 0.05$, FDR-corrected).

Conclusions: Patients with PTSD present with varying levels of anger and aggression, and these differences are strongly associated with global connectivity and excitability in prefrontal cortex, along with changes in fronto-striatal connectivity. These results may help pave the way for circuit-based treatments for anger in PTSD.

Keywords: Trait Anger, PTSD, fMRI Functional Connectivity, TMS-EEG

Disclosure: Nothing to disclose.

M21

Learning Not to Avoid: Effects of Transcranial Direct Current Stimulation on Reversal Learning

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Background: Avoidance of anxiety-provoking situations plays a central role in the maintenance of anxiety- and fear-based disorders, such as obsessive-compulsive and posttraumatic stress disorders. The success of exposure-based psychotherapies for these disorders relies on no longer avoiding anxiety-provoking situations. While initial fear acquisition and avoidance generalizes easily across contexts, subsequently learning to not avoid is context-bound and may not readily generalize. The influence of context on learning is mediated by hippocampal-prefrontal connections and represents a novel target for future treatment development. To this end, transcranial direct current stimulation (tDCS) targeting the prefrontal cortex may disrupt contextual encoding to subsequently facilitate generalization of avoidance-based associative learning to novel contexts. However, prefrontal inhibition due to tDCS should also impair avoidance-based associative learning. Here we tested whether cathodal tDCS applied during a contextually-bound reversal learning task 1) acutely impaired avoidance-based associative learning, and 2) facilitated generalization of avoidance-based associative learning to a novel context indicative of reduced contextual encoding.

Methods: Fifty-seven participants (34F:23M; mean 30.8 years old) completed a contextual reversal learning task with the goal to avoid losing points. During the first phase (initial learning) participants saw two sets of images presented in Context 1 (images A/B) or Context 2 (images C/D). Selecting images A and C resulted mostly in losing points, whereas B and D resulted mostly in no points lost. After participants successfully learned this pattern, participants started the second (reversal) phase. During reversal, image pair A/B now appeared in a new context (Context 3; context-dependent reversal), whereas image pair C/D continued to appear in the originally learned context (Context 2; context-independent reversal). Regardless of context, all contingencies reversed, i.e. images B and D resulted in points lost and images A and C resulted in no loss of points.

Participants received cathodal tDCS (20 minutes, 2mA) over the left dorsolateral prefrontal cortex (DLPFC; $n = 27$) or sham stimulation ($n = 30$) at the beginning of the reversal phase and continuing throughout this phase. After meeting reversal criterion, participants completed a third phase in which they saw previously presented stimuli pairs (A/B and C/D) in never before seen Context 4. Participants were asked to select the image they preferred most. In order to test generalization of previous learning and effects of tDCS on generalization of reversal learning, no accuracy feedback was provided during this test phase.

Results: Analyses showed that the groups did not perform significantly different during the initial training ($p = .60$), suggesting no baseline differences in associative learning. Although participants made more errors during context-independent reversal versus context-dependent reversal across groups ($p = .002$, $d = .83$), the active tDCS group performed significantly worse compared to the sham group ($p = .03$, Cohen's $d = .61$) on reversals overall. This effect of tDCS was particularly pronounced in the context-dependent reversal condition ($p = .027$, $d = .60$). There also was a near reduced performance for the active tDCS group vs and sham group on context-independent reversal, but this did not reach for statistical significance ($p = .06$, $d = .51$).

Examination of preferences during the third phase, i.e. generalization, revealed a significant main effect of context ($p = .04$, $d = .54$). Namely, during the third phase, participants continued to avoid the image that was associated with a loss of points during context-dependent reversal, and instead preferred the image that was associated with a loss during initial learning ($p = .001$, $d = .44$). No such preference was observed for images after context-independent reversal ($p = .25$). There was also no significant main effect of active vs. sham group or interaction

between context and group on preferences ($p = .69$ and $p = .27$, respectively).

Conclusions: These results suggest that cathodal tDCS targeting the left DLPFC may function to impair avoidance-based associative learning. Moreover, this finding was prominent during context-dependent reversal, where context provided additional information indicating changing associations. Indeed, across both groups, context-independent reversal, where no additional contextual information was provided, was more difficult than context-dependent reversal, consistent with the importance of context in associative learning. These data support the notion that contextual information from hippocampus to prefrontal cortex can be disrupted with tDCS targeting prefrontal regions. Moreover, the pattern of responses during the test phase in the novel (i.e., never seen before) context suggest that context-dependent associations learned during reversal generalized to a novel context while context-independent reversal associations did not. Yet, tDCS had no effect on the generalization of learned avoidance associations regardless of the presence of contextual information. Ultimately, these findings may provide insight into how tDCS can be combined with exposure-based psychotherapies in which contextual information and avoidance play a critical role.

Keywords: Transcranial Direct Current Stimulation, Reversal Learning, Context, Hippocampal-prefrontal, Anxiety & PTSD

Disclosure: Nothing to disclose.

M22

Investigating Fatty Acid Amide Hydrolase Levels in Social Anxiety Disorder: A PET Study Using [C-11]CURB

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Background: Social anxiety disorder (SAD) is one of the most common psychiatric illnesses in the world, with lifetime prevalence rates ranging from 3.0% to 12.1% among North American adults. Despite ongoing clinical efforts response rates to conventional pharmacotherapies remain low, constituting a need for the investigation of new neurobiological mechanisms. Preclinical evidence suggests that deficient signalling of the major endocannabinoid neurotransmitter, anandamide, through upregulated activity of its enzyme, fatty acid amide hydrolase (FAAH), may be associated with the pathophysiology of anxiety-spectrum disorders. However, there are no clinical in-vivo studies investigating FAAH status in SAD. The aim of this study was to determine whether whole brain FAAH levels are elevated in individuals with SAD compared to healthy controls using positron emission tomography (PET) imaging with the novel FAAH radioligand, [C-11]CURB.

Methods: Participants meeting DSM-5 diagnostic criteria for SAD completed PET imaging procedures with arterial blood sampling. An irreversible two-tissue compartment model with plasma input function was used to estimate λ_{k3} , an index of brain FAAH levels. [C-11]CURB λ_{k3} was investigated in 11 regions of interest (ROI) using a repeated-measures ANCOVA, controlling for genetic variability known to affect [C-11]CURB binding (FAAH rs324420 C > A). Individuals with SAD also completed the Social Interaction Anxiety Scale and the Liebowitz Social Anxiety Scale to assess symptom severity of SAD.

Results: Six participants with SAD ($n = 6$; M/F=1/5; 25.83 ± 3.76 years old; FAAH rs324420: 5 CC, 1 AC) and 49 healthy controls ($n = 49$; M/F = 21/28; 27.84 ± 10.62 years old; FAAH rs324420: 30 CC, 16 AC, 3 AA) completed the study. Preliminary findings revealed that compared to healthy controls, individuals with SAD had a trend for 11.1% elevated [C-11]CURB λ_{k3} across ROIs ($p = 0.08$). Moreover, there was a significant interaction between ROIs X Group ($p = 0.04$). Post-hoc pairwise comparisons show significance in regions including the insula, hippocampus and striatum ($p < 0.05$). We did not find that [C-11]CURB λ_{k3} was related to symptom severity of SAD.

Conclusions: This is the first in-vivo human study investigating brain FAAH levels in SAD. Our early data suggest a trend for modestly elevated whole brain FAAH levels in individuals with SAD compared to healthy controls, as inferred from [C-11]CURB binding. A finding of elevated FAAH levels would support up-regulated FAAH activity as a potential neurobiological mechanism in SAD and could inform future development of FAAH-targeted interventions.

Keywords: Social Anxiety Disorder, Positron Emission Tomography Imaging, Fatty Acid Amide Hydrolase, Endocannabinoid System

Disclosure: Nothing to disclose.

M23

Escitalopram in Adolescents With Generalized Anxiety Disorder: A Double-Blind, Randomized, Placebo-Controlled Study With Pharmacogenomic and Pharmacokinetic Measures

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Background: While selective serotonin reuptake inhibitors represent the first-line pharmacotherapy for pediatric anxiety disorders, including generalized anxiety disorder (GAD), the SSRI, escitalopram, has not been systematically evaluated in adolescents with GAD. Additionally, in pediatric anxiety disorders, there are limited data to aid clinicians in determining which patients will respond to which SSRI. With these considerations in mind, we examined the efficacy and tolerability of escitalopram in adolescents with GAD as well as pharmacogenomic predictors of the magnitude and trajectory of escitalopram-related improvement (e.g., serotonin transporter promoter gene variants and 5-HT2A receptor gene [HTR2A] polymorphisms and CYP2C19 metabolizer status). Additionally, we sought to examine the impact of CYP2C19 phenotype on escitalopram pharmacokinetics.

Methods: Patients were treated with escitalopram (forced titration to 15 mg/day, then optional flexible titration to 20 mg/day) ($n = 26$, mean age: 14.8 ± 1.7 yrs) or placebo (PBO, $n = 25$, mean age: 14.9 ± 0.16 yrs) for 8 weeks. Outcomes were the change in the Pediatric Anxiety Rating Scale (PARS) score and Clinical Global Impressions (CGI) scales as well as vital signs and adverse events. Variants in HTR2A and serotonin transporter (SLC6A4) genes and cytochrome P450 2C19 (CYP2C19) phenotypes were examined with regard to response trajectory and magnitude. For categorical outcomes (e.g., adverse events), treatments were compared with the use of Pearson's chi squared tests or beta posterior probabilities and logistic regression, as appropriate. For continuous outcomes, logarithmic mixed effect models were employed to determine predicted mean outcome values at weeks 0, 1, 2, 4, 6 and 8 (or early termination) to examine group differences. Mixed-effects models included indicator

variables for week and treatment as fixed effects. Each model was created with limited covariates (e.g., age, sex) and refined based on model fit statistics to obtain the most parsimonious response model. Change from baseline CGI-S and CGI-I were examined using the same approach. Plasma escitalopram and desmethylescitalopram concentrations were determined in 18 youth (70% of escitalopram treated patients), including poor (n = 1), intermediate (n = 7), normal (n = 6), rapid (n = 3) and ultrarapid (n = 1) metabolizers. In patients randomized to escitalopram, the relationship between CYP2C19 phenotype and plasma escitalopram AUC0-24 and CMAX at 10 mg/day and 15 mg/day as well as the relationship between CYP2C19 phenotype and escitalopram: desmethylescitalopram ratios were examined using ANOVA tests.

Results: Of 79 individuals screened, 51 were randomly assigned to treatment, took at least one dose of escitalopram (or placebo) and were included in the analyses (escitalopram n = 26; placebo, n = 25). The trajectory of improvement over the 8-week trial and improvement at week 8 was significantly greater in escitalopram-treated patients compared to those receiving placebo (both p-values <0.001). At week 8 (LOCF), the mean change in PARS score in escitalopram-treated patients was -8.65 ± 1.31 compared to -3.52 ± 1.06 in patients receiving placebo (95% CI: -8.57 to -1.70, p = 0.005). The trajectory of improvement in anxiety (escitalopram vs. placebo) over time—as measured by the PARS score—was not associated with age (p = 0.478), age-by-time (p = 0.155), sex (p = 0.870) or sex-by-time (p = 0.708) but was associated with baseline PARS score (p < 0.001). In a logistic response model (CGI-I ≤ 2), SLC6A4 (short/short vs. short/long or long/long, p = 0.005) and HTR2A genotype (presence or absence of GG genotype, rs6311, p = 0.037) as well as CYP2C19 phenotype (intermediate vs. normal, p = 0.015) were significantly associated with CGI-I response. Escitalopram AUC0-24 significantly decreased with increased CYP2C19 metabolism at 10 mg/day and 15 mg/day (ANOVA test for linear trend, p = 0.0496 and p = 0.042, respectively). CMAX trended towards being higher in slower metabolizers, relative to faster metabolizers, at the 15 mg/day (ANOVA test for trend, p = 0.070), but not at 10 mg/day (p = 0.170). Desmethylescitalopram: escitalopram ratios were increased in patients with faster CYP2C19 metabolism relative to those with slower metabolism (p < 0.001). Vital signs, QTc and adverse events were similar in the escitalopram-treated adolescents compared to those receiving placebo.

Conclusions: Consistent with most studies of SSRIs in pediatric anxiety disorders, escitalopram was superior to placebo in reducing anxiety symptoms. In fact, the NNT (2.7) in this study is similar to other studies of SSRIs in pediatric anxiety disorders and placebo response was relatively low (24%) which is similar to most federally-funded trials of SSRIs in pediatric anxiety disorders. Pharmacogenomic variables—including pharmacodynamic and pharmacokinetic genes—influenced the trajectory and magnitude of improvement. Finally, variation in CYP2C19 metabolism accounts for pharmacokinetic variation of escitalopram in adolescents, raising the possibility that—like in adults—CYP2C19 phenotype should be considered when dosing escitalopram.

Keywords: Anxiety Disorders, Escitalopram, SSRI, Pediatric

Disclosure: Myriad Genetics, Consultant, Neuronetics, Grant, Allergan, Grant, Otsuka, Grant, Self, CMEology, Honoraria, UpToDate, Honoraria

M24

PTSD Re-Experiencing and Arousal Indices are Associated With Volumetric Measures of Thalamic Nuclei

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Background: Re-experiencing symptoms are core to posttraumatic stress disorder (PTSD) and are often dominated by the recollection of sensory-perceptual elements of the trauma (Brewin et al 2014). This phenomenology points to involvement of the thalamus, a key hub for relaying and modulating sensory information across brain networks. In PTSD, functional activation and connectivity of the thalamus has been implicated in flashbacks and processing of trauma-related cues (e.g., Rauch et al 2016). However, less research has examined trauma re-experiencing in relation to thalamus anatomy. Moreover, despite substantial evidence that thalamic nuclei are anatomically and functionally heterogeneous, it is unknown whether re-experiencing and other symptoms of PTSD are differentially related to particular nuclei.

In this study, we examined re-experiencing symptoms in relation to volumes of thalamic nuclei implicated in sensory information processing. We hypothesized that re-experiencing, controlling for other PTSD symptoms, would be significantly associated with volumes of the pulvinar nuclei that are involved in visual salience, of the ventroposterolateral thalamic (VPL) nuclei that mediate somatosensory functions, and of the lateral and medial geniculate nuclei (LGN, MGN) that relay primary visual and auditory information. We also predicted that re-experiencing symptoms would not be significantly associated with the aggregate volume of remaining, predominantly non-sensory, thalamic nuclei.

Methods: Participants were 42 trauma-exposed adults (20 female), 29 with DSM-IV PTSD. All were interviewed with the Clinician Administered PTSD Scale (CAPS) and underwent 3T magnetic resonance imaging. Freesurfer was used to estimate volumes of thalamic nuclei and intracranial volume (ICV; Iglesias et al 2018). CAPS scores were derived for current re-experiencing, anxious arousal, dysphoric arousal, emotional numbing, and active avoidance symptoms (Pietrzak et al 2015). Three hypothesis-based analyses were run using 1) total pulvinar volume/ICV, 2) total ventroposterolateral volume/ICV, and 3) total LGN + MGN volume/ICV as dependent variables, and using a Bonferroni-adjusted p-value of .0166. A fourth analysis had as the dependent variable total non-sensory thalamus/ICV, with non-sensory thalamus defined as: total thalamus minus the pulvinar, VPL and LGN/MGN volumes. The independent variables in all four analyses were the five CAPS symptom dimensions entered as simultaneous predictors, with age and sex as covariates.

Results: Re-experiencing symptoms were positively associated with pulvinar/ICV (p = .005), LGN + MGN/ICV (p = .007) volumes, and VPL/ICV volumes (p = .02), the latter falling short of Bonferroni-adjusted significance. Anxious arousal was significantly negatively associated with pulvinar/ICV (p = .005), LGN + MGN/ICV (p = .002), as well as VPL/ICV (p = .002) volumes. Re-experiencing and anxious arousal were not significantly associated with non-sensory thalamus/ICV.

Conclusions: Re-experiencing and anxious arousal symptoms were associated with volumes of thalamic nuclei that have a central role in processing primary sensory information, as well as modulating and integrating sensory information within large-scale cortical and subcortical networks. In a cross-sectional study, we cannot differentiate whether these correlations suggest that individual differences in thalamus volumes confer vulnerability to developing re-experiencing and hyperarousal symptoms, or whether PTSD symptoms may induce changes in the morphology of thalamic nuclei. Overall, these results encourage further examination of specific thalamic nuclei whose functional

differentiation may be mechanistically relevant to trauma intrusions in PTSD.

Keywords: PTSD, Magnetic Resonance Imaging, Thalamus, Traumatic Memories, Hyperarousal

Disclosure: Nothing to disclose.

M25

The Role of Ventral Hippocampal Parvalbumin Interneurons During Anxiety-Like and Reward-Seeking Behaviors

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Background: The hippocampus is functionally heterogeneous across the dorsal-ventral axis in rodents and the anterior-posterior axis in humans, with the ventral/anterior portion more involved in emotional processing. In mice, the output region of the ventral hippocampus, the ventral CA1 (vCA1), has direct connections to both fear and reward circuits and mediates expression of innate and learned fear as well as reward-seeking behavior. Parvalbumin (PV)-expressing interneurons help regulate outputs from the vCA1, and altered functioning of this cell population has been implicated in stress models and psychiatric disease states. In these studies, we determine the behavioral and local network activity effects of PV cell inhibition using optogenetics and immediate early gene quantification. We also examine in vivo PV cell activity during anxiety-like and reward-seeking behaviors using calcium imaging with miniature microscopes.

Methods: Male and female PV-Cre c57BL/6J mice are used for all experiments. For optogenetic studies, adeno-associated virus (AAV) containing Cre-dependent eNpHR3.0 was injected bilaterally into the vCA1, followed by fiber optic implants. For calcium imaging studies, AAV containing Cre-dependent GCaMP6f is injected unilaterally into the vCA1 followed by implantation of a gradient index (GRIN) lens. Mice then freely explored various environments that included innately anxiogenic zones, fear-conditioned contexts, or palatable rewards in familiar or novel environments. During behavioral tests, inhibitory opsins on PV cells were stimulated by timed light delivery in optogenetic experiments. For calcium imaging experiments, calcium signals from PV cells was imaged using miniaturized microscopes. At the end of optogenetic experiments, mice explored either an open field or sucrose gustometer while light was delivered to active or control opsins. Animals were perfused 90 minutes later and brain tissue was collected. Local vCA1 activity levels during eNpHR3.0 stimulation of PV cells was assessed using immunohistochemical staining and quantification of cFos expression.

Results: Optogenetic inhibition of vCA1 PV cells increased avoidance of innately anxiogenic environments, such as the center of an open field. PV inhibition decreased expression of learned fear after contextual fear conditioning, as measured by decreased time spent freezing in the fearful context one day after conditioning. Inhibiting PV cells had no effect on general locomotor activity ($n = \sim 6/\text{group}$). When mice had access to variable sucrose concentrations, delivered in a random order and with a cue signaling the end of the intertrial interval, PV cell inhibition increased reward-seeking, as measured by decreased latency to lick the spout delivering sucrose reward. Comparisons of PV cell inhibition effects on latency versus consumption behavior (i.e., licking) are ongoing. vCA1 cFos expression and task-related in vivo calcium activity from vCA1 PV cells will also be presented.

Conclusions: Our findings demonstrate that acute manipulation of PV interneuron activity in the vCA1 is sufficient to alter

anxiety-related behavior. Inhibiting PV interneurons would be presumed to disinhibit local excitatory pyramidal cells. Consistent with this prediction, the behavioral effects of PV inhibition in innate and learned anxiogenic conditions were in the opposite direction from the effects of optogenetically inhibiting vCA1 pyramidal cells. Here, we show disparate effects of PV cell inhibition on innate versus learned fear expression. We also examine the effects of PV inhibition on different components of reward ('wanting' versus 'liking') during sucrose consumption. These studies suggest how PV cell dysfunction in the vCA1 might affect anxiety-related and reward-seeking behavior.

Keywords: Ventral Hippocampus, Parvalbumin Neurons, Optogenetics, in vivo Calcium Imaging, Anxiety Circuitry

Disclosure: Nothing to disclose.

M26

Inhibition of Phosphodiesterase 2 Alleviates Anxiety-Like Behavior and Fear Memory in Mouse Model of Post-Traumatic Stress Disorder

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Background: Post-traumatic stress disorder (PTSD) is characterized primarily by a dysregulated fear response and memory, with some anxiety and panic episodes, depression and avoidance coping symptoms. Current treatments are effective for some individuals but are limited for most patients. Thus, it is imperative to develop new therapeutics for the treatment of PTSD-like symptoms by understanding the pathological changes of this disorder. Dysfunction of phosphodiesterase (PDE) enzyme is involved in PTSD-related anxiety-like behavior. However, whether PDE2 inhibition could rescue PTSD-like symptoms such as anxiety, depression and fear memory disorder are still unknown.

Methods: The present study determined whether PDE2 inhibition could rescue PTSD-like symptoms such as anxiety and fear memory disorder. Male mice were separated into 7 groups (10 mice/group) and subjected to single prolonged stress (SPS). They were treated with Bay 60-7550 (0.3, 1, or 3 mg/kg, i.p.) from the next day of SPS for 10 days. The behavioral tests such as open field, forced swimming test, elevated plus maze, and contextual fear paradigm were conducted to determine the effects of the selective PDE2 inhibitor Bay 60-7550 on SPS-induced depression- and anxiety-like behavior and fear memory deficits. Results were analyzed statistically by one-way analysis of variance (ANOVA), followed by Dunnett's t-test. All test results that yielded a P-value less than 0.05 are considered statistically significant.

Results: The results suggested that Bay 60-7550 increased the time spent in the center zone time, the open arm entries and time spent onto the open arms in the open field and elevated plus maze tests, which were reduced in mice subjected to SPS. Bay 60-7550 also reversed SPS-induced increase in the immobility time in forced swimming test and the percentage of freezing time in the contextual fear paradigm. Moreover, Bay 60-7550 prevented SPS-induced changes in the adrenal gland index, synaptic proteins synaptophysin and PSD95 expression, PKA, PKG, pCREB, and BDNF levels in the hippocampus and amygdala. These effects were completely prevented by PKG inhibitor KT5823. While PKA inhibitor H89 also prevented Bay 60-7550-induced pCREB and BDNF expression, but only partially prevented the effects of Bay 60-7550 on PSD95 expression in the hippocampus.

Conclusions: These findings suggest that Bay 60-7550 protects animals against SPS-induced traumatic injury by activation of

cGMP- or cAMP-related neuroprotective molecules, such as synaptic proteins, pCREB and BDNF.

Keywords: PTSD, Phosphodiesterase-2, Bay 60-7550, PKA/PKG, BDNF

Disclosure: Nothing to disclose.

M27

Neurostructural Heterogeneity in Youth With Internalizing Symptoms

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Background: Internalizing disorders such as anxiety and depression are common psychiatric disorders, frequently begin in youth, and exhibit marked heterogeneity in treatment response and clinical course. Given that symptom-based classification approaches do not align with underlying neurobiology, an alternative approach is to identify neurobiologically-informed subtypes based on brain imaging data.

Methods: We used a recently developed semi-supervised machine learning method (HYDRA) to delineate patterns of neurobiological heterogeneity within youth with internalizing symptoms using structural data collected at 3T from a sample of 1,141 youth. Data were analyzed using generalized additive models with penalized splines to capture both linear and nonlinear developmental effects. Age, sex, and data quality were included as covariates. To account for multiple testing, we controlled the False Discovery Rate (FDR, $q < 0.05$).

Results: Using volume and cortical thickness, cross-validation methods indicated two highly stable subtypes of internalizing youth ($ARI = .66$; permutation-based $p_{fdr} < .001$). Subtype 1, defined by smaller brain volumes and reduced cortical thickness, was marked by impaired cognitive performance and higher levels of psychopathology than both Subtype 2 and typically developing youth. Using resting-state fMRI and diffusion images not considered during clustering, we found that Subtype 1 also showed reduced amplitudes of low-frequency fluctuations in fronto-limbic regions at rest and reduced fractional anisotropy in several white matter tracts. In contrast, Subtype 2 showed intact cognitive performance, greater volume, cortical thickness, and amplitudes during rest compared to Subtype 1 and typically developing youth, despite still showing clinically significant levels of psychopathology.

Conclusions: We identified two subtypes of internalizing youth differentiated by abnormalities in brain structure, function, and white matter integrity, with one subtype showing poorer functioning across multiple domains. Identification of biologically-grounded internalizing subtypes may assist in targeting early interventions and assessing longitudinal prognosis.

Keywords: Brain Structure, Brain Development, Anxiety Development, Human Neuroimaging

Disclosure: Nothing to disclose.

M28

Structural Covariance Analyses of Cortical Thickness and Surface Area Measures: - A Multisite ENIGMA - PGC Study

Abstract not included.

M29

Neuroticism Polygenic Association Score and Dopaminergic Systems Correlates: Investigations With [18F]-FDOPA, [11C] NNC-112, and [18F]Fallypride PET

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Background: Neuroticism is a heritable trait characterized by a proclivity towards anxiety, depressed mood, and stress-sensitivity. It is correlated with severity and prognosis of a variety of psychopathological conditions, including mood, anxiety, and psychosis. Despite its clinical significance, the neurobiological underpinnings of neuroticism are poorly understood. Prior studies suggest inverse correlations between measures of neuroticism and D2/3 receptor availability as well as dopamine synthesis capacity in the dopamine-rich striatum. Whether these potential neural phenotypes are meaningfully linked to newly identified genetic contributors to this trait remains untested.

Methods: Three overlapping cohorts of healthy adults underwent PET scans in separate sessions as follows: a 90 min scan after IV administration of up to 16 mCi of [18F]-FDOPA radiotracer to assess presynaptic dopamine synthesis capacity (Ki) [$n = 182$, mean age = 35.6 ± 0.8 , 93 female], a 90 min scan after IV administration of 20 mCi of [11C]NNC-112 radiotracer to assess D1-receptor binding potential (BPND) [$n = 113$, mean age = 38.1 ± 1.0 , 57 female], and a four hour scan after IV administration of 5 mCi [18F]fallypride radiotracer to assess D2/3 binding potential (BPND) [$n = 105$, mean age = 38.5 ± 1.1 , 48 female]. Specific tracer uptake constant ([18F]-FDOPA-Ki) and binding potentials ([11C]NNC-112-BPND and [18F]fallypride-BPND) were calculated with Gjedde-Patlak and SRTM modeling using cerebellar reference regions in PMOD. Additionally, participants were genotyped and, using data derived from a meta-analysis of GWAS studies examining neuroticism in 449,484 individuals (Nagel et al, 2018), a series of neuroticism polygenic association scores were derived using a range of p-value thresholds, and the first principal component of these scores (PAS) was carried forward for voxel-wise analyses within the striatum. Exploratory whole brain analyses were also conducted ($p < 0.005$, uncorrected).

Results: Striatal voxel-wise analyses revealed a negative correlation between the neuroticism PAS and [18F]-FDOPA-Ki in the right putamen ($p = 0.002$) and [18F]fallypride-BPND in the left putamen ($p = 0.003$). There were no striatal findings with [11C]NNC-112. Whole-brain analyses revealed a positive correlation with [18F]-FDOPA-Ki in the right inferior frontal gyrus ($p = 0.00005$), and with clusters in the bilateral medial temporal lobes (MTL) (p 's < 0.0004). A negative correlation was seen in the left orbitofrontal cortex with [11C]NNC-112 ($p = 0.002$) and in the left amygdala with [18F]fallypride-BPND ($p = 0.001$).

Conclusions: Individuals with greater polygenic risk for neuroticism may have lower presynaptic dopamine synthesis and dopamine receptor availability in regions important for mood, anxiety, and reinforcement learning, consistent with the literature on neuroticism itself, but also increased presynaptic dopamine synthesis in limbic temporal lobes. These results lend support for associations between putative molecular foundations of neuroticism and dopamine systems in the healthy human brain and merit further study in patient populations.

Keywords: Neuroticism, Dopamine, Polygenic Risk Scores, Positron Emission Tomography Imaging

Disclosure: Nothing to disclose.

M30

Neural Markers of Successful In-Scanner Worry Reappraisal in Older Adults: Open-Label Neural Target Engagement Using Intermittent Theta-Burst TMS

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Background: Severe worry is a transdiagnostic symptom that is associated with increased risk of conversion to Alzheimer's disease and increased risk of cardiovascular events, including stroke. While treatments like cognitive-behavioral therapy are effective at reducing overall anxiety, they are largely ineffective in reducing worry severity in older adults. In this study we investigate the neural basis of both induction and reappraisal of worry using an in-scanner personalized worry task. We identified several regions that were associated with worry severity and conducted an open-label treatment using intermittent theta-burst stimulation (iTBS) [a form of transcranial magnetic stimulation (TMS)] of an identified target in 5 participants.

Methods: We recruited 36 individuals age 50 and older, with varying degrees of worry severity (as measured by the Penn State Worry Questionnaire, PSWQ). Based on the clinical interview with each participant, we built a participant-specific list of worry induction and worry reappraisal sentences. These sentences (one per block) were presented to participants during functional magnetic resonance imaging (fMRI) with fixation in between blocks. Worry induction blocks were followed either by neutral blocks (control condition, included random factual sentences – e.g., “fish live in the ocean”) or by worry reappraisal blocks. Following each block, participants rated their worry severity (1-5). After motion correction, spatial normalization to a standard anatomical space, and smoothing we conducted mass-univariate general linear modeling to model worry induction, reappraisal, and neutral blocks. We then parametrically modulated each block by the in-scanner worry severity rating in order to identify regions of activation associated with worry ratings following induction/reappraisal. We also modeled 6 parameters of motion and the mean, along with an autoregressive, AR(1), model to account for unmodelled/aliased noise and a discrete high-pass filter (1/128Hz) to account for drift. Using statistical non-parametric mapping (SnPM12), we performed a paired t-test to identify regions that were significantly more parametrically modulated during worry induction compared to worry reappraisal. We also conducted paired t-tests to identify regions that were activated more during worry induction and reappraisal compared to neutral. SnPM computes non-parametric p-values which are then corrected using a cluster-wise inference method (cluster forming threshold of $p < 0.001$) that controls the family wise error rate (FWE) at $\alpha = 0.05$.

We identified several regions that had activation that increased parametrically with in-scanner worry severity. We targeted the right supramarginal gyrus (see results). We recruited 5 participants from our sample with PSWQ > 55 to participate in an open-label treatment with iTBS (120% of the motor threshold, triplet 50 Hz bursts, repeated at 5 Hz; 2 s on and 8 s off; 400 pulses per session with a ramp up block of 400 pulses; total duration of 5 min; 10 total sessions). Neuronavigation software was used to target the right supramarginal gyrus identified in our study.

Results: Compared with the neutral condition, worry induction and reappraisal were associated with significantly higher activation in multiple regions including the visual cortex, caudate, the cingulate and prefrontal cortex, hippocampus/parahippocampus, insula, and supramarginal gyrus. The parametric modulation

analysis showed that the dorsal anterior cingulate (dACC: $x = 6$, $y = -4$, $z = 42$, $T_{peak} = 4.7$, 171 voxels) and the bilateral temporoparietal junction (supramarginal gyrus SMG: $x = -48/56$, $y = -38/-30$, $z = 22/16$, $T_{peak} = 4.5$, 258/165 voxels) had greater activation associated with higher in-scanner worry severity rating following worry induction. The same regions had greater activation associated with lower in-scanner worry severity rating following worry reappraisal.

Stimulation of SMG using iTBS significantly decreased worry severity (PSWQ, $t(4) = 2.4$, $p = 0.0779$). Parametrically worry-modulated activation in both the dACC and SMG decreased [$t(4) = 2.3$, $p = 0.0822$ and $t(4) = 1.6$, $p = 0.176$]. Improvement in worry (PSWQ) was associated with increased worry-modulated activation during reappraisal relative to worry induction blocks [$r(4) = 0.4$, $p = 0.5617$].

Conclusions: The dACC and the temporoparietal junction showed greater activation during worry induction when participants reported higher levels of worry at the end of worry-induction blocks. This result may indicate an increased level of affective mentalizing (SMG) coupled with a more sustained effort toward implicit-controlled regulation (dACC) in participants who exhibit higher level of worry following induction.

In contrast, the activation in these regions was greater during reappraisal if the participants reported lower levels of worry at the end of worry-reappraisal blocks. This result may be interpreted as a marker of successful reappraisal and points toward role of both dACC and SMG in cognitively regulating worry severity. These results indicate potential targets for future interventions designed to alleviate severe worry in older adults (e.g. multimodal interventions such as CBT plus device-based interventions).

TMS of the TPJ seems to show potential for reducing worry-modulated activation and while not significant (due to small sample sizes), there is potential for its development as a treatment for severe worry.

Keywords: Worry, TMS, Target Engagement, Anxiety, Theta Burst Transcranial Magnetic Stimulation

Disclosure: Nothing to disclose.

M31

Differential Neural Activation During Contextual and Cue Reversal Learning in Humans

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Background: The ability to flexibly respond to changes in the environment is critical for adaptive behavior. Reversal learning is a form of associative learning that tests the ability of an organism to change responses when contingencies are altered. For example, conditions that were once rewarding may become threatening and vice versa. Successful reversal learning is thought to be mediated by cortico-striatal and medial temporal lobe (MTL) systems. Interestingly, a previous study in patients with confirmed MTL atrophy reported specific deficits in reversal of contextual associations, but intact reversal of cue associations, independent of the outcome valence. Reversal learning of contextual versus cue associations may rely on different components on this system; however, this has not been investigated.

Methods: 34 adults (mean age 24.09 ± 4.92 years; 21 females) completed a validated cue-context reversal learning paradigm adapted for use with functional magnetic resonance imaging (fMRI). Pairs of cues (objects) and contexts (background color) were presented with one of two outcomes (money or bomb), and

then the outcome association of either the cue or context was reversed, requiring participants to form new associations between the context or cue and outcome. Neural activation during contextual versus cue reversal learning was compared in MTL subregions (e.g., hippocampus, parahippocampal cortex), striatum, and prefrontal regions, including the orbitofrontal cortex (OFC)/insula and dorsolateral prefrontal cortex (dlPFC) based on previous reversal learning studies. Significance was determined in each region of interest using a small-volume family-wise error-corrected threshold $pFWE < 0.05$.

Results: Relative to cue reversal, contextual reversal learning was associated with increased activation in the anterior hippocampus [35 voxels, $pFWE = 0.002$, $Z = 4.07$, $x = -26$, $y = -10$, $z = -20$] and anterior parahippocampal cortex [13 voxels, $pFWE = 0.04$, $Z = 3.03$, $x = -24$, $y = -34$, $z = -20$]. In contrast, relative to contextual reversal, cue reversal learning was associated with increased activation in the striatum [47 voxels, $pFWE < 0.001$, $Z = 4.34$, $x = -12$, $y = -6$, $z = 6$], lateral OFC/insula [60 voxels, $pFWE < 0.001$, $Z = 4.21$, $x = 46$, $y = 16$, $z = 0$], and dlPFC [56 voxels, $pFWE = 0.004$, $Z = 3.63$, $x = 50$, $y = 18$, $z = 36$].

Conclusions: These findings support previous evidence that the MTL is critical for the reversal of associations that involve contextual (but not cue) information. Furthermore, our results implicate cortico-striatal regions in the reversal of cue-based associations.

Keywords: Associative Learning, Hippocampal-Prefrontal, Corticostriatal Circuit, fMRI, Reversal Learning

Disclosure: Nothing to disclose.

M32

Circulating Cell-Free MtDNA (ccf-MtDNA) in Healthy Young Adults With and Without Early Adversity Following Acute Psychosocial Stressor: A Pilot Study

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Background: Mitochondria are responsive to both acute and chronic exposure to psychosocial stress. A few recent studies suggest that circulating cell-free mitochondrial DNA (ccf-mtDNA) may rise with psychopathology and acute psychosocial stress. No prior work has examined ccf-mtDNA in relation to adversity or trauma exposure. This study assessed ccf-mtDNA at baseline and in the context of an acute stressor in a sample of healthy young adults, with or without a history of parental loss and maltreatment.

Methods: Healthy young adults ages 19-40 ($N = 40$) were recruited via community advertisements and assessed for eligibility via phone interview. Cases ($N = 20$) experienced parental loss before age 11 and childhood abuse/neglect. Controls ($N = 20$) had no early stress or psychiatric history. Standardized interviews and self-reports assessed demographics, medical/psychiatric history, childhood adversity, health behaviors. Participants had no acute/chronic medical conditions, current medications, bipolar or psychotic disorders. Blood samples were collected for ccf-mtDNA and nuclear DNA (ccf-nDNA) at baseline visit as well as before and after acute psychosocial stress, using the Trier Social Stress Test (TSST). Quantitative PCR was performed in triplicate using ccf-DNA extracted from centrifuged plasma samples and copy numbers of ccf-mtDNA and ccf-nDNA were normalized to plasma volume.

Results: In response to the psychosocial stressor, both groups demonstrated a significant increase in ccf-nDNA levels ($p < .05$),

but no significant change in ccf-mtDNA levels ($p = .33$). There was no significant time by group interaction for ccf-nDNA ($p = 0.32$) or ccf-mtDNA ($p = 0.10$). The ratio of ccf-mtDNA to ccf-nDNA demonstrated a significant effect of time ($p < .01$) and of trauma-related disorders ($p < .05$).

Conclusions: These preliminary findings add to the evidence that cell-free DNA may be involved in the response to acute psychosocial stressors. Because elevations in ccf-mtDNA are known to be elevated in physiologic stress, such as exercise or illness, elevations in response to a psychosocial stressor may indicate the role of mitochondrial signaling in broader systemic responses to psychosocial stress. Higher ratio of ccf-mtDNA to ccf-nDNA was also linked to current trauma and stressor-related disorders, suggesting the possibility of mitochondrial changes may be associated with underlying psychological functioning.

Keywords: Mitochondrial DNA, Early life Stress, Trauma and Stress Disorders

Disclosure: Nothing to disclose.

M33

Cross-Species Analysis of Motor Activity as Window Into Determinants of Rhythm Disturbances in Mood Disorders

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Background: Convergent evidence suggests that mood disorders are associated with irregular wake/sleep rhythms and altered motor activity. Human studies using actigraphy-based tracking in real time have revealed the central role of disturbances in motor activity in mood disorders, particularly Bipolar Disorder (BD) that is characterized by dysregulation of patterns of motor activity. Likewise, the atypical subtype of depression, characterized by overeating and oversleeping is associated with disrupted patterns of both activity and sleep based on objectively tracked assessments). At present, there are well-established methods for measuring motor activity in different species, such as non-human primates and rodents. However, the widespread variability of tools limits comparisons across these studies as well as applying the findings to human research. This presentation will examine the use of actigraphy in humans and monkeys as a first step in developing cross species approaches to examine patterns of motor activity. Second, we present data on the definitions, measures and correlates of motor activity in rodents based on infrared motion detectors.

Methods: Patterns of motor activity based on accelerometry using a Phillips accelerometry device was collected across two-week period in humans ($n = 339$) (65% female and mean age of 41) and one week in adult female rhesus monkeys ($n = 35$; 16-24 years of age). The human sample comes from the NIMH Family Study of Mood Spectrum Disorders that is a community-based family study of the mood disorder spectrum. The monkey data are derived from a sample of adult female rhesus monkeys from a study of predictors of degeneration of nigrostriatal dopaminergic neurons.

Results: First, we asked how similar were daily activity patterns in monkeys and humans? fPCA showed that the first four components of the daily activity patterns were very similar in humans and monkeys. Moreover, the 4 highest loading components of monkeys explained 85.44% of the variability in PC1 in humans, and 76.44% of PC2. Conversely, the 4 highest loading components from human explain 85.32% of the variability in PC1 of monkeys, and 83.85% of the variability in PC2 of monkeys. Second, we asked if there were detectable differences in activity

patterns in humans with Bipolar disorder? People with Bipolar disorder exhibited altered circadian activity patterns relative to controls with lower average activity in mid to late afternoon (p vs controls < 0.01), and higher day-to-day variation in their activity level (p vs other disorders < 0.03). In addition, the general activity of single-housed adult male mice was monitored using both infrared motion detectors mounted to the top of the cages and wheels. Patterns of motor activity in response to light were examined to identify whether there were particular light conditions that led to depression like behaviors.

Conclusions: These findings demonstrate the value of studying a core component of behavior across humans and animals. The use of motor activity as a core behavioral measure of depression can in part minimize the limitation of current behavioral models of depression in basic science. Harmonization of measures that distinguish motor activity from exercise in humans, and motor activity from motivation in animals will be critical to provide valid comparisons both within basic science and between basic and human studies.

Keywords: Motor Activity, Actigraphy, Mood Disorder Subtypes, Cross Species, Circadian Rhythm

Disclosure: Nothing to disclose.

M34

Understanding Development and Dysfunction of the Human Prefrontal Cortex Through the Lens of Evolution

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Background: The human prefrontal cortex (PFC) is a region of the cerebral cortex expanded in primates and associated with higher-order cognitive function including abstract thinking, moral reasoning, and language. Dysfunction in the PFC has been implicated in various neuropsychiatric diseases including ASD and schizophrenia. Comparative analyses of the PFC have identified human-specific changes in cell number, morphology, and types as well as microcircuitry and long-range connections, which are thought to underlie human advanced cognitive capabilities and possibly, susceptibility to disease. These changes in circuitry are likely mediated by divergent changes in spatiotemporal patterns of gene expression and cis-regulatory element activity, most evident during early fetal and mid-fetal stages. Linking these genomic changes with human-specific changes in cortical anatomy will be integral to modeling and understanding development of human and primate-specific cortical circuitry, as well as how dysfunction in this circuitry lead to neuropsychiatric disease. However, this process has been arduous due to difficulty accessing high-quality human and primate expression data. To remedy this problem, our laboratory along with multiple other laboratories have created a spatiotemporal atlas of gene expression in humans and macaques spanning from embryonic ages to adulthood, as part of the PsychEncode initiative, called BrainSpan. Using this database, we are able to identify candidate genes differentially expressed in the human and primate PFC during important periods of development, which can be then be studied in animal models to identify their role in development and dysfunction of the cerebral cortex.

Methods: We used the BrainSpan database to identify genes and signaling pathways enriched in the frontal lobe during mid-fetal development, a crucial developmental period for the formation of neuronal circuits with an enrichment of expression

of genes implicated in schizophrenia and ASD. Expression of select candidate genes was further profiled in various species including mouse, macaque, and human using In Situ Hybridization and RNA-sequencing. In addition, we utilized comparative genetics combined with both in vitro and in vivo assays to identify non-coding elements that underlie these species-specific shifts in expression. Finally, we studied the function of select candidate genes and regulatory elements in mouse using CRISPR-Cas9 technology and in utero electroporation combined with a diverse set of assays including diffusion tensor imaging (DTI) and RNA-sequencing to study the functional significance of this network.

Results: We identified an enrichment of genes involved in synaptogenesis and axon development in the frontal lobe during human mid-fetal development (PCW 16-22). Pathway analysis identified that many of these enriched genes are regulated by the same upstream signaling pathway, whose function in later cortical development has not been explored. Reduction in activity of this signaling pathway in the early postnatal mouse medial frontal cortex leads to selective loss of reciprocal thalamocortical connectivity both through DTI (135.3 fibers in WT to 14 fibers in Mutants; $p < 0.001$, $n = 5$) and lipophilic tracing ($n = 3$) at both P5 and P30. Furthermore, expansion of this signaling pathway in mouse frontal cortex, to replicate the lateral expansion of activity of this signaling pathway seen in humans, leads to an increase in the thickness of layer IV in motor regions. In addition, we identified an enhancer regulated by this pathway with a hominini-specific (human, great apes) deletion. Targeted replacement with the human enhancer in mouse leads to increased excitatory synapses of both upper (39.5%; $P < 0.05$; $n = 5$) and deeper layers at P0 (47.9%; $P < 0.0005$; $n = 5$) and selectively in the deeper layers at P30 frontal cortex (21.4%, $P < 0.005$; $n = 3$).

Conclusions: Using the BrainSpan database, we were able to identify genes enriched in the human PFC which are co-regulated by a signaling pathway previously linked to schizophrenia. Using mouse models, we identified that this signaling pathway is required for mediodorsal thalamus innervation and density of excitatory synapses in the PFC. Connections between the mediodorsal thalamus and PFC are implicated in working memory, behavioral flexibility and goal-directed behaviors and are disrupted in schizophrenia. Together, we have identified a possible mechanistic link between a previously described dysregulation in a specific signaling pathway, cognitive dysfunction, and schizophrenia.

Keywords: Cortical Development, Gene Regulation, Fetal Brain Development, Cortical Circuit Function, Schizophrenia

Disclosure: Nothing to disclose.

M35

Amelioration of Autism-Like Social Deficits by Targeting Histone Methyltransferases EHMT1/2

Abstract not included.

M36

Gray Matter Volume Correlates of Behavioral Activation and Inhibition System Traits in Children: A Voxel-Based Morphometric Study of the ABCD Data

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Background: Behavioral activation system (BAS) and behavioral inhibition system (BIS) traits play a central role in determining individual variations in behavioral disposition and vulnerability. Structural brain imaging provides information on regional gray matter volumes (GMV), as may be related to BAS and BIS traits. With voxel-based morphometry (VBM) Li et al., 2014 examined sex differences in the correlations between regional GMVs and BIS/BAS scores in 353 healthy young adults (~ 20 y/o). The results showed a negative correlation between BIS trait and rGMV in the parahippocampal gyrus (PHG), as well as positive correlations between BAS sensitivity and rGMV in the ventromedial prefrontal cortex (vmPFC) and inferior parietal lobule (IPL) in women, whereas men showed the opposite pattern. These findings suggest sex-linked neuroanatomical correlates of BIS and BAS.

In the current work, we took advantage of the large data set from the NIH Adolescent Brain Cognition Development (ABCD) project, and employed VBM to examine GMV correlates of BAS/BIS and potential sex differences in these trait correlates. In addition to whole-brain analyses, we also conducted ROI analyses to replicate the findings of Li et al. 2014.

Methods: We used a cohort of 7057 subjects (3361 girls) from the ABCD-NP Challenge 2019, (<https://sibis.sri.com/abcd-np-challenge/>). We only considered subjects for which raw images were available. This cohort is part of the ABCD Curated Annual Release 2.0 (March 26, 2019). Structural magnetic resonance imaging (MRI) was acquired using optimized protocol for 3T machines (including Siemens Prisma, GE 750 and Philips) with voxel size 1 mm isotropic. We used the ABCD Youth Behavioral Inhibition/Behavioral Approach System Scales (BIS/BAS), as adapted from Pagliaccio et al., 2016. This modified version shortens the Reward Responsiveness subscale but includes the BAS Fun known to be a reliable predictor of substance involvement in older samples.

Voxel-based morphometry or VBM is used to identify differences in the local composition of brain tissue and its association with behavioral and cognitive measures, while discounting large scale differences in gross anatomy and position. We performed VBM using the Computational Anatomy Toolbox (CAT 12 r933) toolbox (<http://dbm.neuro.uni-jena.de/cat/>) packaged in Statistical Parametric Mapping 12 (Wellcome Department of Imaging Neuroscience, UCL, U.K.). CAT12 provides several components optimized for morphometry, including an internal interpolation, affine preprocessing (affine registration of bias-corrected images), partial volume segmentation, denoising, DARTEL normalization, local adaptive segmentation, skull-stripping, an adaptive maximum a posteriori (AMAP) segmentation, and a final clean-up. We used the ABCD raw images as opposed to the preprocessed data (supposedly optimized to be used with Freesurfer) in order to avoid any interference with the CAT12 preprocessing pipeline.

In group analyses, we conducted multiple regression on the GM maps for the whole-brain with both BAS and BIS scores as regressors and age (in months) as a covariate for girls, boys and girls + boys combined, in separate models. In regions of interest analyses, we focused on the left parahippocampal gyrus, ventromedial prefrontal cortex, and right parietal cortex, regions that were identified from Li et al. 2014.

Results: Boys and girls were significantly different in BIS and BAS scores. Girls showed higher BIS score than boys (7.459 ± 3.654 vs. 7.040 ± 3.515 ; $t = 4.908$, $p = 9.4e-07$, two-tailed two sample t-test). In contrast, girls demonstrated lower BAS score than boys (20.206 ± 6.826 vs. 21.293 ± 6.888 ; $t = -6.645$, $p = 3.3e-11$). Additionally, BIS and BAS scores were significantly correlated across groups (all: $r = 0.360$, $p = 2.0e-214$; girls: $r = 0.370$, $p = 1.4e-109$; boys: $r = 0.362$ and $p = 4.9e-115$, Pearson regression).

In multiple regressions based on a threshold of voxel $p < 0.005$ uncorrected and AlphaSim cluster corrected, regions that correlated positively with BAS included the bilateral insula and the left somatosensory motor cortex for boys + girls, and bilateral insula/caudal putamen, right superior frontal gyrus (SFG), ventral

striatum, and the ventromedial prefrontal cortex (vmPFC) for girls. Regions that correlated positively with BIS included the cuneus and left superior and inferior frontal gyri for boys + girls, and the left SFG and vmPFC for girls.

In regions of interest analyses, we replicated the finding that the vmPFC GMV was significantly correlated with BAS for girls and the difference in regression slope was significant between girls and boys ($t = 2.27$, $p = 0.0232$).

Conclusions: The GMV of the vmPFC, in the area of the medial orbitofrontal cortex, correlated positively with both BAS and BIS trait, suggesting a neural basis of the intensity of personality traits in girls. Further, replicating Li et al. 2014, the findings suggested that this vmPFC correlate remains stable through young adulthood. Further, the GMV of the right and left SFG each correlated with the BAS and BIS trait in girls, suggesting hemispheric functional differentiation of this prefrontal cortical structure. In sum, with a moderate effect size BAS and BIS traits are associated with distinct cerebral GMV in children.

Keywords: ABCD Study, Voxel-Based Morphometry (VBM), BAS, BIS, Children

Disclosure: Nothing to disclose.

M37

Cortical Inputs of the 'Social Striatum' in Monkey

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Background: The striatum is associated with goal-directed behaviors and has been mapped extensively in primate models. It receives unidirectional inputs from the cortex which dictate its functional organization in 'sensorimotor', 'cognitive', and motivational ('limbic') sectors along a dorsolateral to ventromedial gradient. Newer work shows that there is a broad overlap of 'limbic' and cognitive inputs to the primate striatum, consistent with massively expanded cortical regions devoted to cognitive function in this species.

One complex limbic-cognitive task for both human and nonhuman primates is living in social groups. In humans, specific striatal sectors in humans are activated by social interactions ranging from gazing at 'beloved' child or romantic partner, performing altruistic acts, tasks requiring learning to trust another, and the experience of rejection. We assessed the literature to determine striatal sites implicated in these functions in humans, and used Macaques to place retrograde tracer injections in each region. Our goal was to assess the range and combinatorial profile of cortical inputs across striatal zones associated with social activity.

Methods: Regions of interest were chosen from a meta-analysis of fMRI studies that assessed a variety of social behavior in the human (Baez-Mendoza and Schultz, 2013). We then localized sites of interest using structural MRI in 6 Macaques who were 3-4 years of age, and male ($n = 5$) and female ($n = 1$). We used individual coordinates in each animal to place 8 small injections of different retrograde tracers into the following striatal regions under stereotaxic guidance: the 'core' of the nucleus accumbens, ventromedial and central body of the caudate nucleus, central and ventromedial caudal putamen, and interstitial nucleus of the anterior commissure, (IPAC). Following a 2-week survival period, animals were sacrificed, and the brains were prepared for visualization of tracer using immunocytochemical and histologic techniques. In the current study we mapped retrogradely labeled cells in the prefrontal cortex, insula, and amygdala.

Results: All injection sites resulted in labeled cells in labeled cells in Brodmann's areas 12, 45/44, and 8, which mediate multimodal sensory processing, particularly of auditory and visual information associated with faces. A subset of these injection sites additionally resulted in high concentrations of labeled cells in the agranular, dysgranular, and in some cases posterior (granular) insula. Finally, a subset of injection sites that resulted in labeled cells in visual/multimodal prefrontal cortex and insula, also had labeled cells in area 32 on the medial wall (a region associated with 'social monitoring'). The latter sites also had many retrogradely labeled cells in the amygdala.

Conclusions: Striatal injections in monkeys matched to sites associated with social responding in humans produced labeled cells in cortical areas linked to the 'social brain.' A common input to all sites derives from the ventrolateral PFC in the region of 12L and 45, and associated frontal eye fields. These regions form a critical part of the 'what' visual/auditory pathway, and have recently been implicated as the rostral-most 'face patch' in humans and monkeys. In addition to these inputs, striatal regions in the caudal ventral striatum receive strong inputs from the insula, area 32, and the amygdala, regions implicated in many affiliative behavioral paradigms. Together, these data suggest networks to the caudal ventral striatum that code for behavioral responses involving social stimuli.

Keywords: Amygdala, Conspecific, Insula, Connectivity, Nonhuman Primates

Disclosure: Nothing to disclose.

M38

Prenatal Disruption of D1R-SynGAP Complex Impairs GABAergic Interneuron Migration and Causes Behavioral Deficits in Adulthood

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Background: The dopamine D1 receptor (D1R) plays a role in GABAergic interneuron migration. However, the molecular mechanism underlying this process and the pathophysiological consequences that occur when it is disrupted during prenatal development remain unclear. Synaptic Ras-GTPase activation protein (SynGAP) has been found to regulate GABAergic innervation. In this study, we investigated a potential protein-protein interaction between D1R and SynGAP, and its role in GABAergic interneuron migration and physiological consequences in the behaviors of adulthood.

Methods: Co-immunoprecipitation and GST pull-down were carried out to investigate the D1R-SynGAP interaction and its regulation of D1R signaling. An interfering peptide (TAT-D1Rpep) was developed and injected into pregnant mice during the occurrence of GABAergic interneuron migration. Immunofluorescent staining was used to analyze the distribution of GABAergic interneurons at various developmental stages. Locomotor, pre-pulse inhibition, visual discrimination and social behaviors were assessed to determine whether the prenatal impairment of GABAergic interneuron migration caused behavioral deficits in adulthood.

Results: We found a novel protein-protein interaction between the D1R and SynGAP, which facilitates D1R membrane expression, and D1R-mediated downstream signaling. These effects were blocked by TAT-D1Rpep, which specifically disrupts the D1R-SynGAP interaction. Interestingly, disrupting this complex during embryonic development resulted in pronounced GABAergic

interneuron tangential migration deficits, possibly due to altered actin and microtubule dynamics. More importantly, administration of TAT-D1Rpep to pregnant mice led to abnormalities in locomotor activity, pre-pulse inhibition, sociability and visual discrimination in the offspring.

Conclusions: Our study discovered a novel protein-protein interaction between D1R and SynGAP, and this interaction plays a critical role in the prenatal GABAergic interneuron migration and development of important behaviors in adulthood.

Keywords: D1 Dopamine Receptors, Synaptic Ras-GTPase Activation Protein, GABAergic Interneuron Migration, Discrimination Learning, Sociability

Disclosure: Nothing to disclose.

M39

Therapeutic Effects of the 8-Week ORADUR®-Methylphenidate on Drug-Naïve Children With Attention-Deficit/Hyperactivity Disorder: A Counting Stroop Functional MRI Study

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Background: Methylphenidate has been used to treat patients with attention-deficit hyperactivity disorder (ADHD) since the 1960s with significant effects on clinical symptoms and functional improvement. The data on the neural substrates for brain activity changes after treatment with methylphenidate is limited. ORADUR® is a new medication of extended-release methylphenidate. This study aims to investigate neural correlates for brain activity changes with the treatment of ORADUR®-Methylphenidate for eight weeks in drug-naïve children with ADHD in Taiwan.

Methods: We recruited 28 drug-naïve children with DSM-5 ADHD and 28 typically developing (TD) children with similar distributions of age, sex, and IQ. All the participants completed the counting Stroop task within the functional MRI (fMRI) scan, and ADHD participants had the 2nd fMRI assessment after 8-week treatment with ORADUR®-Methylphenidate. Both ADHD (before and after treatment) and TD groups were assessed with the Rapid Information Processing and Continuous Performance Test. The initial daily dose of ORADUR®-Methylphenidate was 22 mg, and the last average dose was 29.97 mg (standard deviation, 9.70).

Results: ORADUR®-Methylphenidate significantly decreased overall clinical and ADHD symptoms (Cohen's *d*, 0.98 to 2.32). Regarding the counting Stroop task, there were significant main effects of treatment in reaction time ($F(1, 27) = 9.88, p = .004$) and condition effects in accuracy ($F(2, 54) = 4.39, p = .02$) and reaction time ($F(2, 54) = 10.99, p < .001$). For the incongruent versus congruent condition, we found less activation in the right inferior frontal gyrus (rIFG) in the pre-treatment ADHD group than the TD group, greater activation in the dorsal anterior cingulate cortex (dACC) and the right dorsolateral prefrontal cortex (rDLPFC) from pre-treatment to post-treatment, and greater activation in the dACC and rDLPFC in the post-treatment ADHD group than TD. Partial correlation analyses showed the beta values of the dACC, rDLPFC and rIFG were significantly correlated with CCPT response style ($r = .45, p = .025$), CCPT perseveration ($r = .47, p = .019$), and A' (target sensitivity) of RVP ($r = .45, p = .023$) and CCPT response style ($r = .45, p = .026$), respectively, after controlling the effect of overall clinical and ADHD symptoms.

Conclusions: Treatment with ORADUR®-Methylphenidate may increase the brain activity in the dACC, rDLPFC, and rIFG corresponding to improving focused attention, impulsivity, and inhibition control in drug-naïve children with ADHD. These brain

regions might play a role as biological markers for the treatment effectiveness of methylphenidate.

Keywords: Methylphenidate, Attention-Deficit/Hyperactivity Disorder, Counting Stroop Functional MRI (fMRI), Rapid Information Processing, Continuous Performance Test

Disclosure: Nothing to disclose.

M40

Impact of Baclofen on EEG Markers in Fragile X Syndrome Across Mouse and Human Study

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Background: Fragile X Syndrome (FXS) is the most common single gene cause of autism spectrum disorder (ASD) and the most inherited form of developmental disability. Despite significant efforts in the past decade to foster translational medicine efforts in FXS, no approved treatments for FXS exist and numerous drugs have failed to meet primary outcomes in Phase II and III study. One challenge in FXS treatment development is a lack of rigorous, synchronized outcome readouts across animal and human study. We have developed across the mouse (fmr1 KO) and human FXS lab EEG evaluations that 1) present stimuli in a similar manner; 2) have EEG data processed using the same analysis methods across mouse and human study; and 3) show baseline results indicating a similar aberrant patterns of brain activity across mouse and human study. Specifically, we have noted consistent excessive resting state gamma band activity in both humans with FXS and in the fmr1 KO mouse model of FXS. We now will evaluate the sensitivity of EEG abnormalities in humans with FXS and in the mouse model of FXS to change following a single-dose treatment regimen. In FXS, disturbance of the balance of glutamatergic and GABAergic signaling has been consistently reported. Specifically, potentiation of GABA B signaling via treatment with the GABA B selective agonist arbaclofen has been extensively studied in FXS across murine and human study. Racemic baclofen has been extensively evaluated in the mouse model of FXS and racemic baclofen is readily available for immediate use in human FXS study. We now report on evaluation of single-dose racemic baclofen on EEG signatures in FXS across mouse and human study.

Methods: Fmr1 KO mice (n = 10) and 10 wild type mice (both C57BL/6 background) were implanted with a commutator and headstage based 30-channel multi-electrode array EEG probe. Following surgical implantation at 80-85 days and then two days to recover the mice underwent pre-dose 5 minutes of resting EEG followed by 300 trains of the up chirp auditory entrainment paradigm. Then a racemic baclofen 5mg/kg i.p dose was given and 1 hour post-dose the EEG paradigms were repeated. For chronic daily dosing, the mice received the same baclofen dose daily for 2 weeks and then EEG paradigms were repeated after repeated dosing. In humans, thirteen 15 to 55-year olds years with full mutation received 30mg of oral racemic baclofen or matching placebo with a two week washout period between dosing days. Prior to drug dosing the patients completed resting state and auditory chirp EEG paradigms consistent with the EEG work in the mice. Four hours post-dose the humans repeated all EEG measures. In addition, the humans completed pre- and post-dose clinical measures including the Woodcock Johnson auditory attention subscale, the Repeatable Battery of Neuropsychiatric

Status (RBANS), the KiTap computer-based continuous performance testing, and eye tracking.

Results: In mouse study, baclofen single dose 5mg/kg i.p was associated with significant reduction (rescue) in gamma band power as measured by multi-electrode array. The reductions in gamma band power were consistent across left and right frontal, medial, and temporal brain regions. Following chronic daily baclofen dosing over 2 weeks, the changes in gamma band power remained consistent with the impact of single dosing. Additionally, single and chronic baclofen treatment in the fmr1 KO mouse was associated with enhanced (increased) entrainment to a modulated up chirp auditory stimuli in the gamma region. Single 30mg baclofen dosing was well tolerated in the human study without significant adverse effects noted. In 13 humans with FXS, single dose baclofen compared to placebo administration was associated with significant reduction in excessive gamma band brain activity in the resting condition. Work continues to evaluate the impact of single dose baclofen in humans with FXS using the chirp auditory paradigm. Additionally, work continues to evaluate potential correlations between gamma reduction and clinical change in the human subjects.

Conclusions: Racemic baclofen exhibits a consistent signature across mouse and human FXS study of rescue of aberrant/elevated resting state gamma band activity. Chronic dosing studies are underway in the mouse as are lower dose baclofen studies. EEG may be useful in future baclofen study in FXS to profile which humans may best respond to this treatment while also working to show target engagement early during the treatment course. Future work will evaluate potential clinical correlation between baclofen EEG impact and clinical measures. Future work is additionally report on the impact of baclofen of other EEG measures including auditory evoked potentials and auditory entrainment paradigms.

Keywords: Fragile X Syndrome, Drug Repurposing, EEG Biomarkers

Disclosure: Stalicia, Advisory Board, Lenire Biosciences, Advisory Board, Confluence Pharmaceuticals, Advisory Board, Allergan, Consultant

M41

Neuroimaging Features and Trauma Predict Subsequent Depression in Healthy Adolescents

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Background: One in five teens suffer from major depression before they reach adulthood. Early identification of adolescents at risk for depression is critical to prevention efforts. Adults with depression show smaller volumes of the amygdala, orbitofrontal cortex, anterior cingulate cortex, and hippocampus, yet larger volumes of the cerebellum and lateral ventricle. Whether these neural features are present in adolescents with depression, or develop after its onset, is unclear. The additive role of trauma and stress is also important to consider. The National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA) provides an ideal design to longitudinally study these neural features in adolescents who develop depressive symptoms compared to non-affected peers, with a cohort of 831 youth initially assessed at ages 12-21 years, half at elevated risk for mental health and/or substance use during adolescence, all followed annually.

Methods: We prospectively examined whether risk factors could be identified to predict major depression using machine learning in healthy adolescents ($n = 643$) of the NCANDA cohort. We hypothesized that subsequently depressed compared to continuously healthy youth would exhibit smaller volumes of the orbitofrontal cortex, amygdala, anterior cingulate cortex, hippocampus, and basal ganglia, and that stress and trauma would exacerbate these effects.

Results: We identified 22 neuroimaging features and 1 metric reflecting childhood trauma that were most predictive of transitioning from a healthy to depressed state in adolescence, with a specificity of 0.73 and sensitivity of 0.28 using half the dataset for training and half for validation. Thicknesses and volumes were found to be lesser in several of these brain regions in depressed subjects compared to non-depressed subjects after a transition from a non-depressed baseline, consistent with lesser brain maturation and supporting our hypothesis.

Conclusions: Generally smaller frontal and limbic structures, and greater childhood trauma predicted increased probability of transitioning into major depression in adolescence. Results may point to neural systems that could be explored as targets of early prevention programs.

Keywords: Adolescent Depression, Magnetic Resonance Imaging, Childhood Trauma, Early Life Stress, Artificial Learning

Disclosure: Nothing to disclose.

M42

Epigenetic Modifications of the Oxytocin Receptor Gene and Autism Spectrum Disorders

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Background: The role of the neuropeptide oxytocin in social cognition has attracted tremendous interest in social neuroscience. Autism spectrum disorders (ASD) has been characterized by deficits in oxytocin function and the exogenous application of oxytocin has been shown to improve social symptoms in ASD. However, little is known about the role of epigenetic variations of the oxytocin receptor gene (OXTR) in symptom severity and brain function in ASD.

Methods: Here, we investigated the difference of OXTR methylation levels between adult men with autism spectrum disorders ($N = 40$) and healthy adults ($N = 62$). The analysis was specifically conducted in the MT2 region of the OXTR gene given its direct relevance to transcription. We also examined the associations between OXTR methylation and social responsiveness and resting-state functional connectivity (rsFC) between networks involved in social cognition and reward processing in ASD. The data used in this study in relation to rsFC was collected as part of a larger clinical trial that is registered on clinicaltrials.gov. The rsFC data used in this study is only related to placebo intake. This is a basic experimental study and therefore the predictions/hypothesis were not part of the registered clinical trial.

Results: First, we found that adults with ASD show higher OXTR methylation levels within the exon 1 of MT2 region, as compared to healthy controls (after correcting for age, gender and race) ($P < 0.0001$). Second, we found that OXTR methylation predicts ASD social responsiveness and repetitive behaviors. Higher methylation levels are associated with more ASD symptom severity. Third, we found that OXTR methylation is associated with rsFC between theory of mind networks and reward networks.

Conclusions: These findings provide first evidence for the implication of OXTR methylation in ASD symptom severity particularly regarding social motivation, restricted interests and reward processing. Future longitudinal studies can reveal whether these epigenetic modifications are genetically inherited or acquired through environmental exposures.

Keywords: Oxytocin Receptor Gene Methylation, Autism Spectrum Disorders, Social Responsiveness, Resting State Functional Connectivity, Theory of Mind and Reward Functional Networks

Disclosure: Nothing to disclose.

M43

Prelimbic Medial Prefrontal Cortex Disruption During Adolescence Increases Susceptibility to Helpless Behavior in Adult Rats

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Background: Major depressive disorder (MDD) is the most prevalent mental disorder worldwide. Several stress animal models have been used to study the neurobiology of this disorder, including learned helplessness (LH), in which susceptible animals show a failure to escape a noxious stimulus along with a downregulation of ventral tegmental area (VTA) dopamine (DA) system activity. In LH, the prelimbic portion of the prefrontal cortex (pPFC) is known to regulate stress and anxiety, and as such plays an important role in the modulation of helpless behavior, but so far there is no evidence indicating that its developmental disruption alters susceptibility to this behavior. The aim of this study was to investigate the impact of pPFC lesion, performed at adolescence or adulthood, on the susceptibility to helpless behavior and its corresponding effects on VTA DA system activity in rats.

Methods: Male adolescent (PND 31-33) and adult (PND 70-72) Sprague-Dawley rats were submitted to pPFC lesion surgery (ibotenic acid injection) and during adulthood ($> PND 65$) or 1 week later were evaluated in the elevated plus-maze. Two days after, the rats were submitted to the LH model to evaluate helpless behavior. Electrophysiology recording of DA neurons in the VTA in adult rats was performed after four days of LH. All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Results: Whereas adult pPFC lesion ($n = 9$; $n = 6$ controls) induced neither anxiety responses (% Time and Entries in open arms; $p > 0.05$, t-test) nor increased susceptibility to helpless behavior (Number of escape failures and Latency to escape; $p > 0.05$, t-test), adolescent pPFC lesion ($n = 18$) induced an anxiety response, decreasing the % of Time ($t = 4.01$, $p < 0.05$, t-test) and Entries ($t = 3.25$, $p < 0.05$, t-test) in open arms compared to controls ($n = 24$). The adolescent pPFC lesion also increased the proportion of animals showing helpless behavior in LH at adulthood (adolescent pPFC lesion: 92.3% of helplessness, 12 of 13 total rats; control group: 42.1% of helplessness, 8 of 19 total rats), increasing the Latency to escape ($t = 2.92$, $p < 0.05$, t-test) and the number of escape failures ($t = 2.75$, $p < 0.05$, t-test). Moreover, the adolescent pPFC lesion ($n = 11$), submitted to LH, decreased the number of spontaneously active DA neurons compared to the control ($n = 16$) and the groups not submitted to LH (Saline, $n = 5$; Ibotenic Acid, $n = 5$) in the VTA ($F_{3,33} = 5.18$, $p < 0.05$, ANOVA, Tukey's test). No effect of condition was found

on firing rate ($p > 0.05$, ANOVA) and the percentage of spikes in bursts ($p > 0.05$, ANOVA). Helpless animals with adolescent pPFC lesion ($n = 10$) showed a decreased DA population activity in the VTA ($F_{5,31} = 5.83$, $p < 0.05$, ANOVA, Tukey's test) compared to naive rats ($n = 5$) and nonhelpless rats of the saline group ($n = 11$). The adult pPFC lesion ($n = 9$; $n = 6$ controls) did not affect the number of spontaneous DA neurons, firing rate and percentage of spikes in burst ($p > 0.05$; t-test) in the VTA.

Conclusions: These data suggest that the disruption of pPFC activity during adolescence increases susceptibility to helpless behavior in adult rats. Therefore, a predisposition or early life adverse events that impair pPFC activity may enhance susceptibility to depression in adulthood. Financial support: MH101180 to AAG.

Keywords: Adolescence, Depression, Prefrontal Cortex, Animal Models

Disclosure: Nothing to disclose.

M44

Early Life Adversity and Maturation of Dorsal Raphe Synapses

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Background: Early life is a critical period in the development and refinement of the central nervous system. Experience during this early life period plays an important role in shaping the maturation of the brain. Exposure to early life adversity is implicated in increased susceptibility to many neuropsychiatric disorders such as depression, anxiety and addiction. These processes are themselves linked to dysfunction of neuromodulatory systems. In particular, the serotonergic neurons of the dorsal raphe nucleus (DRN) exert widespread and complex neuromodulatory effects that are necessary for fine-tuning neural circuit formation. While it is known that serotonin regulates a wide range of brain functions and behavior, it is not clear how their circuits regulating serotonergic function are modulated by development and early-life experience.

Methods: Here, we used slice electrophysiology to characterize the maturation of synaptic inputs received by serotonergic and GABAergic neurons in the DRN from juvenile to adult developmental stages in mice. To examine the consequences of early life stress, we also subjected mice to a limited bedding and nesting protocol from PND 4-11. We then performed electrophysiological recordings from DRN serotonergic and GABAergic neurons.

Results: We found that the strength of excitatory transmission increased over the course of the juvenile and adolescent period and were maintained through early adulthood. Additionally, we determined that within the juvenile and adolescent developmental stages the AMPA/NMDA receptor-mediated current ratio in serotonergic neurons is higher than that found in GABAergic neurons, suggesting differential patterns of synaptic maturation in DRN serotonergic and GABAergic neurons.

To better understand the impact of early life experience on maturation of DRN synapses, we implemented a limited bedding model to induce early life stress (ELS) from PND4 to PND11. We then used slice electrophysiology to assess the maturation of DRN synaptic inputs. Our results indicate a decrease in excitatory neurotransmission (mean spontaneous excitatory postsynaptic current frequency ELS $1.11 \text{ Hz} \pm 0.1$, $n = 3$ cells vs

control $2.43 \text{ Hz} \pm 0.1$, $n = 3$ cells) onto DR serotonergic neurons immediately following stress that persists into adulthood

Conclusions: Our results demonstrate that adverse experience during early life can cause persistent alterations in the synaptic architecture of the DRN. We anticipate that our findings will be a starting point to understand the impact of adverse experiences on synaptic maturation of the DRN and to highlight targets for novel treatments for stress-related disorders.

Keywords: Early Life Stress, Serotonin, Dorsal Raphe

Disclosure: Nothing to disclose.

M45

Compulsive Behavior to Both Natural Rewards and Psychostimulants in a Mouse Model of Neurodevelopmental Disorders

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Background: Neurodevelopmental disorders, especially autism spectrum disorders, are associated with multiple etiological mechanisms and symptom presentations. However, a core symptom across autism-spectrum disorders are repetitive locomotor behaviors. Autism is a highly genetic disorder, and a frequently associated genetic variant with autism is 16p11.2 hemideletion. In a mouse model of 16p11.2 hemi-deletion, we have previously observed male-specific deficits in simple reward-guided learning and molecular function in the striatum. We therefore questioned whether molecular dysfunction in the striatum in these animals may be a common mechanism leading to both simple repetitive behaviors or stereotypies, as well as more complex compulsive behaviors in reward-guided decision making tasks.

Methods: In male and female mice modelling 16p11.2 hemideletion, we assessed inflexible behaviors in two tasks. In our first cohort, we measured delay discounting to assess compulsive choice in reward guided decision making. In our second cohort, we measured amphetamine-induced locomotor sensitization to assess striatal dopaminergic function and resulting behavioral hyperactivity.

Results: In delay discounting, 16p11.2 hemideletion males show a strong and persistent preference for a large reinforcer, even at very long delays. In contrast, wildtype males and females of both genotypes show strong discounting as delay lengths for a large reward increase. This is despite similar levels of trials completed and overall motivation, suggesting that the flexible expression of changes in preference is impaired in 16p11 hemideletion males. In amphetamine locomotor sensitization, wildtype mice increased their distance traveled, reflective of psychostimulant-induced hyperactivity, but this was suppressed in 16p11.2 hemideletion. Instead, 16p11.2 hemideletion males performed a significant number of small motor stereotypies in response to amphetamines that were not seen in response to saline control and was not seen in wildtype males.

Conclusions: In a mouse model of an autism-associated genotype, repetitive and inflexible behavior patterns were elicited in response to behavioral challenges (reward delay) and pharmacological challenges (amphetamine) that were not seen in these mice at baseline. These suggest that translationally relevant simple stereotypies/repetitive behaviors and more complex inflexible responding in reward-guided motivated behaviors may need to be measured using a behavioral challenge or "press", rather than at baseline. Further, these two behaviors

may result from a common molecular and circuit neurodevelopmental adaptations, possibly centered around dopamine release in the striatum.

Keywords: Delay Discounting, Amphetamine, Autism Spectrum Disorder and Related Syndromes, Repetitive Behavior, Mesolimbic Reward Circuitry

Disclosure: Nothing to disclose.

M46

Linking Habenula Functional Connectivity and Psychiatric Symptomatology in Adolescents

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Background: Adolescence is a critical period of development when symptoms of many psychiatric illnesses, including depression, anxiety, and behavioral disorders, first emerge (Belfer ML, 2008). These nascent clinical symptoms frequently entail dysregulation of the reward system, manifestations of which include anhedonia (i.e. inability to experience pleasure) and impulsivity. A growing body of literature indicates that the habenula (Hb), a small nucleus bordering the dorsomedial thalamus, plays a critical regulatory role by inhibiting dopaminergic reward signaling (Boulos LJ et al, 2016). In animal models, Hb stimulation induces depressive phenotypes, whereas Hb inhibition and lesions result in impulsive behaviors (Proulx CD et al, 2014). Despite these promising preclinical findings, the human Hb remains poorly understood due to its small size, which makes it difficult to study with traditional fMRI approaches. Nevertheless, high-resolution fMRI work by our group and others has reported altered Hb responses to aversive task outcomes (Lawson RP et al, 2017) and resting-state functional connectivity patterns (Ely BA et al, 2016) in people with depressive symptoms. Building on our recent study mapping whole-brain Hb connectivity in healthy young adults using optimized Hb seeds and neuroanatomically accurate cortical surface-based analysis (Ely BA et al, 2019), we examined Hb connectivity and its association with psychiatric symptomatology in adolescents.

Methods: Subjects consisted of 87 adolescents (age $\pm$ SD = 15.2 ± 2.1 , range = 12 - 20): 19 healthy controls and 68 with significant clinical symptoms, primarily related to anxiety, depressive, and/or behavioral disorders. Diagnoses were confirmed by clinician via semi-structured interview (K-SADS-PL). All subjects completed self-reported questionnaires to assess depression (BDI), anxiety (MASC), and anhedonia (SHAPS) severity. MRI was performed on a 3T Siemens Skyra with a 16 channel head coil using sequences similar to the Human Connectome Project (HCP) Lifetime protocols, including 0.9mm isotropic anatomical T1w MPRAGE and T2w SPACE scans as well as 10 minutes of resting-state fMRI (2.3mm isotropic, TR = 1s, 600 volumes, multiband factor = 5).

Preprocessing followed standard HCP pipelines (Glasser MF et al, 2013). Whole-brain resting-state data were denoised using ICA-FIX, CompCor, spatial smoothing (FWHM = 4mm), and bandpass filtering (0.1 - 0.01Hz). For each subject, the Hb was semi-automatically segmented at anatomical resolution in native space using T1w, T2w, and T1w/T2w ratio images (Kim J-w et al, 2016); individual Hb seed ROIs were then generated at functional resolution in MNI template space using an optimized interpolation approach that significantly increases Hb BOLD sensitivity while maintaining good specificity (Ely BA et al, 2019).

Hb ROI timeseries were extracted from unsmoothed, denoised fMRI data in MNI space using the CONN toolbox. Subject-level whole-brain Hb connectivity was calculated in CIFTI grayordinate space (i.e. combined 2D cortical surfaces + 3D subcortical volumes) using Connectome Workbench. Nonparametric group-level statistics (1-/2-sample t-tests, correlations) were performed using FSL PALM. Correlations with symptom scales were controlled for the other two scales. Both unthresholded and familywise error (FWE) corrected ($p < 0.05$) connectivity maps were evaluated.

Results: Significant positive Hb connectivity in the full adolescent cohort reproduced nearly all the features we previously described in healthy young adults from the HCP (Ely BA et al, 2019), encompassing monoamine nuclei, the salience network, and early sensory/motor areas. However, the full adolescent cohort and each major clinical subset (i.e. mood, anxiety, behavior) also exhibited significant positive Hb connectivity with the posterior cingulate and ventral anterior cingulate within the task-negative default mode network, areas where we consistently observe negative Hb connectivity in healthy adolescents and young adults with negative Hb connectivity in the subset of healthy control adolescents and in our previous study of healthy young adults (Ely BA et al, 2019). The unthresholded contrast of clinical over healthy control adolescents further underscored this pattern, revealing elevated Hb connectivity throughout the entire cortical default mode network as well as associated subcortical areas. Moreover, Hb connectivity with these default mode areas showed a highly specific positive correlation with anxiety scores in the full adolescent cohort, whereas stronger Hb connectivity with the salience network and early sensory cortex was associated with higher anhedonia severity; Hb connectivity was minimally correlated with overall depression severity.

Conclusions: Our results indicate that Hb connectivity is well-developed by adolescence and is associated with specific psychiatric symptoms early in their development: atypical positive Hb connectivity with the default mode network was correlated with anxiety, whereas hyperconnectivity with "canonical" salience network and early sensory targets was linked to anhedonia. These findings also strongly support the feasibility of our approach for studying the human Hb in clinical populations, reproducing all of the major Hb connectivity features we reported using high-quality fMRI data from the HCP (Ely BA et al, 2019) in an independent sample of mixed clinical and healthy subjects scanned under typical laboratory conditions.

Keywords: Habenula, Resting State Functional Connectivity, Anxiety, Depression, Adolescent

Disclosure: Clicks Therapeutics, Consultant

M47

Familial Patterns of Sensorimotor Issues in Autism Spectrum Disorder

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Background: Sensorimotor disturbances are common features of autism spectrum disorder (ASD) and they are present in some unaffected first-degree relatives. Family studies assessing the extent to which different clinical issues are present in unaffected biological parents hold promise for clarifying trait dimensions that track with distinct pathophysiological processes. We examined sensorimotor behavior in family "trios" including individuals with ASD (i.e., probands) and their biological mothers and fathers. Two

sensorimotor behaviors implicated in ASD were examined: ballistic eye movements reflective of feedforward motor processes and visually guided precision gripping behavior dependent upon sensory feedback processes.

Methods: Forty family trios were studied. Probands and parents were examined and compared to two separate age, sex and IQ-matched control groups: 33 “proband controls” and 39 “parent controls”. Participants completed visually guided saccade and precision gripping tasks. During the saccade test, participants made rapid eye movements towards peripheral targets appearing at +12 or 24 deg. During the precision gripping test, participants pressed with their thumb and index finger on separate load cells while viewing a white FORCE bar on a screen that moved upwards with increased force toward a fixed green TARGET bar. Participants completed trials at 15, 45 and 85% of their maximum force for 8 seconds. For both tests, the accuracy and variability of motor output were examined.

Results: Probands showed reduced saccade accuracy ($p = .005$) and greater trial-wise variability of saccade accuracy relative to proband controls ($p = .011$). Analysis of ASD parent saccade accuracy indicated a sex $\times$ group interaction reflecting reduced accuracy in ASD mothers but not ASD fathers relative to parent controls ($p = .002$). No differences in trial-to-trial variability of saccade accuracy were found between ASD parents and parent controls. Trial-wise variability of saccade accuracy was correlated between probands and parents ($p = .046$). During precision gripping, probands ($p = .015$) and parents ($p = .008$) showed increased force variability compared to proband and parent controls, respectively. Elevations in force variability tended to be inter-related among probands and ASD parents ($p = .051$).

Conclusions: Sensorimotor alterations were seen in ASD probands and their parents relative to healthy controls. Associations of sensorimotor alterations among probands and parents suggests that they are familial and may represent distinct pathophysiological processes affecting a subgroup of ASD families. Our findings that probands and ASD parents show reduced feedback control of sensorimotor behavior implicate cortical-cerebellar networks involved in reactively adjusting motor behavior in response to sensory feedback. Results indicating that cortical-cerebellar dysfunctions are familial therefore build on histopathological and gene expression studies implicating the cerebellum in the pathophysiology of ASD. Overall, our findings suggest that sensorimotor alterations represent promising intermediate phenotype features for advancing gene discovery in ASD.

Keywords: Autism Spectrum Disorder, Sensorimotor, Endophenotype

Disclosure: Nothing to disclose.

M48

Local Genetic Influences on Cortical Folding Characterized With In Vivo Brain MRI: A Possible Mediator of Neuropsychiatric Genetic Risk

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Background: Major gaps remain in our understanding of the mechanisms that underlie the folding of the cortical surface of the

human brain. Recent progress in deciphering the mechanisms of cortical folding—which are likely relevant to psychopathophysiology as folding is subtly altered in many neuropsychiatric illnesses—leave unexplained whether spatially-patterned genetic influences contribute to folding. Stereotyped folding in specific cortical locations could be explained by a corresponding pattern of genetic influences mediated by molecular gradients, and alterations to such gradients could plausibly mediate genetic risk for disease, but no direct evidence supports this explanation.

Perhaps surprisingly, this gap in knowledge can potentially be addressed using in vivo brain MRI of adult participants. Recent imaging studies suggest that shared genetic influences during development are expected to create co-variability of cortical thickness in adult neuroanatomy. Several features of cortical thickness do track folding patterns, and MRI can be used to estimate genetic correlations in inter-regional cortical thickness (co-variability due to shared genetic factors in family-based studies). Progress has been hampered by the fact that shared genetic influences related to folding patterns are likely to operate at a sub-centimeter scale that is much more local than that addressed in prior neuroimaging studies, requiring the development of novel methodological approaches to examine local genetic influences on cortical thickness.

Methods: We analyzed high resolution brain MRI from two genetically informative datasets: the Genetics of Brain Structure and Function Study (GOBS; 1,443 individuals in extended pedigrees) and the Human Connectome Project (HCP; 1,113 individuals including more than 200 pairs of twins). FreeSurfer (version 5.3) estimated cortical thickness—the distance between the gray-white and pial surface—at vertices located at approximately 10,000 points across the cortical surface. We characterized the pattern of shared local genetic influences between the cortical thickness of a vertex and its anatomical neighbors, quantified by the genetic correlation of their thicknesses. Mean curvature at each vertex was used to measure of cortical folding.

To determine if the cortical pattern of shared genetic influences on thickness was related to folding, we tested the anatomical correspondence between maps of mean curvature and maps of local genetic correlations, using a recently developed randomization test (the ‘Spin Test’, PMCID: PMC6095687). We also tested the correspondence between maps on opposing cortical hemispheres, as a high degree of left-right symmetry would be expected if these maps emerge during early development. Further, the directionality of shared genetic influences was characterized relative to the local sulcal axis at each vertex, defined as the axis of minimum change in sulcal depth: axial correlations were parallel to (“along”) this axis, while tangential correlations were perpendicular to (“across”) this axis. Finally, given the large number of potential confounds and divergent methodological choices involved in any neuroimaging study, replicability across datasets and imaging processing procedures was considered a necessary indicator of biological validity.

Results: The strength of anatomically local co-variability in cortical thickness was predictive of patterns of folding, genetically mediated, symmetric between cortical hemispheres and consistent across independent datasets. We found that such influences were markedly heterogeneous in strength, and in some cortical areas were notably stronger parallel to gyri or sulci. Critically, there was high anatomical correspondence between results based on analyses of HCP or GOBS datasets (Pearson’s correlation, $r = 0.55$, $P_{spin} < 0.001$). The overall, phenotypic local correlation was largely driven by shared genetic factors and was highly symmetric between left and right cortical hemispheres ($r = 0.8$, $P_{spin} < 0.001$). Furthermore, the degree of local cortical folding related systematically with the strength of local correlations, which tended to be higher in gyral crests and lower in sulcal fundi ($r = 0.3$, $P_{spin} < 0.001$). The relationship between folding and local correlations was stronger in primary sensori-motor areas (such as the

central cortex) and lower in association areas (such as prefrontal cortex), consistent with reduced genetic constraints on the structural topology of association cortex.

Conclusions: Collectively, our results suggest that patterned genetic influences, measurable at the scale of in vivo MRI, may be a causal factor in the development of cortical folding and its disruption in developmental neuropsychiatric disorders. This work provides novel evidence for patterned genetic influences on cortical thickness, which are shared within local cortical neighborhoods less than one centimeter apart on the cortical surface and correspond anatomically with cortical folding in the adult human brain. These patterned maps may reflect local signaling gradients that provide a spatial template for cortical folding patterns during early development, by influencing differential expansion and the biomechanical properties of local tissue. Disruption of these gradients may in turn underlie folding disruption in developmental neuropsychiatric disorders.

Keywords: Neuroanatomy, MRI, Cortical Folding, Neurogenetics, Cortical Thickness

Disclosure: Nothing to disclose.

M49

Hippocampal Brain Activation During a Spatial Memory Task in Healthy Adolescents: Moderating Effects of Vulnerability and Resilience Factors

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Background: Adolescence is characterized by significant structural and functional remodeling, particularly in brain regions including prefrontal cortex and mesolimbic systems, given associated roles in inhibitory control and reward-seeking, respectively. More recently, examination of developing hippocampal circuitry has become a point of focus, in order to probe the effects of substance use initiation, as well as emotion regulation and adaptive learning. The objective of this study was to evaluate relationships between hippocampal activation, and vulnerability and resilience factors in healthy adolescents, prior to the onset of substance use or the presence of depression or anxiety.

Methods: Functional magnetic resonance imaging data were acquired at 3Tesla during performance of a virtual water maze task in 72 alcohol and drug naïve healthy adolescents recruited locally to participate in a three-year longitudinal study of brain development. Baseline task fMRI data from the full sample are presented, as well as longitudinal findings from a subgroup of adolescents who also completed a one-year follow up ($n = 32$, 15 female, 13.7 ± 0.8 ; 14.9 ± 0.9 years old). Participants completed tests assessing vulnerability and resilience factors. Vulnerability factors included childhood maltreatment, family history density of an alcohol use disorder, age of initiation of substance use, perceived rejection and stress, depressive and anxiety symptoms, impulsivity, and parental perception of internalizing and externalizing symptoms. Resilience factors were forgiveness, friendship, parental monitoring, school connectedness, and positive affect. Baseline fMRI task data confirm hippocampal activation during memory retrieval.

Results: Greater hippocampal activation was significantly, positively associated with vulnerability factors: higher depression (CES-DC, $p = .006$) and anxiety (MASC, $p = .002$; STAI-state/trait, $p = .020/.023$), perceived stress (NIH Toolbox, $p = .006$), and lower

age of first substance use ($p = .054$). In contrast, hippocampal activation was negatively associated with resilience factors: forgiveness ($p = .01$), friendship (NIH Toolbox, $p = .002$) and positive affect (NIH Toolbox, $p = .034$). Baseline hippocampal activation continued to be significantly positively associated with depressive ($p = .014$) and anxiety ($p = .032$) symptoms, and higher perceived stress (NIH Toolbox, $p = .020$) at one year follow-up.

Conclusions: Associations between hippocampal activation and vulnerability factors such as non-clinical depression and anxiety, and age of first substance use may reflect later maturation and/or differential efficiency of this brain region when performing a memory task. Notably, only higher anxiety was associated with worse task performance. Given robust associations across all vulnerability and resilience factors examined, forgiveness in particular may be an important moderator that could protect against negative outcomes, including depression, anxiety and initiation of substance use, which often manifest during the pivotal stage of adolescent brain development.

Keywords: Adolescence, Hippocampal Function, Childhood Psychiatric Symptoms, Vulnerability, Resilience

Disclosure: Nothing to disclose.

M50

Impact of Parent-Child Relationship Quality on Children's Physiological Reactivity During an Observational Fear Learning Protocol

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Background: Fears can be learned by being directly exposed to a negative situation or by simply observing one's experience. The latter phenomenon refers to observational fear learning and has been shown to occur in various species, including humans. This type of learning is particularly important within the family given that children are sensitive to their parents' emotions. Although various factors, such as parental attachment and the child's gender, have been documented to modulate children's fear reactions, factors modulating the child's sensitivity to observational fear learning within the family environment remain to be examined. This study therefore aimed to examine the impact of the mother-child and father-child relationship security on observational fear learning in children as a function of the child's gender.

Methods: 60 parent-child dyads (children aged 8 to 12 years old) were recruited. The parent was first exposed to a classical fear conditioning procedure where one stimulus was paired with an electric shock (CS + Parent) and another stimulus was not associated with the shock (CS-). An adult stranger was exposed to the same procedure, except that a different stimulus was reinforced (CS + Stranger). Both of these procedures were filmed and then presented to the child, while skin conductance responses were recorded. Afterwards, the child was directly presented with the three stimuli (CS + Parent, CS + Stranger, and CS-) while his/her skin conductance responses (SCRs) were recorded in order to quantify fear responses. To assess the parent-child relationship security, the child filled out the Security Scale questionnaire twice, once for the relationship with his/her mother and once for the relationship with his/her father. The child's SCRs for each stimulus (CS + Parent, CS + Stranger, CS-) was modeled using linear regressions. We first tested whether the parent-child relationship security influenced the child's SCRs to each stimulus, separately for the mother and the father. We then tested the interaction between the parent-child relationship security and the

child's gender. Simple slope tests were used to decompose significant interactions.

Results: The mother-child relationship quality did not predict the child's SCRs for any of the three stimuli (all p s > 0.4) nor did it interact with the child's gender (all p s > 0.09). A significant interaction between father-child relationship security and the child's gender was found ($t(55) = -2.165$, $p = 0.035$; $\Delta R^2 = 0.081$), where girls who had lower security levels towards their father exhibited higher fear levels to the CS + Parent ($B = -0.464$, $p = 0.028$). No effects were found in boys ($B = 0.191$, $p = 0.365$).

Conclusions: These data suggest that a low-quality relationship towards the father has an impact of how girls respond to danger-related cues signaled within the family environment. So far, studies have mostly focused on the mother-child relationship without paying particular attention to gender differences among children. Our results highlight the importance of considering the father-child relationship as well as the child's gender when studying observational fear learning in children. These data could help targeting children who are more at-risk of fear-related psychopathologies and could also contribute to the development of innovative intervention avenues for these children.

Keywords: Observational Learning, Fear Conditioning, Sex Differences, Parent - Child Dyads, Skin Conductance Responses

Disclosure: Nothing to disclose.

M51

Defects of Myelination are Common Pathophysiology in Autism Spectrum Disorder

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Background: Autism spectrum disorder (ASD) affects approximately 1:68 individuals and has uncountable burdens on affected individuals, their families, and health care systems. While the genetic contributions to idiopathic ASD are heterogeneous and largely unknown, the causal mutations for syndromic forms of ASD, including truncations and copy number variants, provide a genetic footing with which to gain mechanistic insights. Models of these syndromic disorders have been used to better characterize the downstream molecular and physiological processes disrupted by these mutations with the expectation these phenotypes will translate to idiopathic forms of ASD. To begin to identify pathophysiology underlying ASD, we studied a mouse model and patient-derived induced pluripotent stem cells (iPSCs) derived from patients with Pitt Hopkins Syndrome (PTHS) which is caused by autosomal dominant mutations in Transcription Factor 4 (TCF4) gene.

Methods: RNA sequencing was performed on five independent mouse models of PTHS syndrome (Tcf4+/tr, Tcf4R579W, Tcf4del5740579, Actin-Cre::Tcf4+/floxed, Nestin-Cre::Tcf4+/floxed) and differentially expressed genes were used in subsequent gene ontology enrichment and cell type-specific expression analysis (CSEA). In addition, we analyzed differentially expressed genes obtained from PTHS mouse models with two other ASD mouse models, harboring mutations in MeCP2 and Pten. We then compared our PTHS mouse models with sporadic human ASD by comparing DE genes to Simons Foundation Autism Research Initiative (SFARI) ASD risk gene

database and we also searched for overlap using weighted gene co-expression network analyses (WGCNAs) from postmortem human ASD RNA sequencing data. Biological validation of transcriptional signatures from the PTHS mouse model was performed using a variety of techniques including immunohistochemistry, transmission electron microscopy, and electrophysiology. Human iPSCs from four PTHS patients were reprogrammed from fibroblast and all contain single point mutations within the basic helix-loop-helix domain of TCF4. iPSCs were differentiated into oligodendrocytes for downstream immunocytochemistry and qPCR studies.

Results: Gene and cell-type enrichment analyses of these DEGs highlighted oligodendrocyte dysregulation and we confirmed the myelin-associated transcriptional signature in two additional mouse models of syndromic ASD (Ptenm3m4/m3m4, Mecp2tm1.1Bird). We subsequently validated oligodendrocyte deficits in our Tcf4 mouse model which showed inefficient oligodendrocyte maturation in both an isolated oligodendrocyte in vitro cell culture system ($N = 16$, $p < 0.0001$) and ex vivo at day 24 (P24; $N = 10$, $p = 0.0016$) and day 42 (P42; $N = 10$, $p = 0.0055$). Furthermore, we used transmission electron microscopy (TEM) to visualize myelination in the corpus callosum (CC) of Tcf4+/tr and Tcf4+/+ littermates, observing a significant decrease in the proportion of myelinated axons in the CC of Tcf4+/tr mice compared to Tcf4+/+ littermates ($N = 9$, $p = 0.046$). When comparing compound action potentials (CAP) using electrophysiology, we show the ratio of N1/N2 is significantly reduced in the Tcf4+/tr mice compared to Tcf4+/+ littermates ($N = 30$; $p = 0.0012$), indicative of a greater proportion of CAP traveling down unmyelinated axons. Moreover, we integrated syndromic PTHS mouse model DEGs with human ASD genes (SFARI) and human idiopathic ASD postmortem brain RNA-seq, and found significant enrichment of overlapping DEGs and common biological pathways associated with myelination. Remarkably, we show that DEGs from syndromic ASD mouse models can be used to identify human idiopathic ASD cases from controls ($p = 0.044$). Lastly, using patient-derived iPSCs we optimized oligodendrocyte differentiation protocols and assays to determine if oligodendrocyte phenotypes observed in PTHS mouse models translate to human models.

Conclusions: Here, we attempt to address several fundamental questions about the relevance of animal models for the study of human ASD and attempt to identify a common pathophysiology that bridges across the ASD spectrum. To address these questions, we performed integrative transcriptomic analyses of seven independent mouse models covering three syndromic forms of ASD generated across five laboratories, and assessed dysregulated genes and their pathways in human postmortem brain from patients with ASD and unaffected controls. These cross-species analyses converged on shared disruptions in myelination across both syndromic and idiopathic ASD, and we biologically validate OL and myelination defects using ex vivo and in vitro studies in our PTHS mouse model. Together, these results highlight both the face validity of mouse models for these disorders while also identifying novel convergent molecular phenotypes amenable to potential rescue with therapeutics.

Keywords: Autism spectrum disorder and related syndromes, oligodendrocytes, Myelination, Induced Pluripotent Stem Cells (iPSCs), RNA Sequencing

Disclosure: Nothing to disclose.

M52

Systematic Review and Meta-Analysis of Randomized Clinical Trials of Nonpharmacologic Interventions to Reduce Suicide Risk Among Adolescents

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Background: Suicide is the second leading cause of death in youth in the US. As suicidal and non-suicidal self-injurious behaviors and suicidal ideation are factors that strongly predict suicide deaths, extensive effort has been invested in developing suicide prevention interventions that aim to at reduce these factors. However, recent evaluations of the effectiveness of these interventions resulted in contradicting conclusions that may be due to methodological issues. The present study applied a rigorous meta-analytic approach to evaluate randomized controlled trials (RCTs) for effectiveness of interventions to reduce suicidal behaviors and ideation among youth.

Methods: Five search engines (PubMed, EMBASE, PsychINFO, Medline, and the Cochrane Central Register of Controlled Trials) were used to search for RCTs published in English between 2004-2019, concerning psychosocial interventions with a primary outcome of reducing suicidal ideation (SI) and/or self-harming behaviors (SB) in youth aged 10 to 18 years. Studies focusing on a specific clinical population defined by diagnosis, and those with inadequate data for analysis either in the published article or through personal communication were excluded. Statistical analyses were performed in R (v. 3.3.2). For each study, we analyzed SB and SI separately. Here we report on preliminary data for SI only. Treatment effects were estimated as difference in SI = $Y_{trial} - X_{trial}$, where Y_{trial} and X_{trial} represent post-intervention SI values for the treatment and control groups, respectively (assuming equivalent baseline values for the groups). We modeled the occurrence of SI for each subject as a gamma mixture of Poisson processes, estimating the parameters (using method of moments) and Cohen's d. The standard error (SE) was estimated using a parametric bootstrap: Cohen's d was computed for each of 1,000 data sets generated based on the fitted model; the SD of the 1,000 Cohen's d's is our estimate of the SE. Mixed-effect models were used, adjusting for author confounds, study duration, treatment intensity, and baseline exposure. Within-subject correlations were estimated at 0.3.

Results: Thirty RCTs were included in the meta-analysis. Of these studies, nineteen reported on SI as an outcome, involving 12,886 subjects. Seven studies were school-based, including 3 direct psychoeducation, 2 online, 1 screening and 1 individual psychotherapy interventions. The remaining 15 studies were based in a health-care setting, including 9 family therapy, 2 group therapy, 2 youth support team and 2 individual psychotherapy approaches. The overall effect size was Cohen's d = 0.378, p = 0.003, a less than medium effect size. Neither intervention type (family, group, individual) nor setting (educational vs treatment) distinguished more effective from less effective studies (greater/less than the standardized mean). A funnel plot revealed significant publication bias.

Conclusions: Interventions tested thus far were only modestly effective in reducing youth suicidal ideation. However, suicidal ideation is highly temporally variable, difficult to quantify, and a less powerful predictor of suicide risk than suicidal behavior. Meta-analysis of effectiveness of interventions with respect to suicidal behaviors is forthcoming.

Keywords: Suicide Prevention, Clinical Trials, Children and Adolescents, Meta-Analysis

Disclosure: Nothing to disclose.

M53

Training a Large-Scale 3D Convolutional Neural Network Predicting Human Intelligence in Adolescent Brain Cognitive Development Study

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Background: Intelligence is complex, multi-dimensional, and encompasses a number of simpler cognitive processes and governed by the distributed brain circuitry. Literature shows the brain underpinnings of specific aspect of human intelligence; however, this knowledge has hardly led to a prediction of intelligence in an individual human. A promising, but untested, way to investigate the complex relationships between brain and cognition is the artificial intelligence-based data-driven approach with scalability. To test the feasibility of the deep neural network to the large-scale brain data, here we train several deep neural networks on the entire brain structural MRI dataset from the ABCD study in a task to predict fluid intelligence in pre-puberty children.

Methods: For the outcome label, we used fluid intelligence, estimated using the NIH Toolbox

Neurocognition battery with confounding effects (e.g., site, sex, age, race/ethnicity, and maternal education) regressed out. The dataset consists of 3739 subjects in training set, 415 in validation set, and 4515 in test set. T1-weighted MRI were used as the input data. All the data come from the 2019 Medical Imaging Computation and Computer-Assisted Intervention (MICCAI) ABCD challenge (<https://sibis.sri.com/abcd-np-challenge/>).

We used two 3D-CNN architectures: Naive-CNN and ResNet50-3D. The Naive-CNN has 4 convolutional layers, followed by 2 $\times$ max-pooling layers, ReLU layers, and dropouts. The 4 convolutional layers have output channels of sizes 10, 20, 40, and 80 respectively. Then, batch normalization is applied to the mini-batch. All the features from the last convolutional layer are sent to the 3 fully connected layers of sizes 4840, 2420, and 1. The ResNet50-3D architecture is derived from the well-known 2D ResNet-50 model with an added third dimension for the convolutional kernels. In total, the Naive-CNN model has about 527 million parameters and the ResNet50-3D model has about 47 million parameters.

To resolve the GPU memory issue owing to the high resolution 3D MRI, we used two parallel training paradigms: data parallelism (DP) and model parallelism (MP). In DP mode, the model is replicated onto 8 GPUs to which different mini-batch of images are fetched, so a total number of 24 images can be consumed in a single run. In MP mode, we split the neural network model onto 4 GPUs, so that the input features to the 2nd GPU is the output features from the 1st GPU and so forth. MP mode allows an increased mini-batch size since the memory utilization of each GPU is significantly reduced. We ran our experiment using Cori HPC system at National Energy Research Scientific Computing Center (NERSC) and Google Cloud Platform (GCP). In Cori, we used 4 Nvidia Tesla V100 GPUs, each with 1530 MHz and 16 GB of memory, to experiment with the ResNet-50 3D model. In GCP, we used 8 Nvidia Tesla V100 GPUs to experiment with the Naive-CNN model.

Results: The performance metrics of the three neural network models on the validation set are: Naive-CNN Baseline, MSE (mean squared error), 72.13; Naive-CNN Tuned, MSE, 71.51; and ResNet50-3D Baseline, 73.00. The baseline model does not include any pre-processing (i.e., normalization and log transformation). The tuned model includes both of the pre-processing steps along with hyperparameter tuning.

The overall magnitude of the training time – approximately 25–28 minutes per epoch – required for the Naive-CNN model shows that the task of predicting fluid intelligence scores from structural brain MRI can be achieved in a reasonable amount of time. Both versions of Naive-CNN surpass ResNet50-3D in model performance. The tuned version of the Naive-CNN demonstrates a competitive performance relative to the top-scoring model on the ABCD Challenge leaderboard (MSE=67.39). Our training showed a monotonic decrease in the mean squared error of the Naive-CNN Tuned model throughout the iterative epochs of training. The mean squared error decreases from 398.37 in epoch 2 to 71.51 in epoch 8; it is clear that up to epoch 8, each shuffled iteration through the training set adds to the learning in the model.

The code for this 3D distributed deep learning framework, including the training, tuning, and testing scripts, can be found in the following GitHub repository: https://www.github.com/ML4HPC/Brain_fmri.git.

Conclusions: Our study presents a novel application of data-driven AI approach to neuroscience. Our deep neural network trained on 3D brain structural MRI illustrates the feasibility in predicting human fluid intelligence estimated from multiple cognitive tasks. Using distributed deep learning framework on a GPU supercomputer, our framework successfully trained a deep neural network in approximately 4 hours. This scalability and feasibility may lead to more rigorous implementation of deep neural network in human developmental, cognitive, neuroscience research, such as developing an architecture optimized for human brain imaging data or integration of multiple modalities of MRI data to leverage both brain physiological and structural signals.

Future research encompasses two major endeavors: *_x000C_*firstly, improving scalability by combining both data parallelism and model parallelism into the deep neural networks to maximize the use of all the GPUs in a node and to optimize the training time; secondly, applying model interpretability to make neuroscienti_x000C_c inferences as to what brain circuits and features are linked to human cognition and emotion in physiological or pathological/abnormal conditions.

Keywords: Multivariate Approaches, Deep Learning, MRI, ABCD Study, Artificial Intelligence, Children and Adolescents

Disclosure: Nothing to disclose.

M54

Striatal Role of the Fragile X Mental Retardation Protein in Synapse Regulation

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Background: Fragile X syndrome (FXS) in humans results from monogenic loss of expression of the fragile X mental retardation protein (FMRP) and accounts for 5% of autism spectrum disorder (ASD) diagnoses. Multiple behaviors with relevance to fragile X syndrome, several of which overlap with those observed in ASD, are impacted by striatal function, including repetitive behavior and decreased sociability. However, much of our understanding of FMRP, an RNA-binding protein, is based in studies of hippocampal and cortical neurons, where its loss is associated with impaired synapse elimination and increased numbers of immature dendritic spine types (i.e., filopodia, thin). FMRP RNA-binding domains include the RGG box, which attaches to G-quartet structures, and KH domains (e.g., KH1, KH2), which bind kissing complex structures. In hippocampal slice culture, the KH2 RNA-binding

domain, but not the RGG box, is required for FMRP-mediated synapse elimination. In contrast, we have observed a loss of stable (e.g., stubby) dendritic spines across multiple striatal subregions in adult Fmr1 KO mice, suggesting that FMRP's role in striatal cell populations may be different than in cortex and hippocampus. Here we study the role of FMRP and these RNA-binding structures in dendritic spine and synapse regulation in vitro in a striatal culture model.

Methods: Cortical-striatal co-cultured cells were prepared from male and female Fmr1 WT and KO mouse embryos. Synaptic puncta markers (synapsin and/or PSD-95) and dendritic spine number and structure were measured in striatal cells under basal conditions, as well as following transfection of wild-type or mutant forms of FMRP in KO co-cultures (compared to untransfected cells). All experimental procedures were approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Results: Similar to our in vivo observations, we find significantly lower numbers of synaptic puncta and dendritic spines basally, including for the stubby spine type, in Fmr1 KO striatal co-cultured cells. This deficit is time-dependent, appearing at later, but not earlier, timepoints in vitro. Possibly due to the acute availability of FMRP, transfection of our KO cultures elicits a different outcome, where WT-FMRP non-significantly decreases presynaptic marker number in striatal cells compared to KO (untransfected) cells. In this scenario, the KH2-mutant form of FMRP in Fmr1 KO cultured striatal cells drives significant synapse elimination and is not statistically different from the WT-FMRP condition. In contrast, the RGG-mutant form of FMRP phenocopies the KO condition and appears to prevent synapse elimination.

Conclusions: Our observations in vivo and in vitro suggest that FMRP, under basal conditions, serves to stabilize dendritic spines and synapses in striatal brain regions, a process that emerges as cellular connections mature. In contrast, previous work suggests that FMRP stabilizes early hippocampal connections but shifts to driving synapse elimination at more mature time points. While a direct comparison is needed, these results suggest a different basal role for FMRP in these two brain regions, possibly determined by activity levels. Furthermore, our findings show that the process of FMRP-driven synapse elimination in striatal cells relies on a different RNA binding domain than previously reported for hippocampus, suggesting the involvement of different RNA partners and/or cellular processes of FMRP in striatum. Ongoing work is focused on further characterizing these differences.

Keywords: Fragile X Syndrome, Excitatory Synapses, Striatum

Disclosure: Nothing to disclose.

M55

Analysis of EPHB2 Mutations in Autism Risk and Autism-Associated Behavior in Mice

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Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interaction/communication along with restricted and repetitive interests/behaviors. ASD is caused by a complex interaction between genetic and environmental factors. Current mechanistic hypotheses, based on converging findings from clinical neuroimaging and human post-mortem brain analyses, attribute the

pathophysiology of ASD to widespread disruptions in brain connectivity. De novo nonsense (Q857X) and de novo missense (G900S) mutations (Sanders et al, 2012; Kong et al, 2012) in EPHB2 were discovered in two autistic individuals. EPHB2 is a gene coding for a transmembrane receptor tyrosine kinase involved in major axon tract formation, axon guidance and axon pruning, synaptogenesis and synapse plasticity.

Methods: To identify new mutations in EPHB2, whole-exome sequencing of ~800 ASD patients from the Simons Simplex Collection (SSC) and of ~800 unrelated neurotypical individuals from the National Institute of Neurological Diseases and Stroke (NINDS) dataset was performed. Different plasmids expressing each of these mutations were generated in the mouse EphB2 gene, transfected into a human transformed cell line and the mutated proteins were analyzed by western blot. To investigate the role of EphB2 in autism, a battery of autism-associated behaviors, including social interaction, pup ultrasonic vocalizations (USVs), locomotor activity, open field, elevated plus maze, fear conditioning, acoustic startle response, startle response to foot shock, was tested on global EphB2 heterozygous loss-of-function mice. A myelin stain was finally performed on global EphB2 heterozygous brain sections to assess potential malformations of the major axon tracts. Animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: ~30 inherited, rare variant missense mutations were identified in EPHB2. Among the tested mutations, the de novo nonsense mutation, Q857X, and the rare variant missense mutation, V650A, produce truncated EPHB2 proteins and cause a loss of EPHB2 intrinsic tyrosine kinase activity. Regarding the behavior, global EphB2 heterozygous mice show social interaction, USVs and anxiety-like behaviors similar to controls. However, EphB2 heterozygous females, but not males, display increased repetitive behaviors – a core symptom of autism – as well as several common autism-associated behaviors, including motor hyperactivity, learning and memory deficits and increased sensitivity to acoustic stimuli. Finally, EphB2 heterozygous mice present normal corpus callosum, anterior commissure, and cortical thickness.

Conclusions: Our results, together with the de novo mutations found in individuals with ASD, suggest that EphB2 hypofunction can result in several common autism and autism-associated behaviors. To better understand how EPHB2 mutations might increase autism risk in genetically affected individuals, future studies are required to identify the tissue, cell type and developmental processes by which EPHB2 regulates nervous system development and neurotypical behaviors. Since the effect of EphB2 genetic disruption seems to be female-specific, and since the de novo mutation (Q857X) was found in an autistic female, it will be important in the future to explore the sex-specific roles for EphB2 in ASD risk and neurotypical development.

Keywords: Autism, EPHB2, Neurodevelopmental and Behavioral Deficits

Disclosure: Nothing to disclose.

M56

Leveraging Computational Modeling and fMRI to Study the Mechanisms of Interpretation Bias Training in Chronic Irritability

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Background: Clinically anxious and irritable youth demonstrate a hostile interpretation bias, the tendency to appraise ambiguous social cues as threatening (Stoddard et al., 2016; Stuifzand et al., 2017). Studies have started to investigate whether threat-related hostile interpretation biases can be modified and whether modification impacts anxiety- or irritability-related outcomes (Penten-Voak et al., 2013; Stoddard et al., 2016). Interpretation bias training (IBT), a computerized training protocol, is designed to assess and train participant's face-emotion interpretations towards more benign appraisals, possibly by altering bottom-up representational input. No studies have yet employed IBT for youth with chronic, severe irritability (codified as Disruptive Mood Dysregulation Disorder [DMDD] in the DSM-5), who also often present with concurrent anxiety.

Computer-based interventions rely on the patient's ability to learn to update affective associations with new information.

In two studies, we used a bifactor approach to 1) examine links between anxiety, irritability, and learning to update hostile interpretations using a novel computational model (Stoddard et al., in prep) and 2) examine neurobiological threat appraisal and learning mechanisms in relation to both anxiety and irritability in a transdiagnostic sample of youth.

Methods: Study 1: Youth with chronic irritability ($n = 44$) who participated in a double-blind randomized trial of IBT. Study 2: 42 youth with primary anxiety, irritability (DMDD), attention deficit/hyperactivity disorder (ADHD) and healthy volunteers (HVs) completed the face-emotion task in the scanner and the IBT training outside the scanner. A voxel-wise whole brain analysis tested associations between shared and unique factor loadings, learning rate extracted from the IBT task, and activation during the in-scanner face-emotion task. All analyses were whole-brain corrected to $p < .005$.

Across studies, a bifactor model of unique and shared components of anxiety and irritability was applied to a transdiagnostic sample ($N = 134$) across two studies (Kircanski et al., 2018; Cardinale et al., 2019). Factor scores representing negative affectivity (representing shared variance between irritability and anxiety), irritability, and anxiety were extracted for each participant. A computational model measured learning rate and generalization of learning during IBT.

Results: Study 1: In the context of the IBT trial (i.e., in a sample of youth with DMDD), those youth loading high on the shared factor (i.e., youth high in anxiety and irritability) learned more slowly to update their appraisals of ambiguous face-emotions ($r = -.49$, $p < .01$).

Study 2: Activation during the in-scanner face-emotion task predicted learning in the IBT task in interaction with shared factor loadings ($F(1,580) = 19.07$, $p < .001$). Fast learners high in anxiety and irritability showed modulated neural responses in the superior frontal gyrus with increased responses to ambiguous face-emotions ($\chi^2 = 4.88$, $p = .02$). Slow learners did not show a modulated their neural response to ambiguous face-emotions regardless of anxiety/irritability status.

Conclusions: Slower learning of benign face-emotion associations in among youth with DMDD may represent an important mechanism by which hostile interpretations are maintained despite contrary environmental input. Youth high in both anxiety and irritability may be an important clinical group with a specific behavioral and neural deficit.

Keywords: Affective Disorders, Developmental Psychopathology, Face Emotion

Disclosure: Nothing to disclose.

M57

Cannabis use Initiation in Adolescence is Associated With Declines in Verbal Learning and Memory: A Longitudinal Comparison of Premorbid Versus Post-Initiation Neurocognition and its Neural Correlates

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Background: Cannabis is the most commonly used illicit substance in the U.S. For the first time in many decades, use is accelerating among young adults but in the context of decreased perceptions of harm. Numerous case-control studies suggest that cannabis use is associated with relative cognitive deficits in motivated decision making, various aspects of executive function, verbal learning and memory. However, these findings are difficult to interpret. Many studies use cross-sectional designs that do not capture potential pre-existing differences between cannabis users and non-users. Moreover, users are often characterized by high levels of externalizing behaviors, comorbid substance use and psychopathology, thus limiting causal interpretations regarding potential neurotoxic effects of cannabis use. The goal of this study was to assess neurocognitive function and its neural correlates in a typically-developing sample of adolescents and young adults pre-versus post-cannabis use initiation.

Methods: This study utilized data from a five-wave NIDA-funded longitudinal study of adolescent brain development. Typically developing participants ($n = 197$ with mean IQs in the above average range) provided behavioral data and drug-use information at two-year intervals. MRI scans were collected at each assessment at the University of Minnesota's Center for Magnetic Resonance Research to examine regional brain volumes, white matter diffusivity, and intrinsic functional connectivity. At study enrollment, participants ranged in age from 9 to 23 years and were free of psychopathology and neurological impairment. Most participants were substance naïve at baseline enrollment. Participants who initiated use of cannabis over time ($n = 51$) were identified and grouped based on transitions into light (total occasions of use < 5 times) versus moderate (total occasions of use more than 20 times) usage. Performance on the Rey Auditory Verbal Learning Task (RAVLT; a measure of verbal learning/memory) and the Iowa Gambling Task (IGT; a measure of reward-related decision-making) was contrasted between groups and across time, capturing performance prior to, and within two years of, cannabis use initiation. Alternate test forms were used for the longitudinal assessments.

Results: Gender distributions, WASI-estimated IQ scores, and self-reported frequencies of alcohol use were comparable between groups but are associated with cognitive outcomes. After controlling for the number of study visits prior to use initiation, alcohol usage after cannabis use initiation, and IQ, the groups differed significantly post-usage on several aspects of RAVLT performance including overall verbal learning ($F = 4.02$, $p = 0.05$) and delayed recall ($F = 6.49$, $p = 0.01$) with worse performance in the heavier-using group. They did not differ significantly on these metrics prior to cannabis use initiation. On the IGT, there was a trend-level group difference ($F = 3.21$, $p = .08$) with worse performance in the relatively heavy use group regardless of timepoint, but this difference was no longer significant when comorbid alcohol use was controlled. Analyses are underway to associate these behavioral differences with longitudinal differences in neural structure and intrinsic functional connectivity. These findings will be presented.

Conclusions: Potential harms associated with recreational cannabis use are of interest to clinical, educational, and public

health audiences given current advances toward legalization in the United States. Impacts on cognition and motivation are of particular relevance given the strongly replicated finding of decreased verbal learning and memory in cannabis users. Using longitudinal data, we observe that verbal learning and long-term verbal recall, but not reward-related decision making, may be adversely impacted early in the course of cannabis use initiation and that the extent of impairment varies by frequencies/amounts of use. The differences observed for learning/memory versus motivated decision-making suggest that neural systems may be differentially sensitive to cannabis effects in the context of adolescent development.

Keywords: Cannabis Use, Adolescence, Neuropsychology, Longitudinal Imaging

Disclosure: Nothing to disclose.

M58

Adolescent Binge Drinking Leads to Accelerated Age-Associated Decline in Cortical Thickness: A Data Driven Approach

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Background: Cortical gray-matter volume increases during childhood and generally decreases continuously after puberty. These significant developmental changes in brain structure and function from adolescence to early adulthood are vulnerable to a variety of environmental insults. Healthy brain development throughout childhood and adolescence supports optimal neurocognitive performance, and even minor brain changes can affect cognitive, emotional, and social functioning. Youth who initiate heavy drinking exhibit an accelerated frontal cortical gray matter trajectory compared to the non-drinkers whereas moderate drinkers exhibit trajectories in between no/low and heavy drinkers. These findings were derived from global and regional gray matter volumes defined by an anatomical atlas. However, such an approach does not reveal patterns of change in cortical structure that do not obey anatomical boundaries captured by established atlases. Recent data-driven methodologic developments are capable of detecting patterns of coordinated vertex-level cortical change (structural covariance) associated with adolescent drinking using non-negative matrix factorization (NMF) analysis. Unlike traditional dimensionality reduction methods such as principal component analysis, NMF uncovers sparse patterns of coordinated change with positive weights, which are more interpretable and correspond to specific large-scale functional networks. In this study we use NMF to detect vertex-level patterns of coordinated change in cortical thickness trajectory that were associated with adolescent drinking.

Methods: Adolescent participants ($N = 830$) (age range, 12 to 21 years) in the National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA) were assessed longitudinally at four time points from five sites (University of California San Diego, SRI International, Duke University Medical Center, University of Pittsburgh, and Oregon Health & Science University). Structural MRI along with a comprehensive assessment of substance use and psychiatric symptoms were acquired at baseline and three annual follow-ups. Cortical surfaces were obtained from structural MRI images using FreeSurfer 'recon-all' longitudinal pipeline. Spherical registration was performed to normalize the cortical thickness maps of 20,000 vertices in each subject to an average template.

Thickness data was harmonized to remove the site effects with ComBat. NMF was applied to cortical thickness maps from the baseline scans of non-binge-drinking subjects to obtain basis vectors, which represent the structural covariance. The effects of binge drinking on structural covariance were tested with general linear models of coefficients of the basis vectors as a function of age, sex, binge drinking in the past one year, family history of alcohol use, socioeconomic status, trauma exposure, race, interaction of age and binge-drinking, with random effects for site and participant. A Bonferroni correction (0.05/number of basis vectors) was used to correct for multiple comparisons.

Results: Baseline scans of 691 subjects that had not binged at baseline were used to obtain basis vectors representing healthy brain development. Multiple NMF resolutions were examined (2 to 100) with split-sample reproducibility to choose an optimal number of 20 components. The cortical thickness maps of all subjects' longitudinal scans ($N = 2809$) were projected onto the basis vectors to obtain the subject wise coefficients for each basis vector. General linear models fit to the coefficients of each basis vector showed that the interaction between age and binge-drinking was a significant contributor ($p < 0.0025$; Bonferroni corrected) for 18 of the 20 basis vectors. The significant interaction term indicated that the larger difference in cortical thickness in binge drinking compared to normal adolescents was moderated by age. All the other variables in the model were not significant. The 18 basis vectors covered vertices in superior frontal, rostral middle frontal, post central, middle and superior temporal, parietal, superior marginal, paracentral, pars triangularis and medial orbitofrontal cortex.

Conclusions: NMF was able to successfully delineate coordinated patterns of change in cortical thickness associated with binge drinking in adolescents. We found that the middle and superior temporal cortex in addition to confirming previously reported lateral prefrontal, supramarginal, and orbitofrontal brain regions were adversely influenced by adolescent binge drinking. Interestingly, we found that binge drinking was associated with more rapid age-associated reduction in cortical thickness compared to non-binge drinkers.

Keywords: Cortical Thickness, Binge Drinking, Adolescent Alcohol Use

Disclosure: Nothing to disclose.

M59

Neurodevelopmental Impact of Prenatal Drug Exposure and a Child's Perception of Caregiver Acceptance on Emotional Reactivity

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Background: Emotional reactivity as well as its regulation during childhood may set the course of either predisposition or resilience to a later onset of affective psychopathologies in the face of psychosocial stressors and trauma. Dynamic changes occur during neurodevelopment that may account for divergent emotion reactivity as well as the ability to regulate emotions. The size, diversity, and prospective nature of the NIH Adolescent Brain Cognitive Development (ABCD) database provides an opportunity to examine factors which may influence emotional reactivity and then eventually to observe longitudinal divergence in neural development and psychosocial functioning. It is well-understood that childhood attachment and the prenatal

environment affect various domains of thoughts, feelings, behaviors, and functioning.

Although scarce, prior evidence suggests that divergence in neurodevelopmental trajectory of emotion reactivity may be explained by prenatal and perinatal stressors, such as exposure of drugs in utero, as well as by problematic childhood attachment. We hypothesize that children with prenatal drug exposure (PDE) and low perception of caregiver warmth, acceptance, and responsiveness (PCW) will manifest the extremes of aberrant emotion reactivity as assessed by (A) behavioral performance and (B) neuroimaging. We expect to find differences in fMRI activation of "top-down" (cortical regions including vmPFC) and "bottom-up" (limbic regions including amygdala) circuits elicited by the ABCD Emotional N-back task.

Methods: Data from ABCD Data Release 2.0 were analyzed for this study ($n = 8,892$, ages 9-10 years, 49% female). Groups were determined by (A) mothers' self-report of drug use during pregnancy, assessed via the Developmental History questionnaire [PDE, and no prenatal drug exposure (NDE)], and (B) high and low quartile scores on the Children's Report of Parent Behavior Inventory- acceptance subscale (high and low PCW). Emotion reactivity in the fMRI Emotion N-back task was specifically assessed by the positive versus neutral (POS-NEU) and negative versus neutral (NEG-NEU) contrasts within the vigilance condition (0-Back). Task behavior [mean reaction time (MRT) and accuracy rate] and respective fMRI BOLD activations in pre-selected regions-of-interests (ROIs) based on prior literature, were extracted for each emotional condition. Thus far for prenatal exposure, we have analyzed between- and within-group behavioral differences (mixed ANOVA) and between-group differences in ROI activity (independent samples t-test) during the task. Analyses for high versus low PCW are underway and will be included in the ACNP presentation. Additionally, we will qualify a more specific phenotype by statistically accounting for relevant covariates (e.g., demographics, family history, childhood experiences, and parent-behavior) and other psychological outcomes such as self-worth. We will also explore correlations between these variables to further assess the underlying relationships.

Results: Mixed ANOVAs on MRT and accuracy rate showed significant main effects of emotion on accuracy rate [$F(2,17510) = 89.0$, $p < 0.001$] and MRT [$F(2,15284) = 36.4$, $p < 0.001$]. The main effect was further explored using paired samples t-tests: the within-group effect of emotional reactivity on accuracy rate was driven by the negative versus neutral condition (NEG<NEU; [$t(8756) = -12.17$, $p < 0.001$]) whereas the positive and neutral conditions did not differ significantly (POS=NEU; ($p = 0.942$)). The same analysis showed that the within-group effect of emotional reactivity on MRT was driven by longer MRT in both the positive and negative compared to neutral condition (POS > NEU; [$t(7930) = 5.3$, $p < 0.001$], NEG > NEU; [$t(7881) = 9.30$, $p < 0.001$]) as well as longer in negative compared to positive condition (NEG > POS; [$t(7915) = -3.73$, $p < 0.001$]). A group effect of prenatal drug exposure was not significant.

Beta-weights for each ROI were analyzed by independent samples T-Tests of POS-NEU and NEG-NEU contrasts. PDE, compared to NDE, showed higher task activation in left hippocampus [PDE > NDE; $t(8712) = 2.3$, $p = 0.02$] for the POS-NEU contrast; and in left dlPFC [PDE > NDE; $t(8711) = 2.0$, $p = 0.047$], left vmPFC [PDE > NDE; $t(8711) = -2.4$, $p = 0.017$] and right vmPFC [PDE > NDE; $t(8711) = -2.2$, $p = 0.028$] in NEG-NEU contrast.

Conclusions: In regard to our hypothesis, prenatal exposure (1) did not affect performance, but (2) did effect fMRI activation patterns in the expected emotional circuits. Interestingly, the fMRI data suggested that prenatal exposure alters neural recruitment during emotional reactivity, such that PDE showed diminished activation patterns in left hippocampus and bilateral vmPFC.

Differential activation of subcortical limbic structures during emotional reactivity will be further explored. With subsequent analyses of PCW and its interaction with PDE, as well as with accounting for potential confounds, we expect to show more coherent patterns of group differences in brain and behavior. These preliminary results will provide the basis for a better understanding of the impact of prenatal as well as environmental factors on emotional reactivity in children. With more longitudinal data from the ABCD cohort becoming available, we will be able to track neurodevelopmental changes in emotion reactivity and psychological outcomes. These results begin to shed light on responsive and compensatory ways in which the brain responds to these forms of environmental insults and attachment trauma.

Keywords: BOLD Imaging, ABCD Study, Emotional Reactivity, Attachment, Prenatal Drug Exposure

Disclosure: Nothing to disclose.

M60

Ketamine in Adolescent Treatment-Resistant Depression: Secondary Outcomes of a Randomized, Midazolam-Controlled Trial

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Background: Nearly one in five adolescents will experience major depressive disorder (MDD), and suicide is the 2nd leading cause of death in this age group. 40% of adolescents with MDD fail to respond to initial treatment with selective serotonin reuptake inhibitors (SSRIs). Better treatments for adolescent depression are urgently needed. We have previously shown that ketamine has short-term efficacy compared to midazolam for depressive symptoms (measured via MADRS) in a treatment-resistant population of adolescents. In adults, ketamine also reduces implicit measures of mood disturbance (via implicit association tasks (IATs), and specifically reduces the symptom domain of anhedonia. It is currently unknown whether ketamine has similar effects in the pediatric population. Pediatric metabolism of ketamine at sub-anesthetic doses has also not been well characterized.

Methods: We conducted a randomized, midazolam-controlled crossover trial (n=17) to evaluate the effects of ketamine in treatment-refractory adolescent MDD over four weeks. Adolescents (13-17 years old) must have failed at least one adequate trial of a standard antidepressant to enroll. On day 1 and day 14 adolescent received either ketamine (0.5mg/kg over 40 minutes) or midazolam (0.045mg/kg over 40 minutes). Subjects stayed on their psychiatric medications, with stable dosing for the four weeks prior to the trial and the duration of the trial. We have previously reported the primary outcome, MADRS score at 1 day following infusion between midazolam and ketamine; secondary outcomes of the trial included measures of anxiety, anhedonia, and hopelessness. A subset of subjects (n = 12) took IATs at baseline and 24 hours post infusion assessing the following 4 constructs: 1) Depression; 2) Suicide/death; 3) Self-harm; 4) Anxiety. D scores from the 24 hours post infusion IATs for both ketamine and midazolam were compared for all 4 IAT measurements. For pharmacokinetic studies, plasma was isolated from all subjects at baseline and four timepoints post-infusion (40 min, 80 min, 110 min, 230 min).

Results: For implicit association tasks, ketamine treatment reduced D scores compared to midazolam treatment for the depression IAT ($p = 0.053$) with an effect size of 0.65. There was also a trend towards a reduced D score for the suicide/death IAT, with an effect size of 0.50. The self-harm and anxiety IAT results did not appear sensitive to ketamine treatment in this small

sample. Ketamine reduced explicit anxiety measures relative to midazolam, but there was minimal impact on the Children's Pleasure Scale, a measure of anhedonia.

Conclusions: Adolescent treatment-resistant depression is a significant public health problem that is associated with significant morbidity and mortality. The brain undergoes substantial maturation during adolescence, and novel therapeutics must be carefully tested with attention to developmental context. Here we report secondary outcomes of the first randomized controlled clinical trial of ketamine in adolescents with treatment-resistant depression, comparing current findings to the adult literature.

Keywords: Adolescent Depression, Treatment Resistant Depression, Ketamine, Anhedonia

Disclosure: Nothing to disclose.

M61

Dorsal Striatal Response to Risk-Seeking Behavior in Adolescents

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Background: Adolescence is a time of dramatic biological, behavioral and social changes. It is one of the healthiest periods of the life-span, yet morbidity and mortality rates increase 200%, often attributed to natural tendencies to explore and take risks which increase vulnerability to risky and dangerous behaviors. Previous studies have shown that the reward system is stimulated by a rapid increase in dopaminergic activity at puberty, which influences reward-seeking behavior. However, the exact neurobiological deficits remain unknown, especially in African-American youth, who are disproportionately affected by the negative consequences associated with risk-taking behavior in our society.

Methods: In an ongoing investigation, functional magnetic resonance imaging (fMRI) scans were acquired during the Wheel of fortune (WOF) task involving monetary rewards to assess dorsal striatal activation in response to high-risk choices in 66 medication-free, African-American youth (ages 11-14 years) with no personal or family history of psychopathology. Next, the association between behavioral component of the reward-drive (the Behavioral Inhibition System/Behavioral Activation System Scale) and dorsal striatal response was examined.

Results: There was increased blood-oxygen-level-dependent (BOLD) response in the right caudate nucleus during betting on high-risk trials compared to low-risk trials ($p < .05$). Furthermore, reward-drive score positively correlated with BOLD response in the right caudate during betting on high-risk trials ($r = 0.65$, $p < .05$), but not on low-risk trials.

Conclusions: The caudate nucleus plays an important role in reward perception and decision-making, and deficits in this region may contribute to the increased risk-taking behavior observed during adolescence.

Keywords: Adolescence, Risk-Taking, Reward Drive

Disclosure: Nothing to disclose.

M62

Decreased Striatal Response to Monetary Reward in Depressed Adolescents

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Background: Major depressive disorder (MDD) is one of the leading causes of disability worldwide, and it frequently begins in adolescence. Adolescence also is a period of marked changes in brain maturation, and depressive illness during this period might disrupt the developmental processes.

Methods: In an ongoing investigation, functional magnetic resonance imaging (fMRI) scans were acquired to assess blood-oxygen-level-dependent (BOLD) activation changes in the brain in response to monetary rewards in medication-free adolescents with MDD ($n = 27$) and normal controls ($n = 27$) with no personal or family history of psychopathology. Additionally, the Children's Depression Rating Scale (CDRS) was utilized to examine the relationship between depression severity and striatal response.

Results: Normal controls had greater activation in the nucleus accumbens, caudate nucleus, putamen, posterior cingulate gyrus, hippocampus and insular cortex compared to MDD ($p < .05$). Additionally, the BOLD response in the right caudate nucleus was correlated negatively with depressive symptom severity ($r = -0.45$).

Conclusions: Our results are consistent with previous studies in adult populations demonstrating a blunted striatal response to monetary reward in MDD, which also correlated with depressive symptom severity. The nucleus accumbens and caudate nucleus play a significant role in reward processing and these results suggest a relationship between striatal dysfunction and behavioral deficits in MDD.

Keywords: Adolescence, Depression, Striatum

Disclosure: Nothing to disclose.

M63

A Randomized, Double-Blind, Placebo-Controlled Proof-Of-Concept Study of Ondansetron for Alcohol Use Disorder in Outpatients With Bipolar and Related Disorders

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Background: Bipolar disorder is a severe, persistent, and common psychiatric illness that is associated with a staggering 46% lifetime prevalence of alcohol-related disorders. When present in patients with bipolar disorder, alcohol dependence is associated with numerous adverse consequences including increased hospitalization, poor outcome during hospitalization, violence towards self and others, cognitive impairment and treatment nonadherence. Thus, the development of effective treatments for patients with bipolar and alcohol dependence is a major public health concern. However, to date, few placebo-controlled trials have been conducted in patients with bipolar disorder and alcohol dependence. Previous data suggest that ondansetron decreases alcohol use particularly in people with specific single nucleotide polymorphism (SNP) alleles and preclinical data suggest that might have antidepressant properties.

Methods: A 12-week, randomized, double-blind, flexible-dose, placebo-controlled study of ondansetron was conducted in 70 outpatients with bipolar disorder I, II or NOS, cyclothymic disorder, schizoaffective disorder, bipolar type or major depressive disorder with mixed features and early onset alcohol use disorder with active alcohol use. Participants were randomized (1:1) to receive ondansetron (0.5 mg BID) or placebo. At week 4, participants with < 30% reduction in either drinks per week or the 17-item Hamilton

Rating Scale for Depression (HRSD), and who tolerated the medication well had a dose increase to 1.0 mg BID, with an additional increase to 2.0 mg BID in those with < 50% reduction in drinks per week or the HRSD at week 8. If they still had not achieved a 50% reduction in drinks per week and HRSD at week 10, they had dose increase to 4.0 mg BID. At weekly visits, the HRSD, IDS-SR, YMRS, and assessment of alcohol were evaluated. Outcome measures included alcohol use as assessed with the Timeline Followback method, and carbohydrate deficient transferrin (CDT) and γ -glutamyltransferase (GGT) levels, while craving was assessed with the Penn Alcohol Craving Scale (PACS). Mood was assessed with the HRSD, Inventory of Depressive Symptomatology-Self-report (IDS-SR), and Young Mania Rating Scale. Relationships between changes in alcohol use and changes in mood were explored. Genotypes found previously to be predictive of alcohol outcomes (i.e., rs1150226-AG and rs1176713-GG) were compared to all other genotypes. To examine the effect of treatment and genotypes on the eight outcomes of interest, a series of linear mixed-effects regression models with random intercepts were conducted. The first series of models did not include SNPs as predictors. The second series of models included SNP information as described above. Cohen's d effect sizes were calculated. Clinical trials registry NCT02082678.

Results: Of the 70 participants, 35 were randomized to receive ondansetron and 35 were randomized to placebo. Participants had a mean age of 44.9 ± 9.4 years, were primarily male (60.0%), and African American (51.4%). The mean ondansetron dose at exit was 1.62 ± 1.32 mg BID/day, and the mean week 12 dose was 1.91 ± 1.42 mg BID/day. In models without SNP information, no significant between-treatment group differences in alcohol use measures were detected. However, a reduction in depressive symptom severity (HRSD) with ondansetron was observed [$F(1, 64.84) = 4.166, p = .045, \text{Cohen's } d = -0.53$]. Although treatment group effects were non-significant for IDS-SR, the effect size was moderate and in favor of ondansetron ($d = -0.43$). When SNPs were included in the analyses, the treatment group effect for HRSD remained [$F(1, 50.85) = 5.654, p = .021, d = -0.73$] in favor of ondansetron and the effect size for multiple alcohol outcomes increased, compared to those calculated without accounting for SNPs. All were in favor of ondansetron. For alcohol use days, the effect size increased from $d = -0.24$ to $d = -0.28$; for number of drinks from $d = -0.10$ to $d = -0.24$; and for heavy drinking days from $d = -0.15$ to $d = -0.21$. Ondansetron was well tolerated.

Conclusions: This pilot study suggests that ondansetron may be associated with improvement in depressive symptoms in persons with bipolar spectrum illnesses and alcohol use disorder. An antidepressant effect of ondansetron was observed on the HRSD. To our knowledge this is the first study in humans suggesting an antidepressant effect with ondansetron. Alcohol use did not demonstrate a significant between-group difference. However, when SNPs previously associated with a reduction in alcohol use with ondansetron were included in the analysis, effects sizes for alcohol-related outcomes increased. A larger trial is needed to definitively examine the effects of ondansetron on mood and alcohol use. Funded by the Stanley Medical Research Institute.

Keywords: Bipolar Disorder, Alcohol Dependence, Ondansetron, Depression

Disclosure: Otsuka, Grant, Allergan, Advisory Board

M64

Metformin in Schizophrenia Spectrum Disorders and Early Co-Morbid Prediabetes/Diabetes: A Double-Blind Randomized Control Trial

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Background: Patients with severe mental illness (SMI) lose 15-20 years of life due to cardiovascular disease. Much of the metabolic risk, including high rates of type 2 diabetes (T2D) is accrued early on in the illness, highlighting the need for early intervention strategies to target modifiable cardiovascular risk factors. There is however an astounding paucity of studies in SMI examining interventions outside of weight loss. Furthermore, patients with SMI are typically systematically excluded from trials investigating anti-diabetes agents resulting in lack of evidence to guide treatment.

Methods: Thirty participants with schizophrenia spectrum disorders and co-morbid prediabetes or type 2 diabetes were randomly assigned, in a double-blind fashion to 1500mg/ day of metformin or placebo (2:1 ratio; n=21 metformin and n=9 placebo). Patients had to be overweight or obese, within 5 years of psychosis onset or under the age of 40, and receiving a stable dose of antipsychotics. The primary outcome measures were improvements in glycemia (HbA1c, fasting glucose), and insulin resistance index (Matsuda-derived from glucose tolerance tests and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)). Secondary outcome measures included changes in weight, fat mass (MRI quantification of hepatic and visceral fat), improvements in cognition, and hippocampal volume (MRI). Data were analyzed using mixed-models methods, and intention to treat analysis.

Results: Twenty-two patients (n = 14 metformin; n = 8 placebo) completed the 4-month trial. The metformin group had a significant decrease over time in the HOMA-IR (p = 0.043), and fasting blood glucose (p = 0.007) vs. placebo. There were no differences between treatment groups in the Matsuda index or HbA1c or any secondary outcome measures. Interestingly, weight loss in both groups correlated significantly with decreases in subcutaneous, but not visceral adipose tissue measured by MRI. Controlling for baseline BMI and fasting blood glucose did not change any study findings. Exploratory correlations between change in metabolic indices and change in clinical and cognitive parameters did not reveal any significant associations.

Conclusions: Independently of weight loss, metformin effectively improves dysglycemia and insulin sensitivity in a young population with SMI at very high risk for early CV mortality.

Keywords: Schizophrenia Spectrum Disorder, Early Diabetes, Prediabetes, Metformin, Randomized Clinical Trial, Antipsychotics

Disclosure: Alkermes, Advisory Board

M65

Social Isolation Stress Prevents Norepinephrine-Mediated Inhibition of TRPV1 Signaling in Mouse Sensory Neurons

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Background: Stress and pain are inextricably linked. Psychological stress can either suppress or enhance pain. The precise mechanisms of how stress induces analgesia or hyperalgesia is unclear. Similar to chronic pain, stress-related psychiatric disorders such as depression and anxiety have poorly understood pathophysiology. Both chronic pain and stress lead to adaptations sharing significant overlapping physiology such that tricyclic

and serotonin/norepinephrine reuptake inhibitor antidepressants are effective in treating chronic pain. Therefore, norepinephrine (NE) is likely one of the key neurotransmitters regulating pain processing during stress. Recent studies demonstrate that alpha-2 adrenergic receptors in the dorsal root ganglion (DRG) inhibit transient receptor potential cation channel subfamily V member 1 (TRPV1), a polymodal molecular integrator in the pain pathway expressed in A δ and C fiber nociceptors. However, it is unclear whether NE inhibition of TRPV1 signaling is altered during chronic stress.

Methods: We used two models of chronic stress: 1) a 10-day social isolation stress and 2) a three-week chronic mild stress. For social isolation, animals were singly-housed in standard home cages with ad libitum food and water. For chronic mild stress, a series of seven stressful events (removal of nesting for 24 h, 5 min forced swim stress at 15°C, 8 h food and water deprivation, damp bedding overnight, white noise, cage tilt, and disrupted home cage lighting) were randomly shuffled and repeated over a 3-week period. We evaluated mechanical sensitivity with the von Frey assay and thermal sensitivity with a 55°C hot plate. To visualize calcium dynamics in sensory neurons, we cultured DRG neurons from WT mice, loaded the cells with the Ca²⁺ indicator FURA-2, and bath applied a TRPV1 agonist, capsaicin (200 nM), with or without pre-application of NE (10 μ M), the alpha-2 agonist guanfacine (10 μ M), or alpha-2 antagonist atipamezole (10 μ M).

Results: Social isolation stress increases paw-withdrawal thresholds to mechanical and thermal stimuli in both male (p < .01, Student's T-test) and female mice (p < .01, Student's T-test). In contrast, the chronic mild stress paradigm did not alter either mechanical or thermal nociception. Using calcium imaging, we show that social isolation stress blunts TRPV1-mediated calcium mobilization in sensory neurons from male mice (p < .001, Student's T-test). Furthermore, both NE and the alpha-2-adrenergic agonist guanfacine inhibit TRPV1 activation in the DRG of male group-housed mice, but not in group-housed females or singly-housed mice of either sex. These results are reversed by the alpha-2-adrenergic receptor antagonist, atipamezole.

Conclusions: This reduction in TRPV1-dependent calcium signaling may help explain the mechanism for social isolation stress-induced antinociception in male mice, but suggests this phenomenon is NE-independent in female mice. In humans, interestingly, females have twice the lifetime risk for depression and anxiety disorders and have greater pain prevalence compared to their male counterparts. A better understanding of stress-induced analgesia and its sex differences could aid in uncovering new, more targeted therapeutic targets for the treatment of pain and stress-related disorders.

Keywords: Norepinephrine, Pain, Social Isolation Stress, Chronic Mild Stress

Disclosure: Nothing to disclose.

M66

Cognitive Dysfunctions in Anorexia Nervosa and Schizophrenia

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Background: Anorexia nervosa (AN) is a disease characterized by an extreme anxiety about eating, a pursuit of weight loss, and a disturbance of body image¹. Cognitive dysfunction is implicated in development and maintenance of AN in recent years. There are

two subtypes of AN with some similarities and differences in the clinical symptoms: Restricting type (AN-R) and Bingeing/purging type (AN-BP). The MATRICS Consensus Cognition Battery (MCCB), which was designed to evaluate the cognitive functions in patients with schizophrenia. The purpose of this research is a comprehensive evaluation of cognitive dysfunctions in AN and schizophrenia with the use of MCCB and reveal the neurocognitive feature between subtype of AN and schizophrenia.

Methods: We administered MCCB to evaluate the cognitive function for 22 patients with AN-R and 18 patients with AN-BP and 42 patients with schizophrenia and 69 healthy controls at the Kobe University Hospital and 28 healthy controls. Diagnosis were made according to DSM - 5 diagnostic criteria. This research has been approved by the Kobe University Medical Ethics Review Committee.

Results: Compared to the healthy controls, schizophrenia patients had significantly lower scores of all cognitive domains, the AN-R group had significantly lower visual learning and social cognition scores, and the AN-BP group had significantly lower processing speed, attention/vigilance, visual learning, reasoning/problem-solving, and social cognition scores. Compared to the ANR group, the ANBP group had significantly lower attention/vigilance scores.

Conclusions: The AN subtypes differed in cognitive function impairments. The AN-BP patients had same degree of cognitive dysfunctions in social cognition compared to schizophrenia patients. Participants with AN-BP, which is associated with higher mortality rates than AN-R, exhibited greater impairment severities, especially in the attention/vigilance domain, confirming the presence of impairments in continuous concentration. This may relate to the impulsivity, an AN-BP characteristic reported in the personality research.

Keywords: MATRICS Consensus Cognition Battery (MCCB), Restricting Type, Binge-Eating/Purging Type, Social Cognition

Disclosure: Nothing to disclose.

M67

Laparoscopic Sleeve Gastrectomy Enhanced Functional and Structural Connectivity Between the Dorsolateral Prefrontal Cortex and the Anterior Cingulate Cortex, Which Was Associated With Sustained Weight-Loss in Obese Patients

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Background: Bariatric surgery is the most effective intervention for weight reduction in morbid obesity. Laparoscopic sleeve gastrectomy (LSG), one of the surgical procedures, limits caloric intake which is the main driver for the initial weight-loss during the immediate post-surgical period, and it also induces a sustained shift away from consumption of high-fat/sweet food and weight-loss. Though neuroimaging studies have revealed alterations in homeostatic/hedonic/control neurocircuits in obese patients' post-surgery, the neural mechanisms underlying sustained weight loss remain unclear. Here, we test the hypothesis that improved connectivity of prefrontal regions, which are involved with self-regulation and salience attribution post-surgery would be associated with sustained weight loss. For this purpose, we performed fMRI and diffusion tensor imaging (DTI) and used a food-cue reactivity task with high- (HiCal) and low-calorie (LoCal) food-cues to localize the frontal-limbic regions involved in food-cue processing. We used these regions as seed

masks to calculate their structural connectivity (SC) and their association with brain activation and behavior.

Methods: Twenty-five obese patients who underwent LSG (SG) and 30 age- and sex-matched normal weight controls underwent 3T MRI. SG group was tested before (PreSG) and one- (PostSG_1)/six-month (PostSG_6) after LSG. The food-cue reactivity fMRI task was conducted 12-hours after fasting. One-way ANOVA was used to assess LSG time effects on brain responses to HiCal and LoCal food-cues. Regions of interest (ROIs) were identified for subsequent Psychophysiological interaction analyses (PPI) to assess alterations in task-related functional connectivity (FC), and to examine the association with weight loss. For DTI, fractional anisotropy (FA), and mean, axial and radial diffusivity were calculated, and fiber tracking analysis between the ROIs was performed (threshold: PFWE<0.05, minimum cluster size of k=50, cluster-forming threshold P<0.001).

Results: LSG significantly increased FC between dorsolateral prefrontal cortex (DLPFC) and ventromedial anterior cingulate cortex (vmACC) at PostSG_1 and PostSG_6. It also increased FA between DLPFC and ACC at both PostSG_1 and PostSG_6, which correlated with reductions in body mass index (BMI) at PostSG_1 and with reduced craving for HiCal food-cues at PostSG_6. Additionally, LSG decreased activation of the right DLPFC to HiCal versus LoCal food-cues.

Conclusions: We show that LSG increased structural and functional connectivity between prefrontal regions (DLPFC and ACC) at 1 and 6 months post-surgery, that was associated with reduced BMI and food-craving. Our findings provide evidence that improved prefrontal functional and structural connectivity contributes to long term weight loss post-LSG.

Keywords: Bariatric Surgery, fMRI, DLPFC, Anterior Cingulate Cortex (ACC), Structural and Functional Connectivity

Disclosure: Nothing to disclose.

M68

The Role of Obsessive-Compulsive Factors in the Treatment of Binge-Eating Disorders: A Post-Hoc Analysis of a Double-Blind, Placebo-Controlled Trial of Dasotraline

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Background: Binge-eating disorder (BED) is a neuropsychiatric disorder characterized by recurrent episodes of excessive food consumption, accompanied by feelings of loss of control and distress, all in the absence of compensatory behaviors such as purging. The presence of obsessive-compulsive symptoms related to binge-eating has been well described in persons with BED. The objective of this post hoc analysis was to evaluate the role of obsessive-compulsive factors as mediators of the efficacy of dasotraline for reducing binge-eating days in a 12-week, double-blind, placebo-controlled trial in adults with moderate to severe binge-eating disorder.

Methods: Patients meeting DSM-5 criteria for a diagnosis of BED were randomized to 6 weeks of double-blind treatment with flexibly-dosed dasotraline (4, 6, 8 mg/d, N = 155) or placebo (N = 160). The primary efficacy endpoint was the change from baseline in the number of binge-eating days per week at Week 12. Obsessional thinking and compulsive behaviors related to BED were assessed using the clinician-rated Y-BOCS-BE scale. Mediation analysis was based on ANCOVA and MMRM models.

Results: Mediation analyses showed that improvement in obsessive-compulsive symptoms (as assessed by Y-BOCS-BE

change score, indirect effect -0.90; partial correlation between change in Y-BOCS-BE score and BED days per week $r = 0.71$, $p < 0.001$) accounted for 90.7% (0.90 of 0.99 points) of the total effect of dasotraline on reduction of binge-eating days per week (difference vs. placebo, LSM -0.99, $p < 0.001$). There was a significant correlation between changes in obsessive-compulsive symptoms and body weight (partial correlation = 0.37, $p < 0.001$). The combined effects of decreased severity of obsessive-compulsive symptoms and weight decline accounted for 95.4% (0.95 of 0.99 points) of the total effect of dasotraline on BED days per week.

In addition, 64.4% (4.28 of 6.65 points) of the total effect of dasotraline on improvement of obsessive-compulsive symptoms (as assessed by Y-BOCS-BE score, difference vs. placebo, LSM -6.65, $p < 0.001$) was associated with the reduction of binge-eating days (per week, indirect effect = -4.28), while weight decline alone and the combined effects of decreased binge-eating days (per week) with weight decline accounted for 61.0% (4.05 of 6.65 points) and 89.6% (5.96 of 6.65 points), respectively.

The total effect of dasotraline on change in body weight (difference vs. placebo, LSM -5.30 kg, $p < 0.001$) was attributable to indirect effects of dasotraline on decreased severity of obsessive-compulsive symptoms (22.7%, 1.20 of 5.30 kg, $p < 0.001$), and decreased binge-eating days per week (13.3%, 0.70 of 5.30 kg, $p < 0.001$, presumably through its correlation with improved obsessive-compulsive symptoms), or both (23%, 1.22 of 5.30 kg).

Conclusions: In this post hoc analysis of a double-blind, placebo-controlled trial of dasotraline, our findings suggest that the therapeutic benefit of dasotraline 4-8 mg/d for binge-eating disorder was mediated principally through the improvement of obsessive-compulsive symptoms. These findings suggest an effect of dasotraline on the underlying disease process associated with binge eating disorder.

Keywords: Binge Eating Disorder, Obsessive Compulsive Disorder, Dasotraline

Disclosure: Sunovion, Employee

M69

Safety and Effectiveness of Dasotraline in Binge-Eating Disorder: Results of an Open-Label, 12-Month Extension Study

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Background: Dasotraline is a long-acting dopamine/norepinephrine reuptake inhibitor with a PK profile characterized by slow absorption and a $t_{1/2}$ of 47-77 hours, permitting once-daily dosing. In a previous double-blind (DB), placebo-controlled, 12-week, flexible-dose study, dasotraline (4-8 mg/d) demonstrated significant efficacy in the treatment of binge-eating disorder (BED). In a second DB, placebo-controlled, 12-week, fixed-dose study of dasotraline (4 mg/d and 6 mg/d) significant efficacy was confirmed for the 6 mg/d dose. We now present results of a 12-month extension study whose objective was to evaluate the safety and effectiveness of long-term treatment with dasotraline in BED.

Methods: Adult patients with a DSM-5 diagnosis of BED and who had completed either the 12-week flexible- or fixed-dose studies were enrolled in a 12-month, open-label (OL), flexible-dose (dasotraline 4-8 mg/d) extension study. The primary outcome measures were overall incidence of adverse events, events leading to study discontinuation, serious adverse events (SAEs); and frequency and severity of suicidal ideation or behavior. Secondary safety outcomes included change in body weight, metabolic

parameters, ECG, and measures assessing potential for drug withdrawal. Secondary effectiveness outcomes included OL-baseline to week 52 change in the Binge Eating Clinical Global Impression-Severity (BE-CGI S) score, the Eating Disorder Examination Questionnaire modified (EDE-QM) global score. Dasotraline was discontinued abruptly, and potential withdrawal symptoms during the 3-week follow-up period were assessed using the Cocaine Selective Severity Assessment (CSSA), the Discontinuation-Emergent Signs and Symptoms (DESS) scale, the Hamilton Anxiety Rating Scale (HAM-A), and the Montgomery-Asberg Depression Rating Scale (MADRS).

Results: A combined total of 591 patients completed the two 12-week, DB studies, 533 patients entered the OL extension study, and among them, 528 (89.3%) received at least one dose of dasotraline (safety population); 284/533 patients (53.3%) discontinued due to all causes, and 87/533 (16.3%) discontinued due to an adverse event. A total of 21 patients (4.0%) experienced an SAE; there were no deaths in the study. A total of 438 patients (83.0%) experienced at least one adverse event; individual events with an incidence $\geq 5\%$ were insomnia (24.1%), weight decreased (14.6%), dry mouth (13.8%), anxiety (13.3%), headache (9.8%), upper respiratory infection (9.7%), nasopharyngitis (5.7%), irritability (5.1%), and nausea (5.1%). Ten patients (1.9%) discontinued due to treatment-emergent insomnia in the extension study. Treatment-emergent suicidal ideation based on the assessment of C-SSRS was reported by 12 patients, one patient made an attempt, and one patient was rated as having made preparatory acts. Mean week 52 change from DB/OL baseline in weight was -5.8/-3.0 kg. Median week 52 change from DB/OL baseline in metabolic laboratory values (mg/dL) were as follows: total cholesterol (-8.0/-2.5), LDL cholesterol (-4.0/-3.0), and triglycerides (-11.0/-5.0); and median change in hemoglobin A1c was 0.0/0.0%. During OL treatment, 2 patients had a QTcF > 450 (male) or > 470 (female) msec; and no patients had an increase in QTcF ≥ 60 msec compared to both to DB baseline and OL baseline. At OL-baseline, patients who were previously treated with dasotraline ($n = 299$) had a mean BE-CGI-S score of 2.1 (BE-CGI-S ≤ 2 , 61.2%), and the patients who were previously treated with placebo ($n = 229$) had a mean BE-CGI-S score of 2.9 (BE-CGI-S ≤ 2 , 38.9%). After 52 weeks of treatment with dasotraline, the mean BE-CGI-S score was 1.6 (BE-CGI-S ≤ 2 , 80.5%) and 1.8 (BE-CGI-S ≤ 2 , 73.7%) at Week 52 and LOCF-endpoint; the mean change from DB/OL baseline in the EDE-QM global score was -1.34/-0.45 and -1.16/-0.37 at Week 52 and LOCF-endpoint, respectively. After abrupt discontinuation of dasotraline, withdrawal measures (DESS, CSSA, HAM-A, MADRS) did not identify any clinically meaningful concerns indicative of a withdrawal syndrome.

Conclusions: In this open-label extension study, 12 months of treatment with dasotraline (4-8 mg/d) was found to be safe and well-tolerated by the majority of patients with BED. Insomnia was the most common adverse event but was associated with study discontinuation in only 1.9% of patients. Reductions in weight and metabolic laboratories were modest and appeared to attenuate during extension treatment. Global severity of BED was rated as mild or none in 80.5% of patients after 12 months of open label treatment. No withdrawal syndrome was observed when dasotraline was abruptly discontinued at the end of the study.

Keywords: Dasotraline, Binge Eating Disorder, Binge Eating, Binge-Eating Disorder, Long-Term Safety

Disclosure: Sunovion Pharmaceuticals Inc, Employee

M70

Differential Glucose Metabolism in Weight Restored Women With Anorexia Nervosa

Abstract not included.

M71

High Plasma Oxytocin Levels in Men With Hypersexual Disorder

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Background: Hypersexual disorder (HD) integrating pathophysiological aspects such as sexual desire deregulation, sexual addiction, impulsivity and compulsivity was suggested as a diagnosis for the DSM-5. "Compulsive Sexual Behavior Disorder" is now presented as an impulse-control disorder in ICD-11. Recent studies showed dysregulated HPA axis in men with HD. Oxytocin (OXT) affects the function of the HPA axis; no studies have assessed OXT levels in patients with HD. Whether a CBT treatment for HD symptoms has an effect on OXT levels has not been investigated.

Methods: We examined plasma OXT levels in 64 male patients with HD and 38 male age-matched healthy volunteers. Further, we examined correlations between plasma OXT levels and dimensional symptoms of HD using the rating scales measuring hypersexual behaviour: Hypersexual disorder screening inventory (HDSI) and the Sexual Compulsive scale (SCS). A part of the patients (N = 30) completed the manual-based group-administered CBT program for HD and had a secondary measurement of OXT at post-treatment. OXT was measured with Radioimmunoassay (RIA).

Results: Patients with HD had significantly higher OXT (Mean $31.0 \pm SD 9.9$ pM) levels compared to healthy volunteers (Mean $16.9 \pm SD 3.9$ pM) ($p < 0.001$). There were significant positive correlations between OXT levels and the rating scales measuring hypersexual behaviour (Spearman rhos between HDSI $r = 0.649$, $p < 0.001$ and SCS $r = 0.629$, $p < 0.001$) in the study participants combined. Patients who completed CBT treatment had significant reduction of OXT levels from pre-treatment (30.5 ± 10.1 pM) to post-treatment (20.2 ± 8.0 pM) ($p < 0.001$). Patients with HD had a significant positive correlation of their changes in HD:CAS with plasma oxytocin level before and after CBT ($r = 0.388$, p value = 0.0344).

Conclusions: The results suggest hyperactive oxytonergic system in male patients with hypersexual disorder which may be a compensatory mechanism to attenuate hyperactive stress system. A successful CBT group therapy may have effect on hyperactive oxytonergic system.

Keywords: Oxytocin and Addiction, Hypersexual Disorder, Cognitive Behavior Therapy

Disclosure: Nothing to disclose.

M72

Double-Blind, Placebo-Controlled, Single Ascending Dose, Study to Determine the Efficacy, Safety, and Pharmacokinetics of a BXCL 501 (Sublingual Dexmedetomidine) in Agitation Associated With Schizophrenia or Related Disorders

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Background: Dexmedetomidine (DM) is a selective alpha-2

adrenergic agonist currently marketed as an intravenous (IV) formulation for anesthesia. IV studies have shown that doses lower than those needed for anesthesia can reduce agitation in a range of conditions. Based on the pharmacokinetic-pharmacodynamic data from these IV studies, a sublingual formulation of DM (BXCL 501) was developed and tested for its potential efficacy in agitation associated with schizophrenia or related disorders.

Methods: This phase 1B study employed a randomized, double-blind, placebo-controlled, multiple single ascending dose design in separate cohorts of males or female adults (18–65 years of age) with agitation associated with schizophrenia, schizoaffective or schizophreniform disorder as defined by DSM-5. These subjects could be on a wide range of other psychiatric medication if the dose had been stable for at least 2 weeks. Each cohort consisted of 27 individuals assigned 2:1 to either BXCL 501 or an identical appearing placebo. Each participant met commonly used inclusion and exclusion criteria including being agitated at baseline ion defined as a total score ≥ 14 on the 5 items of the PANSS Excited Component (PEC) (i.e., poor impulse control, tension, hostility, uncooperativeness, and excitement) and a score of ≥ 4 on at least 1 of the 5 items. Primary efficacy endpoint was the proportion of subjects in each cohort (BXCL 501 and placebo) who experienced $> 40\%$ reduction in PEC score at 2 hours which is a value that has been used for FDA registration for an anti-agitation indication for other drugs. Safety measures included AEs, clinical laboratory tests, ECG, vital signs (blood pressure and heart & respiratory rate) and level of arousal. Plasma sample were obtained at pre-specified time-points prior to and after dosing for determination of drug concentration. The initial dose was 20 mcg followed by single doses of 40, 60, 80, 120, and 180 mcg in separate cohorts.

Results: BXCL 501 produced a dose dependent reduction in agitation without causing clinically meaningful changes in blood pressure or heart rate based on pre-specified criteria or sedation and was well tolerated. At the highest dose, 91% vs 28% of participants receiving BXCL or placebo, respectively ($p < 0.001$). The pre-defined reduction in agitation was observed at one hour after dose and lasted for approximately 6 hours. Plasma samples are being analyzed with the intent to present the relationship between drug concentration, dose and effect in the poster. There were no apparent drug-drug interactions affecting safety/tolerability.

Conclusions: BXCL 501 produced a rapid (within 1 hour) reduction in agitation which was sustained for up to 6 hours at doses which were well tolerated and safe. The duration of the effect is longer than what would be suspected based on the plasma half-life of the drug. BXCL 501 represents a novel mechanism for the treatment of agitation.

Keywords: Dexmedetomidine, Agitation, Locus Coeruleus, Schizophrenia Novel Treatment

Disclosure: BioXcel Therapeutics Inc, Consultant

M73

Midbrain Dopamine Neuron Activity Predicts Impulsive Actions in Mice

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Background: Impulsivity, or acting without forethought or self-control despite negative consequences, increases the risk of suicidality, substance abuse, and violence. Dopamine (DA) is central to impulsivity, as posited by human neuroimaging data that highlight differences in DA-rich regions between healthy

individuals and those with impulsivity-related disorders. DA is also central to reward processing, learning and motivation. While we understand that motivated behaviors are driven by phasic DA responses to reward information, we have yet to uncover how DA activity could drive the ability to suppress motivated behaviors when they are maladaptive.

Methods: We developed a novel cued-reward lick-withholding task in which head-fixed mice must suppress anticipatory licking during a reward predicting cue (2s) in order to earn water reward. Three different auditory cues that predicted three different reward sizes (big, small, none) were randomly interleaved. The cue was restarted as many times as the animal licked prematurely (before the 2 second cue period was over). The total duration of the tone was used as a trial-by-trial measure of the degree of impulsivity. The task accomplishes the following: 1. It places Pavlovian cue responses in conflict with self-restraint, enabling us to study the neural substrates of action impulsivity. 2. It manipulates the degree of impulsivity by manipulating expected reward value, and 3. It provides a quantitative, trial-by-trial measure of action impulsivity that can be precisely related to neural activity. We measured DA neuron activity using fiber photometry, in which fluorescence emitted from GCaMP6f, a Calcium sensor, is used as a proxy for neural population activity. We specifically targeted DA cells by injecting GCaMP6f in the Ventral Tegmental Area (VTA) of DAT-Cre transgenic mice. An optical fiber was then implanted in the VTA to collect the bulk fluorescence signal.

Results: Our behavioral results show that mice can discriminate between the three trial types and exhibit a learned waiting behavior. In addition, mice behaved more impulsively in anticipation of the larger reward, despite it resulting in a reduced rate of reward receipt. As expected, neural recordings demonstrate that phasic DA encodes expected and received reward value at reward cue and reward onset, respectively. However, when controlling for expected reward size, DA dynamically encodes the failure to suppress conditioned responses throughout the trial structure. First, cue-induced phasic DA activity is predictive of impulsivity level and may explain trial-to-trial variability in impulsivity. Second, DA ramping during the wait period predicts the onset of impulsive actions in a manner that reflects changes in reward expectation over time. Lastly, impulsive actions lead to greater DA reward responses, suggesting a mechanism for the persistence of this maladaptive behavior.

Conclusions: These data suggest that phasic cue-induced DA activity may drive action impulsivity in addition to their role in learning and motivation.

Keywords: Dopamine, Impulsivity, Ventral Tegmental Area (VTA)

Disclosure: Nothing to disclose.

M74

Maternal Depressive Symptoms During Pregnancy and Brain Age in Young Adult Offspring: Findings From a Prenatal Birth Cohort

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Background: Maternal depression during pregnancy is associated with elevated risk of anxiety and depression in offspring, but the mechanisms are incompletely understood. Here we conducted a neuroimaging follow-up of a prenatal birth cohort to test whether deviations from age-normative structural brain development in young adulthood may mediate this link. Based on previous

research of typical brain development which reported pre-adolescent increase in volume/cortical thickness followed by a post-adolescent cortical volume loss and cortical thinning and further research which associated neuropsychiatric disorders with older structural brain age, we hypothesized that more maternal depressive symptoms will be associated with older structural brain age and worse mood and anxiety symptoms in the offspring. Given mixed findings suggesting delayed structural brain maturation may also be detrimental, we further explored the possibility that maternal depressive symptoms and offspring mood disturbance would be associated with greater absolute brain age gap (i.e., either substantially older or younger structural brain age).

Methods: Total of 131 young adults (53% women, age 23-24, all White Caucasians) from the European Longitudinal Study of Pregnancy and Childhood, a prenatal birth cohort born in the South Moravian Region of the Czech Republic between 1991 and 1992, participated in the neuroimaging follow-up Biomarkers and Underlying Mechanisms of Vulnerability to Depression (VULDE). Participants also completed self-report measures of trait anxiety (Spielberger's State-Trait Anxiety Inventory; STAI-T) and mood dysregulation (Profile of Mood States; POMS). Further, mothers of a subset of participants (n = 103, 54% women) answered a self-report questionnaire in 1990-1992 about depressive symptoms during pregnancy.

Structural magnetic resonance imaging (MRI) was done at 3T Siemens Prisma MRI scanner and T1-weighted data were processed using Freesurfer. Structural brain age was calculated based on the Neuroanatomical Age Prediction using R (NAPR), using cortical thickness maps from 2367 healthy controls aged 6 to 89 years as a reference. Finally, we calculated the Brain Age Gap Estimate (BrainAGE) as the difference between this cortical thickness-based estimate of brain age and each participant's chronological age. The absolute brain age gap, describing either substantially older or younger structural brain age, was then calculated as the absolute value of BrainAGE.

All statistical analyses were performed in JMP 10.0.0. First, linear regression assessed whether maternal depression during pregnancy might predict the brain age gap in young adulthood and whether the brain age gap might be associated with anxiety and dysregulated mood in young adulthood. Next, a mediation analysis using bootstrapping evaluated whether the brain age gap might mediate the relationship between maternal depression during pregnancy and anxiety or dysregulated mood in young adulthood.

Results: Maternal depressive symptomatology during pregnancy was associated with higher BrainAGE in the offspring (beta=0.248, p=0.012, R²=0.061), consistent with possible accelerated or premature brain aging. For every standard deviation increase in maternal depressive symptoms during pregnancy, offspring brains in young adulthood were approximately 2.5 years older. Additional analyses demonstrated that maternal depressive symptomatology during pregnancy showed a trending association with higher absolute brain age gap (beta=0.191, p=0.054, R²=0.037), suggesting higher maternal depressive symptoms may result in either accelerated aging or delayed brain maturation. There were no interactions between maternal depressive symptomatology during pregnancy and sex on either the BrainAGE (beta = -0.001, p = 0.992), or the absolute brain age gap (beta=0.015, p = 0.884).

Further analyses revealed that higher absolute value of BrainAGE was associated with more symptoms of anxiety (R²=0.03, p=0.04) and dysregulated mood (R²=0.03, p=0.05) and mediated the relationship between maternal depressive symptoms during pregnancy and anxiety in the young adult offspring (ab = 0.03, 95% CI [0.0003; 0.0697]). There was no similar indirect relationship with dysregulated mood in young adulthood at the 95% confidence level (ab = 0.007, SE=0.006, 95% CI [-0.0004; 0.0211]).

Conclusions: We demonstrated that maternal depressive symptoms during pregnancy were associated with atypical aging of the offspring's brain, which was, in turn, associated with more anxiety and dysregulated mood in young adulthood. We also showed that atypical aging of the offspring's brain mediated the relationship between maternal depressive symptoms during pregnancy and anxiety in the offspring in young adulthood. Our findings suggest that exposure to maternal depressive symptoms in utero may be associated with both accelerated and delayed brain maturation, and that deviations from age-normative structural brain development in either direction may mediate a link between maternal depression during pregnancy and offspring anxiety. Since none of our participants reported any DSM disorder at the time of assessment, we interpret these effects as reflecting probable risk for subsequent psychopathology that may be targetable by early intervention.

Keywords: Maternal Depression, Brain Aging, Anxiety, Magnetic Resonance Imaging, Young Adults

Disclosure: Nothing to disclose.

M75

Epigenetic Mechanisms of Stress Susceptibility and Resilience

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Background: Stressful events are potent adverse environmental factors that can predispose individuals to psychiatric disorders such as depression. Exposure to chronic stress can be differently perceived by individuals and can have persistent sequelae depending on the level of stress, resilience, or vulnerability of the individual. While stress vulnerability may influence depression, the molecular and neural mechanisms underlying the susceptibility and resilience to chronic stress within the brain are poorly understood. Recent studies suggest that stress-induced aberrant synaptic and structural plasticity may be key underlying mechanisms of stress susceptibility. Neuroplasticity is regulated by a complex gene expression program, and multiple lines of evidence implicate aberrant transcriptional regulation of neuroplasticity-associated genes in the pathophysiology of depression. Increasing evidence provides key insights into the biological significance of epigenetic regulation of gene expression in behavioral response to chronic stress. The medial prefrontal cortex (mPFC) is vulnerable to damage from a variety of psychosocial stressors. Aberrant structural and functional changes in brain structure have been implicated in the pathophysiology of depression. However, little is known about the role of epigenetic mechanisms within the mPFC in chronic stress-induced depression-like behavior. In this study, we demonstrated the role of lysine-specific histone demethylase (KDM5C) in the epigenetic regulation of gene transcription associated with behavioral responses to chronic stress.

Methods: Adult male C57BL/6J (B6), BALB/c (BALB), and DBA/2 (DBA) mice were maintained on a 12-h/12-h light/dark cycle with mouse chow and water ad libitum. Eight- to nine-week old mice were used (i.e., stress exposure, stereotaxic surgery). All experimental procedures were performed according to the Guidelines for Animal Care and Use at Kyoto University Graduate School of Medicine. For subchronic social defeat stress (sCSDS), mice were subjected to 5 daily, 5-min defeats by a novel CD1 aggressor mouse and were subsequently housed across a plexiglass divider to allow for sensory contact. For subchronic variable stress (SCVS), mice were subjected to five different stressors over 5 days to

prevent stress habituation. SCVS included 20 random mild foot shocks at 0.45 mA for 10 min (4 mice to a chamber), a tail suspension stress for 1h, restraint stress placed inside a 50 ml conical tube for 6 h within the home cage, fox odor for 2 h, and soiled cage (wet bedding) for 6 h. Behavioral tests (social interaction test and sucrose preference test) were performed during the light phase (9 am to 4 pm), as reported previously (Uchida et al., *Neuron* 2011; Uchida et al., *PNAS* 2011). All behavioral tests were conducted by experimenters who were blind to the treatment conditions of the animal. We used 10-16 mice per group in each behavioral experiment and 5-8 mice per group for biochemical experiments. For virus injections, the AAV vector (0.2 μ l) was injected bilaterally into the mPFC at a rate of 0.05 μ L/min. Mice were allowed to recover for 3 weeks after surgery. For statistical analysis, data were analyzed using an appropriate analysis of variance. Significant effects were followed up with Bonferroni's post-hoc tests. Unpaired t-tests were used for two-group comparisons. In all cases, p values were two-tailed, and the comparisons were considered statistically significant when $p < 0.05$.

Results: We found strain differences in behavioral responses to stress: BALB and DBA mice were behaviorally more vulnerable to sCSDS and SCVS than B6 mice. In addition, we found increased KDM5C mRNA levels in the mPFC of BALB and DBA mice, when compared to B6 mice in naïve (non-stressed) states ($p < 0.001$, BALB vs B6; $p < 0.01$, DBA vs B6), suggesting that KDM5C may be associated with development of stress vulnerability. To test this, we generated transgenic mice overexpressing KDM5C in the forebrain of stress-resilient B6 strain, and found that these mutant mice showed increased depression-like behaviors following sCSDS exposure (social interaction test, $p < 0.05$, non-stressed wild-type mice vs stressed KDM5C mutants). Conversely, focal knockdown of KDM5C expression in the mPFC of stress-vulnerable DBA strain induced stress resilience (sucrose preference test, $p < 0.05$, non-stressed KDM5C knockout mice vs stressed KDM5C knockout mice). Moreover, pharmacological inhibition of KDM5C in the mPFC of DBA mice led to a stress resilient phenotype following stress exposure (sucrose preference test, $p < 0.05$, stressed mice receiving KDM5C inhibitor vs stressed mice receiving vehicle).

Conclusions: Our results suggest that KDM5C-mediated epigenetic regulation of gene transcription is critical for the development of stress vulnerability. More importantly, the KDM5C inhibitor could be a potential therapeutic agent for the treatment of stress-related psychiatric disorders.

Keywords: Stress Models, Epigenetics, Transcription, Medial Prefrontal Cortex, Depression

Disclosure: Nothing to disclose.

M76

Prescription Pattern of Bipolar Disorder in Asian Countries: The REAP Study

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Background: Bipolar disorder is a severe, recurring mental illness and pharmacotherapy plays the major role among treatment. Mood stabilizers and antipsychotics are frequently used in bipolar disorder, in monotherapy or combined medication. Other psychotropics such as anxiolytics and antidepressants are often prescribed as adjunct medication. There is no consensus in the definition of polypharmacy in mood disorder. Research on Asian Prescription Pattern (REAP) is an international collaborative

research in Asia. The objective of REAP bipolar disorder is to compare the prescription pattern of psychotropics medications across Asian countries. Also, the rate of polypharmacy and psychotropic drug load will be analyzed.

Methods: Demographics, prescribed medications, side effects and laboratory data were collected using web-based key-in system. Prescription Patterns were categorized as 1. mood stabilizer monotherapy (MM): only one mood stabilizer used; 2. antipsychotic monotherapy (AM): only one antipsychotic used; 3. simple polypharmacy (SP): one mood stabilizer and one antipsychotic used; and 4. complex polypharmacy (CP): two or more mood stabilizers and/or antipsychotics used. The defined daily dose method is applied to calculate the psychotropic drug load in each patient.

Results: Our study sample consisted of 1747 patients with bipolar disorder (52.3% female, mean age 41.5) from 11 countries. There were 1446 (82.8%) patients received mood stabilizers, 1457 (83.4%) received antipsychotics, and 356 (20.4%) received antidepressants. The distributions of prescription patterns were MM: 14.6%, AM: 12.6%, CP: 51.1%, CP: 20.1%, and none of the above: 1.6%. The average psychotropic drug load of all patients was 2.06 ± 1.34 . There was a wide variety of these results between countries.

Conclusions: Our findings suggest that over 70% of psychotropic regimens among patients with bipolar disorder involved polypharmacy (more than one kind of either mood stabilizer or antipsychotic), which is similar to the high prevalence rate of polypharmacy in bipolar disorder under a permissive criterion (two or more psychotropic drugs) worldwide. Noteworthy, more than 80% of our sample received antipsychotics, which may signal an upward trend of using antipsychotics in the treatment in bipolar disorder.

Keywords: Polypharmacy, Mood Stabilizers, Antipsychotic, Psychotropic Drug Load, REAP

Disclosure: Nothing to disclose.

M77

Cross-Model Transcriptional Profiling of the Stressed Ventral Hippocampus

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Background: The ventral hippocampus is a nexus of information in the limbic system that encodes memory related to stress and emotions. Animal models for stress-related psychiatric disorders have shown that ventral hippocampus regulates anxiety- and depressive-like behavior even when diverse types of stressors are used as a challenge. However, little is known on the genomic signature that characterizes distinct animal models of stress, e.g. types of environmental stressors or genetic predisposition, and their transcriptional commonalities following the response to stress. Access to high throughput technology, such as whole-genome sequencing analysis of discrete cell populations, is a proxy to integrate and analyze diverse data types, including the dissection of brain circuits that respond to stress. Using RNA-sequencing in the ventral hippocampus, we sought to examine a cross-model transcriptomic profile in animal models validated for susceptibility to stress, including mice treated with corticosterone (CORT), mice subjected to chronic social defeat stress, and mice heterozygous for the single-nucleotide polymorphism of the gene encoding for brain-derived neurotrophic factor, BDNF Val66Met.

Methods: One set of wild-type mice or BDNF Val66Met male mice was maintained on chronic oral corticosterone (CORT) or vehicle for two weeks prior dissection of the ventral hippocampus. Three biological replicates were used per experimental group, comprising of RNA pooled from two animals each. The cDNA libraries were sequenced on an Illumina NextSeq 500. Another set of wild-type male mice was raised in an enriched environment or in standard housing. This set of mice also underwent chronic social defeat stress (CSDS) and was subsequently classified into resilient or susceptible groups. RNA was extracted from the ventral dentate gyrus and sequenced using an Illumina 2500. Genes with at least 0.5 counts per million in at least three samples were retained. Differential expression analysis was conducted using the limma-voom package. The effect size (Cohen's D) was calculated for each gene using the differential expression results and this was used as an input in a fixed effects meta-analysis model from which an estimate and associated p-value were calculated. The individual comparisons were also used as inputs to a rank-rank hypergeometric overlap algorithm, which overlaps any two sets of comparisons.

Results: We examined the number of differentially expressed genes (DEGs) across models and found that oral CORT induced an upregulation of 148 genes and 118 genes in BDNF Val66Met and wild type mice, respectively. In addition, 521 genes in BDNF Val66Met and 134 genes in wild type mice were downregulated after oral CORT treatment. When analyzing the effects of CSDS in susceptible versus resilient mice, raised in standard or enriched housing, there was an upregulation of 104 genes and 153 genes in enriched and standard housed mice, respectively. The number of downregulated genes was 124 for mice raised in enriched housing and 130 for mice raised in standard condition ($p < 0.05$, $fc > 1.3$). DEGs found in a cross-model comparison were entered into GO analysis that showed a number of common gene pathways, including glutamate/GABA transmission, circadian rhythm, TGF- β signaling, and epigenetics. Among the genes that were commonly affected across models ($p < 0.01$, $fc > 1.3$), we identified Fkbp5, a co-chaperone of hsp90 that regulates glucocorticoid receptor sensitivity and plays a crucial role in the pathophysiology of affective disorders.

Conclusions: Common DEGs across multiple models of stress show that this type of integrated analysis allows us to highlight common genomic processes influenced by stress in discrete brain regions, such as the ventral hippocampus. Construct validity of animal models for stress-related psychiatric disorders spans from genetic vulnerability to exposure to environmental challenges. Thus, cross-model analyses using distinct types of stressors, may integrate the multifactorial origin of stress-related psychiatric disorders, uncovering biomarkers for prevention and treatment in populations at risk.

Keywords: RNA-Sequencing, Ventral Hippocampus, Stress Models

Disclosure: Nothing to disclose.

M78

Assessing Relationships Among Impulsive Sensation-Seeking, Reward Circuitry Activity, and Predisposition to Bipolar Disorder: An fMRI Replication and Extension Study

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Background: High trait impulsive sensation seeking (ISS), or the

tendency to engage in behavior with little forethought and to seek out new and extreme experiences, is associated with increased risk for bipolar disorder. Previous work has indicated a positive relationship between reward expectancy (RE)-related blood oxygen level dependent (BOLD) activity and ISS. In this study, we aimed to replicate previous findings of a positive relationship between RE-related left ventrolateral prefrontal cortex (L vIPFC) and bilateral ventral striatum (VS) activity and ISS and to test for statistical mediation effects of ISS components on the relationship between RE-related activity and measures of affective symptoms denoting presence of and/or predisposition to bipolar disorder.

Methods: A transdiagnostic community sample of 127 young adults ages 18–25 who were either healthy or seeking treatment for psychological distress completed a card-based guessing game functional magnetic resonance imaging (fMRI) task. Participants completed self-report measures of ISS previously shown to be associated with RE-related activity in the L vIPFC and bilateral VS: the Barrett Impulsiveness Scale (BIS) Attention and Motor Impulsivity subscales, the Behavioral Inhibition/Activation Fun Seeking subscale (BIS/BAS), and the Urgency, Premeditation (lack of), Perseverance (lack of), Sensation Seeking, Positive Urgency Impulsive Behavior, (UPPS-P) Positive and Negative Urgency subscales. Outcome variables included RE-related L vIPFC and bilateral VS activity. Significant findings were confirmed by combining the replication sample with the original sample data for a total $n = 225$. In this combined sample, we performed analyses to test for statistical mediation effects of ISS components on the relationship between regional RE-related BOLD signal and Mood Spectrum (MOODS) factor scores associated with predisposition to bipolar disorder (Psychomotor Activation, Mixed Instability, and Suicidality factors). To confirm the specificity of the findings, we tested for mediation effects of ISS on the relationship between RE-related activity and the Hamilton Rating Scale for Depression (HAM-D). Results were considered significant at $p < 0.02727$ (FDR corrected).

Results: We replicated a significant positive relationship between RE-related L vIPFC activity and UPPS-P Negative Urgency, a component of ISS ($\beta = 0.28$, $t = 2.44$, $p = 0.0169$). There were no significant findings for the other four ISS components identified in the original paper or in the VS regions of interest. We confirmed the association between RE-related L vIPFC and negative urgency in the combined sample ($\beta = 0.27$, $t = 2.41$, $p = 0.0184$). Negative urgency statistically mediated the relationship between RE-related L vIPFC activity and Psychomotor Activation and Mixed Instability MOODS factor scores, such that the indirect model was associated with a 67.98% reduction in the contribution of RE-related L vIPFC activity to the MOODS Psychomotor Activation factor score and an 81.67% reduction in the contribution of RE-related L vIPFC activity to MOODS Mixed Instability factor score relative to the direct model. RE-related L vIPFC activity did not mediate the relationship between negative urgency and MOODS scores, highlighting the specific direction of the mediation effect. There were no significant findings for the HAM-D or the MOODS Suicidality factor.

Conclusions: We replicated findings showing that RE-related L vIPFC activity is a biomarker for Negative Urgency, a component of trait ISS. Negative urgency, the tendency to react with frustration or impatience under distressing conditions, largely explains the relationship between RE-related L vIPFC activity and behavioral measures associated with predisposition to bipolar disorder. Importantly, there was no significant relationship between RE-related L vIPFC activity and depression symptoms (HAM-D, MOODS Suicidality factor), suggesting that RE-related L vIPFC activity may have utility for assessing specific risk or predisposition for bipolar disorder via its relationships with negative urgency. Our findings thereby improve understanding of the neural mechanisms underlying a specific dimension of psychopathology that predisposes individuals to bipolar disorder,

and highlight the importance of RE-related L vIPFC activity as a potential biomarker and target for interventions to reduce negative affective symptoms associated with BD risk. Future studies designed to assess longitudinal changes in hypo/manic symptoms will further clarify the relationship between L-vIPFC activity, ISS, and bipolar disorder.

Keywords: Bipolar Disorder, Impulsive Sensation Seeking, Replication, fMRI, Risk Factors

Disclosure: Nothing to disclose.

M79

FKBP5 Regulates HPA Axis Activity by Fine Tuning the Mineralocorticoid/Glucocorticoid Receptor Balance in the Hippocampus

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Background: Mood and anxiety disorders represent a major disease and social burden worldwide, but the underlying molecular mechanisms are still poorly understood. In recent years, an imbalance between central glucocorticoid (GR) and mineralocorticoid (MR) receptors has been proposed to underlie the hypothalamic-pituitary-adrenal (HPA) axis dysregulation that is associated with the susceptibility to psychopathology such as posttraumatic stress disorder and major depression. The GR and MR are members of the ligand-dependent transcription factor family, and represent the main mediators of the negative feedback control of the HPA axis. This occurs primarily at the levels of the hypothalamus and pituitary, but also within the hippocampus. MRs have a 10-fold higher affinity for glucocorticoids than GRs, suggesting different roles for each receptor in the regulation of HPA axis activity. In addition, MRs are largely occupied under basal corticosterone conditions, whereas GR occupancy is increased when corticosterone levels rise during circadian peak or following stress. Moreover, the co-chaperone and psychiatric risk factor FKBP5 is a negative regulator of GR activity, and at the same time a direct target of the GR. Recent evidence also points to a potential regulation of FKBP5 via MR signaling. An imbalance between central GR and MR receptor signaling is proposed to underlie the HPA axis dysregulation that associates with susceptibility to psychopathology. Here we assessed whether FKBP5 modulates HPA axis activity by fine tuning the MR/GR balance in the hippocampus.

Methods: We conducted immunohistochemistry (IHC), in situ hybridization (ISH) and RNAscope to map the expression pattern of the MR, GR and FKBP5 in hippocampal sub-regions of C57/B6 mice. In addition, we generated different cell type-specific GR and MR knockout mouse lines to investigate the impact of receptor depletion on hippocampal FKBP5, MR or GR mRNA expression, as well as peripheral corticosterone levels under baseline and acute stress conditions. Mice lacking the GR in glutamatergic forebrain neurons (GR-CKONex-Cre), and mice with a conditional deletion of the MR in hippocampal CA2/CA3 neurons (MR-KOAmigo2-Cre) or forebrain glutamatergic neurons (MR-KKOCamk2a-Cre) were used. In addition, hippocampal FKBP5, GR and MR mRNA levels were assessed via qPCR following acute treatment of the MR antagonist Spironolactone in C57/Bl6 mice.

Results: IHC, ISH and RNAscope analyses in the hippocampus of C57/Bl6 mice revealed a similar expression pattern between the MR and FKBP5. In contrast, the expression patterns of the GR and

FKBP5 were more distinct. This was further supported by a positive correlation of FKBP5 and MR mRNA levels in all hippocampal sub-regions. Interestingly, no changes in FKBP5 mRNA expression were observed in GR-CKONex-Cre mice. In contrast, FKBP5 mRNA levels were significantly decreased in MR-CKOAmigo2-Cre mice (2way ANOVA: genotype x hippocampal sub-region interaction $F(3, 16) = 7.66, p < 0.01$), while GR mRNA expression was increased (2way ANOVA: genotype x hippocampal sub-region interaction $F(3, 16) = 8.76, p < 0.001$). Along these lines, deletion of the MR from glutamatergic forebrain neurons (MR-CKOCamk2a-Cre) resulted in significantly decreased FKBP5, and increased GR expression under baseline conditions without affecting circulating corticosterone levels. Acute restraint stress induced a significant increase in hippocampal FKBP5 mRNA levels, which was even more pronounced in MR-CKOCamk2a-Cre mice compared to controls. Interestingly, this stressor resulted in significantly decreased GR mRNA levels in MR-CKOCamk2a-Cre mice, an effect that was not observed in stressed control animals. Although restraint stress increased corticosterone levels in both genotypes, this effect was significantly dampened in MR-CKOCamk2a-Cre mice compared to controls (2way ANOVA: genotype x restraint interaction $F(1, 19) = 6.23, p < 0.05$). Overnight treatment with the MR antagonist Spironolactone (via drinking water) led to significantly reduced hippocampal FKBP5 mRNA levels in C57/Bl6 mice ($T(19) = 2.11, p < 0.05$), while MR and GR mRNA expression remained unaffected.

Conclusions: Our findings demonstrate that MR signaling regulates GR sensitivity in the hippocampus by modulating FKBP51 expression during the initial stress response. This provides additional insights into the molecular mechanism underlying the GR/MR balance hypothesis. Importantly, our data further underline the important, but largely unappreciated role of MR signaling in stress-related psychiatric disorders.

Keywords: HPA Axis, Glucocorticoid Receptor, Mineralocorticoid Receptor, FKBP5, Hippocampus

Disclosure: Nothing to disclose.

M80

Neural Markers of Reward Learning and Frustration in Pediatric Irritability: A Preliminary Study

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Background: Irritability is a common psychiatric symptom in youth, often associated with significant impairment (reviewed in Vidal-Ribas et al., 2016). Irritability typically manifests in the context of frustrative nonreward, i.e., the omission of an expected reward or blocking of a goal (Amsel, 1958; Cuthbert & Kozak, 2013). Based on these observations and supportive fMRI data in severely irritable youth (e.g., Adelman et al., 2011; Deveney et al., 2013), alterations in reward processing and frustrative nonreward have been proposed as key neurobiological mechanisms mediating pediatric irritability (Brotman et al., 2017; Leibenluft, 2017). Reward processing is a multifaceted construct; within this construct, reward learning and its neural substrates have not yet been examined in relation to irritability. In addition, it is unclear whether frustration as an affective context modulates the neural substrates of reward learning in irritability. Here, we developed a novel, child-friendly fMRI paradigm to assess probabilistic reward learning, before and after a frustration induction in the scanner.

We piloted the paradigm behaviorally and are now conducting it in the scanner; data collection is ongoing. Preliminary data include significant behavioral and brain correlates of irritability.

Methods: The “Carnival” paradigm was developed and piloted for feasibility behaviorally with a transdiagnostic sample of 23 youth (M age=13.1 years; 65% male; $n = 16$ with disruptive mood dysregulation disorder [DMDD] and/or attention-deficit/hyperactivity disorder [ADHD] and $n = 7$ healthy volunteers). The paradigm presents participants with a series of 2-armed bandit reinforcement learning tasks (80:20 reward ratio), displayed as games at a virtual carnival. In each game, participants learn to select between pairs of stimuli to maximize their monetary rewards, delivered via trial-by-trial feedback. Participants use hand-held grip force devices to make their selections. Halfway through the paradigm, unbeknownst to participants, a separate, rigged carnival game occurs to induce frustration. Participants are debriefed after the task. We are now acquiring fMRI data from an independent transdiagnostic sample of youth varying in irritability (M age=12.9 years; 64% male; current $n = 22$ youth with DMDD, ADHD, and/or symptoms of irritability and $n = 3$ healthy volunteers). Level of irritability is quantified using the Affective Reactivity Index (ARI; Stringaris et al., 2012), completed by youth and parents. As data collection is ongoing, preliminary data were analyzed here with respect to task effects and neural markers of irritability and affective context (pre-frustration vs. post-frustration) during reward learning. Whole-brain linear mixed effects analyses in AFNI examined level of irritability and affective context in relation to neural activity during reward stimulus selection, anticipation of feedback, and receipt of feedback ($n = 17$ with usable fMRI data; voxelwise threshold $p < .005; k > = 15$ [234 mm³]).

Results: The fMRI paradigm was successful in inducing frustration ($p = .002, \eta^2 = .29$): participants’ subjective frustration ratings were higher during the rigged game than during both the pre-frustration ($p = .01$) and post-frustration ($p = .01$) reward learning games. In addition, higher irritability was associated with greater subjective frustration following the rigged game ($p = .02, r = .53$). Irritability was not significantly associated with behavioral accuracy during reward learning, i.e., preferential selection of high- vs. low-reward stimuli, at either pre- or post-frustration. Preliminary fMRI analyses indicated robust task effects, including recruitment of primary visual cortex (V1), supplementary motor area, prefrontal cortex (PFC), and hippocampus during reward stimulus selection; striatum, amygdala, midcingulate cortex, and orbitofrontal cortex (OFC) during anticipation of feedback; and V1, OFC, and insula during receipt of feedback (all $ps < .005$, all $ks > 15$). Further, there were significant associations of irritability and affective context with neural activity. In particular, during reward stimulus selection, irritability as a main effect was associated with differential activity in the striatum, insula, and IFG, whereas affective context as a main effect was associated with differential activity in the dorsomedial PFC (all $ps < .005$, all $ks > 15$). During receipt of feedback, irritability was associated with differential activity in the posterior cingulate ($p < .005, k = 15$). Finally, irritability and affective context interacted during receipt of feedback in relation to activity in the dorsolateral PFC ($p < .005, k > 15$).

Conclusions: These preliminary fMRI and behavioral data support the feasibility of assessing neural markers of reward learning, before and after an acute frustration induction, in youth along a spectrum of irritability severity. The paradigm successfully induced frustration in the scanner, and fMRI analyses supported robust task effects in neural circuitry known to be engaged in reinforcement learning. Further, irritability and the affective context of frustration modulated neural activity in the striatum, posterior cingulate, and PFC. As data collection is ongoing, additional analyses in the larger sample will utilize a computational reinforcement learning approach to examine neural correlates of expected value and prediction error.

Keywords: Functional MRI (fMRI), Children and Adolescents, Irritability, Reward Learning, Frustrative Non-Reward

Disclosure: Nothing to disclose.

M81

Inflammation, Depression Severity, and Response to Ketamine in Treatment-Resistant Depressed Patients: Sex Differences

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Background: Inflammation plays a role in the pathophysiology and treatment responsiveness of depression. However, the effects of inflammation on depressive symptoms and treatment response may vary by sex and treatment modality. For example, cross-sectional studies in depressed patients and suicide attempters show negative associations between the pro-inflammatory cytokine, interleukin (IL)-8, and severity of mood and anxiety symptoms. We have also previously found that increases in IL-8 occurring over a course of electroconvulsive therapy (ECT) are linked with greater antidepressant response in women, but not men. Like ECT, subanesthetic ketamine administration can also elicit robust and rapid clinical effects in treatment resistant depression. In the current study of treatment resistant depressed patients, we aimed to evaluate whether inflammation, as measured by IL-8 levels, is associated with depressive symptom severity at baseline, whether changes in IL-8 are related to ketamine response, and whether these relationships differ by sex.

Methods: Relationships between plasma IL-8 concentration and depression severity as measured by the Hamilton Depression Rating Scale (HDRS), were evaluated with linear regression analysis in a cross-sectional sample of patients with treatment resistant depression ($n = 108$; 58 men, 50 women; mean age 43). Evaluation of sex as a moderator of this relationship was also evaluated. A subset of this sample ($n = 45$; 16 females, 29 males; mean age 41) went on to receive an open label infusion of ketamine (0.5 mg/kg, infused over 40 minutes). In addition to the cross-sectional measurements obtained at baseline, these participants also completed measurements of plasma IL-8 concentration and depression severity (HDRS) 24 hours following ketamine infusion. Some ($n = 22$) went on to receive additional infusions of ketamine, but only baseline and 24-hour time points were evaluated in the current study. Linear mixed effects models were used to evaluate whether IL-8 change following ketamine infusion varied according to sex, responder status (defined as $> 50\%$ reduction in HDRS), or both combined (using two way and three way interaction terms). Linear regression analyses were utilized to follow-up findings from linear mixed models.

Results: Among the cross-sectional sample of 108 depressed patients at baseline, IL-8 concentration was negatively associated with HAM-D score (standardized $\beta = -0.200$, $p = 0.038$). When sex was evaluated as a moderator of this relationship, the p value for the interaction term was $p = 0.057$. In analyses stratified by sex, there was a highly significant negative relationship between IL-8 concentration and HAM-D severity in women (standardized $\beta = -0.364$, $p = 0.009$), but not in men (standardized $\beta = -0.015$, $p = 0.909$). IL-8 change in response to ketamine infusion ($n = 45$; 16 females, 29 males) did not vary by sex ($p = 0.70$) or responder status ($p = 0.90$), evaluated separately using two way interaction terms in linear mixed effect models (interaction terms were: sex by time, and responder status by time). However, the three-way

interaction term (sex by responder status by time) evaluating the combined effects of sex and responder status on IL-8 concentration change was highly significant ($p = 0.006$). In follow-up univariate linear regression analyses stratified by sex, IL-8 change was positively associated with HAM-D percentage change in women (standardized $\beta = 0.536$, $p = 0.03$), and negatively associated with HAM-D percentage change in men (standardized $\beta = -0.429$, $p = 0.02$).

Conclusions: At baseline, IL-8 was negatively associated with depression severity in women only. Increase in IL-8 in response to ketamine was associated with improvement in depression in women, but the inverse was found in men. Lower IL-8 concentrations may be uniquely related to greater depression severity in women, with treatment-related increase in IL-8 concentrations associated with depression improvement in women only, across at least two different treatment modalities: ketamine and electroconvulsive therapy (previously reported).

Keywords: Inflammation, Depression, Ketamine, Sex Differences

Disclosure: Nothing to disclose.

M82

Roles and Mechanisms of Neuroinflammation in Stress and Depression

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Background: Clinical studies suggest increased inflammatory responses in the brains and bloods of depressive patients. Rodent studies have shown roles of inflammation-related molecules for chronic stress-induced behavioral changes as a rodent model for the study of depression. Since microglia is a major cellular source of these molecules in the brain, it has been hypothesized that chronic stress activates microglia, from which inflammation-related molecules are released and induces neuronal and behavioral changes. However, despite extensive research about neuroinflammation in stress and depression, its roles and mechanisms remain poorly understood.

Methods: We have begun to investigate roles of innate immune receptors Toll-like receptor (TLR) 2/4 and their putative endogenous ligands in repeated social defeat stress, a mouse model for the study of depression. We use systemic and conditional knockout mice of these molecules as well as local infusion of neutralizing antibodies, and perform behavioral, histological and brain region- and microglia-specific transcriptome analyses.

Results: Our published results show that TLR2/4 mediates repeated social defeat stress-induced microglial activation in the medial prefrontal cortex, thereby leading to neuronal and behavioral changes through microglia-derived TNF α and IL1 α . New, unpublished data in this study, using conditional knockout mice and local infusion of neutralizing antibodies, have shown roles and regulations of HMGB1, a nuclear protein that is secreted from cells upon cellular stress and may act as an endogenous TLR2/4 ligand, in prefrontal neurons for repeated social defeat stress-induced behavioral changes. Our data show that repeated social defeat stress induces the release of HMGB1 selectively from the nuclei of prefrontal neurons and causes microglial activation and its neuronal and behavioral consequences through TLR2/4.

Conclusions: This study shows for the first time that chronic stress triggers direct innate immune signaling from the nuclei of

prefrontal neurons to microglia, a key step for translating chronic stress-activated neuronal signaling to neuroinflammation. We are currently investigating mechanisms underlying chronic stress-induced HMGB1 release from prefrontal neurons as well as roles of other putative TLR2/4 ligands upregulated by chronic stress. The goal of this study is to understand molecular and cellular mechanisms underlying chronic stress-induced neuroinflammation that might promote our understanding of stress-related pathology and development of therapeutic strategies for mental illness.

Keywords: Social Defeat Stress, Depression Inflammation Cytokine, Innate Immune Response, Microglia and Neurons

Disclosure: Nothing to disclose.

M83

Subchronic Exposure of Cuprizone Results in Mild Neuroinflammation and a Distinct Set of Behavioral Alterations Related to Psychiatric Disorders

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Background: Cuprizone, a copper chelating agent, is often used to model multiple sclerosis by exposing rodents over a 6-8 weeks period, which results in pathogenesis reminiscent to multiple sclerosis such as oligodendrocyte apoptosis, microglia activation, astrogliosis, and demyelination in the CNS. These cellular alterations parallel to robust changes in gene expression in cytokines and a core component of inflammasomes, and markers for activated microglia in the brain, indicative of inflammation. In contrast to chronic cuprizone exposure, several studies have reported that subchronic treatment of cuprizone resulted in cognitive impairment and augmented locomotor in response to amphetamine challenge. Here we investigated the effect of subchronic cuprizone in a wide array of behavioral paradigms.

Methods: C57BL/6J male mice were exposed to cuprizone by feeding diet containing 0.2% cuprizone for 10 days, in which the duration is not sufficient to cause demyelination. On day 11, the diet was switched back to normal diet. Starting from day 8, the mice were subjected to an array of behavioral testing consisted of home cage locomotor, zero-maze, Y-maze, 3 chamber social interaction, novelty object recognition, and prepulse inhibition tests. The behavioral tests were completed on day 13 and whole hippocampi were collected on day 14 to analyze molecular markers related to inflammation.

Results: The mice fed with cuprizone had no changes in weight and gross appearance. However behaviorally, they exhibited less anxiety-like phenotype in zero-maze test without altering locomotor in home cage environment in comparison to those fed with control diet. The 3-chamber social interaction test demonstrated lack of sociability in the mice treated with cuprizone. We also observed increases in prepulse inhibition without changes in startle response. In contrast, cognitive function of the mice fed with cuprizone was normal as evidenced by Y-maze and novelty object recognition tests. In addition to behavioral phenotyping, we investigated gene expression changes in molecules related to neuroinflammation. In hippocampus, mRNA expression of GFAP, IL-1 α , and CD68 were significantly elevated in cuprizone-fed mice compared with those with control diet.

Conclusions: Taken together, we found a unique set of behavioral alterations from subchronic exposure of cuprizone.

That is impaired sociability, altered sensory responses, and impulsiveness, which may represent some but not all core symptoms seen in psychiatric disorders including schizophrenia and autism. Considering clinical heterogeneity of psychiatric disorders, we speculate this paradigm would represent a subpopulation of patients with a component of neuroinflammation in pathogenesis, providing a unique opportunity for drug discovery research.

Keywords: Neuroinflammation, Behavior, Autism, Neuropsychiatric Disorders [Schizophrenia, Parkinson's Disease, Major Depressive Disorder], Cuprizone Short-Term Exposure

Disclosure: Astellas Research Institute of America, Employee

M84

Modulation of the Inflammatory Response Benefits Treatment-Resistant Bipolar Depression

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Background: Although bipolar disorder (BD) is characterized by both depression and mania, depressive symptoms are predominant throughout the entire course of the illness. Bipolar depression (BDD) can be very difficult to treat as many bipolar depressed patients are slower to respond and less likely to achieve remission than patients going through a manic phase. With untreated or treatment-resistant bipolar depression (TRBDD) often leading to detrimental, and sometimes even fatal, consequences to those afflicted, it is critical to investigate efficacious and safe therapeutic methods. A growing body of evidence suggests that mood disorders, including bipolar depression, are linked to immune activation and an elevated inflammatory status. In this study, we sought to enhance treatment response in patients with TRBDD by modulating inflammatory status through the adjunctive use of celecoxib (CBX), a specific cyclooxygenase 2 inhibitor. While we assessed a panel of various pro-inflammatory biomarkers, this poster highlights our findings on C-Reactive Protein (CRP), an acute phase reactant and indicator for non-specific inflammation. We sought to determine whether blood levels of CRP could be identified as a potential biomarker of inflammation and predictor of treatment response in a cohort of TRBDD patients.

Methods: In this double-blind, placebo-controlled trial, we recruited study participants who had been diagnosed with BD I or II and who had failed to respond to at least two adequate trials of antidepressants and/or antipsychotic or mood stabilizing medications (N = 47). Treatment resistance was quantified using the Maudsley Staging Method. All participants underwent at least a 2-week washout phase and 1-week placebo (PBO) run-in phase. Participants were then randomized into one of the two arms of the study for an 8-week treatment phase. In one arm of the study, participants (N = 27) received CBX, an anti-inflammatory medication, with escitalopram (ESC), an antidepressant. In the other arm, participants (N = 20) received PBO along with ESC. Blood was drawn in all study participants at baseline, week 4, and week 8 of treatment, which was later analyzed using a Zymutest High Sensitivity CRP enzyme-linked immunosorbent assay (ELISA) kit.

To quantitate mood symptoms, the Hamilton Depression Rating Scale 17 Item (HAM-D 17) and Hamilton Anxiety Rating Scale (HAM-A) were measured at baseline and weeks 1, 2, 4, and 8 of treatment. During these weeks, safety and tolerability were assessed through monitoring of bleeding diathesis and an adverse events inventory, as well determination of prothrombin time,

complete blood count and activated partial thromboplastin time. Independent Sample Student T-tests were used to compare depression severity at different weeks of treatment between the two arms of the study. A mixed ANOVA test was conducted to compare symptom severity throughout the entire 8 weeks. To compare remission and response rates, Chi-Square tests were also conducted.

Results: Compared to the PBO arm, the CBX arm had significantly faster response times, showing significant decreases in HAM-D 17 scores ($p < 0.004$) and HAM-A scores ($p < 0.036$) after only week of treatment. The HAM-D 17 scores were found to be different between the two arms through the entire duration of the study ($p < 0.040$). The CBX arm produced higher remission rates ($p < 0.0005$) and response rates ($p = 0.021$) to treatment as compared to the PBO arm. Combination treatment was well tolerated and no adverse events were reported. After analysis, the baseline CRP levels were significantly higher in TRBD patients as compared to healthy control subjects ($p = 0.044$). We found no significant difference of baseline CRP levels between study participants in the PBO arm versus CBX arm ($p = 0.156$). Yet, after 8 weeks of treatment, the participants in the CBX arm had significantly lower CRP levels as compared to those in the PBO arm of the study ($p = 0.003$).

Conclusions: The use of CBX, an anti-inflammatory agent, in combination with a standard anti-depressant medication accelerates and enhances treatment response and increases remission rates in patients with TRBD. Furthermore, the results of this study, in spite of the relatively small sample size, suggest that the use of CBX as adjunctive medication could reverse treatment resistance in patients with TRBD in a safe and effective manner. This supports the clinical potential of anti-inflammatory agents for adjunctive treatment and more effective management of mood disorders. Further studies should investigate the use of CBX for psychiatric indications. Consistent with other studies of BD, we found higher levels of CRP in patients with TRBD as compared to healthy controls. This indicates that CRP could potentially be a useful biomarker for TRBD. Additionally, in this study, the CBX group had significantly lower CRP levels than the PBO group at the end of the 8 weeks. This reduction of inflammatory status could be at least partially responsible for the beneficial outcomes in response and remission that we observed in patients who received the adjunctive CBX treatment.

Keywords: Inflammation, Treatment-Resistance, Bipolar Depression, Celecoxib, CRP

Disclosure: Nothing to disclose.

M85

Validation of the Chronic Restraint Stress Model of Depression in Rats and Investigation of Standard vs Accelerated rTMS Treatment

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Background: Depression is a debilitating neuropsychiatric disorder with significant morbidity and mortality due to the risk of suicide. Antidepressants are typically a first line treatment for depression. However, up to one third of adults have treatment-resistant depression (TRD) that does not respond to pharmacotherapy. Repetitive transcranial magnetic stimulation (rTMS) has been used clinically for TRD for over a decade. However, the mechanisms underlying the therapeutic effects of rTMS remain

poorly understood. Additionally, depression is a complex condition and it is now widely accepted that besides changes in behaviour, depression in humans also involves disruptions in intrinsic functional connectivity of the brain, measured by resting-state functional MRI (rs-fMRI), neurometabolite levels, measured by proton magnetic resonance spectroscopy (1H-MRS), and hippocampal volumes, measured by anatomical MRI. Animal models are an indispensable tool for studying etiology, progression, and treatment of depression. However, there is still controversy regarding the validity of using rodent models of human neuropsychiatric disorders. The chronic restraint stress (CRS) model of depression in Sprague Dawley rats has been shown to exhibit similar behavioural, genetic and protein changes that are established in depression in humans. The continuous and predictable nature of this stress closely mimics the every-day stress that people experience, such as daily repetition of a stressful job and social, financial or familial stresses.

The aim of this study was to use brain imaging in rats to investigate the validity of the CRS model of depression in terms of functional, neurometabolic and structural changes. We also used elevated plus-maze test (EPM) and forced swim test (FST) to determine the level of anxiety and depression-like behaviours in animals following CRS and following treatment with low-intensity rTMS (LI-rTMS). Here we report preliminary findings on the validity of the depression model and short-term effects of LI-rTMS treatment.

Methods: CRS involved placing the animals in individual transparent plexiglass tubes for 2.5 h daily for 13 days. Rats subsequently received 10 min of 10 Hz LI-rTMS treatment daily for 4 weeks (standard) or three times daily for 2 weeks (accelerated). 30 male Sprague Dawley rats were used in total and randomly allocated to one of six groups for LI-rTMS ($n = 5/\text{group}$): 1) active standard treatment, 2) sham standard treatment, 3) active accelerated treatment, 4) sham accelerated treatment, 5) depression control with no treatment, and 6) healthy control with no treatment. Animals were imaged using a 9.4 T pre-clinical MRI to acquire rs-fMRI, 1H-MRS and T2-weighted anatomical data before and after CRS ($n = 25$). The behavioural tests were conducted before CRS, after CRS, and mid-treatment (after 1 week for accelerated LI-rTMS and after 2 weeks for standard LI-rTMS).

Results: CRS induced the following significant changes in behaviour: 1) during the EPM test, animals displayed increased anxiety-like behaviours including increased head dips used for risk-assessment, increased grooming and decreased entries and exits in the centre and open arms of the maze; and 2) during the FST, animals showed increased learned helplessness including an increase in immobility, a decrease in climbing, and a decrease in latency to first immobility ($P < 0.001$; 123 ± 12 s to 69 ± 6 s). Significant brain changes after CRS were also detected by MRI: 1) rs-fMRI data revealed hypoconnectivity within the salience and interoceptive networks and hyperconnectivity between the cingulate cortex and cortical and limbic regions; 2) a decrease in sensorimotor cortical glutamate ($P < 0.05$; 1.41 ± 0.02 to 1.35 ± 0.02), glutamine ($P < 0.05$; 0.53 ± 0.01 to 0.50 ± 0.01) and combined glutamate-glutamine levels ($P < 0.05$; 1.94 ± 0.02 to 1.85 ± 0.03) was detected by 1H-MRS; and 3) a decrease in hippocampal volume was detected by volumetric analysis of T2-weighted anatomical MRI data ($P < 0.05$; 5.873 ± 0.004 % to 5.851 ± 0.011 %). Depression control animals which underwent CRS but no treatment did not show any improvement in behaviours, even 2 weeks after end of CRS, suggesting this protocol provides a relative durable animal model of depression. Following standard LI-rTMS treatment, animals receiving active treatment did not show any significant differences in behaviours compared to animals receiving sham treatment. This may be due to the small sample ($n = 5/\text{group}$). However, in the accelerated LI-rTMS group, significant differences in behaviour were detected compared to sham treatment (also $n = 5/\text{group}$). Specifically, animals receiving active

treatment showed less stretching during EPM and less immobility, greater latency, and increased climbing behaviours during FST.

Conclusions: Our study is the first to demonstrate significant changes in functional connectivity, glutamate and glutamine levels and hippocampal volume in an animal model of depression. Our findings also suggest that accelerated LI-rTMS rescued depression-like behaviours in rats more effectively than the standard treatment. Overall, the substantial concordance of the present findings with the human literature presents a unique opportunity for the integration of behavioural and molecular changes in CRS model of depression in rats with changes in functional connectivity, neurometabolite levels and hippocampal volume that may be translated to the human disorder and therefore improve treatment strategies.

Keywords: Depression, Animal Model, Translational Research, Proton Magnetic Resonance Spectroscopy, Resting-State Functional MRI

Disclosure: Nothing to disclose.

M86

Efficacy and Safety of Seltorexant as Adjunctive Therapy in Patients With Major Depressive Disorder: Results From a Phase 2b, Randomized, Placebo-Controlled, Parallel-Group, Adaptive Dose-Finding Study

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Background: Major depressive disorder (MDD) is often a difficult-to-treat illness with less than half of patients achieving $\geq 50\%$ response with initial treatment, and many patients need a treatment change or an adjunctive treatment for managing treatment failure. Available adjunctive atypical antipsychotic therapies (e.g. aripiprazole, quetiapine) are effective although their use is limited by their side effect profiles; hence, high clinical need exists for effective and better tolerated antidepressants (ADs). Earlier exploratory efficacy results showed that seltorexant, a selective antagonist of human orexin-2 receptors (OX2R), displayed AD effects in patients with MDD regardless of sleep impairment, and also improved sleep efficiency in patients with insomnia. This phase 2b study aimed to investigate efficacy and safety of various doses of seltorexant as adjunctive therapy in patients with MDD who had inadequate response to current AD therapy.

Methods: This multicenter, double-blind (DB), parallel-group, placebo-controlled, 6-week adaptive dose-finding study (NCT03227224) was conducted in men or women (aged 18-70 yr) who met DSM-5 criteria of MDD and who had an inadequate response to 1-3 selective serotonin/serotonin-norepinephrine reuptake inhibitors of sufficient duration and dose in the current episode. Patients also had Montgomery-Asberg Depression Rating Scale (MADRS) total score ≥ 25 at screening. Eligible patients were initially randomized (2:1:1) to receive placebo or seltorexant (20 mg or 40 mg) once daily. After an interim analysis (6 weeks post-randomization of 160 patients), newly recruited patients were randomized to receive placebo or seltorexant 10 mg or 20 mg, with allocation ratio 3:3:1, while 40 mg dose was no longer assigned. Randomization was stratified based on regions (US, Europe, Japan) and by baseline Insomnia Severity Index (ISI) score ≥ 15 vs < 15 . Primary endpoint: change from baseline MADRS total score at week 6 (day 42). Secondary endpoints included change from baseline MADRS total score at week 6 in patients with

baseline ISI score ≥ 15 vs ISI score < 15 ; proportion of patients achieving response ($\geq 50\%$ in improvement in baseline MADRS total score), and remission (MADRS total score of ≤ 12). Other than Multiple Comparison Procedure-Modeling (MCP-Mod), no multiplicity adjustment was done and 90% CI for least square mean (LSM) difference and p-value were calculated for each seltorexant dose vs placebo. Efficacy analyses were based on full analysis set and safety assessments on safety analysis set.

Results: A total of 287 patients were randomized (n = 4 not dosed) and 251 (87%) completed the DB phase. Of 283 dosed patients (placebo = 137, seltorexant 10 mg = 33, 20 mg = 61 and 40 mg = 52), 54% were women; median age was 52 yr (range: 18 to 70 yr). Based on the MCP-Mod analysis, there was no significant dose-response relationship associated with pre-specified models in change from baseline MADRS total score at week 6. The sigmoid Emax model had the best fit of the four models, with 1-sided p-value = 0.083 (prespecified threshold 1-sided p = 0.05). Based on mixed model for repeated measures (MMRM) analysis, a greater improvement (2-sided nominal p < 0.1) in MADRS total score was observed in the seltorexant 20 mg group vs placebo at week 3 and 6, with LSM difference (90% CI): -4.5 (-6.96; -2.07), p = 0.003 and -3.1 (-6.13; -0.16), p = 0.083, respectively. The improvement in MADRS score at week 6 was greater in patients with baseline ISI ≥ 15 than with baseline ISI < 15 , with LSM difference (90% CI) vs placebo of -4.9 (-8.98, -0.80) and -0.7 (-5.16, 3.76), respectively. Response rates were numerically higher for seltorexant 20 mg (41%) and 40 mg (39%) vs placebo (29%) or seltorexant 10 mg (24%) group. Similarly, remission rate was numerically higher for seltorexant 20 mg (30%) and 40 mg (27%) vs placebo (19%) and seltorexant 10 mg (15%). Treatment-emergent adverse events (TEAEs) were reported in 55/146 (37.7%) of patients in seltorexant groups (all doses) vs 56/137 (40.9%) in placebo group with no serious adverse events in seltorexant treatment groups. The most common (> 5% in any group) TEAEs (seltorexant groups vs placebo, %) were somnolence (6 vs 5), headache (6 vs 7), nausea (5 vs 3), and diarrhea (1 vs 5), respectively. No deaths occurred in the study.

Conclusions: A significant and clinically meaningful reduction of depressive symptoms was observed for seltorexant 20 mg. In the subpopulation of MDD patients with insomnia (ISI ≥ 15), a larger treatment difference between seltorexant 20 mg and placebo was observed, which is of interest and warrants further investigation. No safety concerns were observed following seltorexant treatment (10, 20, and 40 mg). Seltorexant with its novel mechanism of action has the potential to be an AD with acceptable safety profile, particularly for inadequately-treated patients with sleep disturbances.

Keywords: Adjunctive Therapy, Major Depressive Disorder (MDD), Orexin, Seltorexant

Disclosure: Janssen Research & Development, Employee, Janssen Research & Development, Stock / Equity

M87

Comparison of Different Duration Regimens for Intravenous Racemic Ketamine: 100-Minute Versus 40-Minute Infusions for Refractory Depression

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Background: Meta-analytic data demonstrate that IV ketamine is effective for treatment-refractory depression (TRD), usually using 40-minute infusions. Precise biomarker or clinical predictors of response have not been identified. As one potential variable,

different response and adverse event patterns could occur with rapidity of ketamine administration. The mammalian target of rapamycin (mTOR) signaling pathway serves as a central regulator of cell metabolism, growth, proliferation and survival, and is implicated in ketamine treatment.

Methods: To examine mTOR response and evaluate other biomarkers, we are conducting a multi-site clinical trial of IV ketamine for TRD, administering 3 acute infusions within 11 days. Remission was defined as MADRS scores less than 9. Both 100-minute and 40-minute infusions have been administered, enabling comparison of efficacy, side effects, safety, and tolerability.

Results: To date, 56 of a proposed 100 subjects have completed the 3 acute phase infusions. Twenty-one participants have had additional maintenance infusions, yielding 154 individual infusions of 100-minutes and 98 individual infusions of 40 minutes. Participants have a mean age of 43.85 years (SD $\pm$ 13.45) and most are female (68%). Mean MADRS score at screening was 29. Change in MADRS score at the end of acute series was 11. Comparison of side effects between the two infusion types revealed noted differences, with the 100-minute infusion appearing more tolerable. In a subsample of 30 infusions, rates of cardiac and psychotomimetic side effects were 8% and 13.8%, respectively.

Conclusions: Preliminary efficacy data suggests lower response after a single 100-minute infusion compared to a single 40-minute infusion, but similar response after 3 infusions. These unique data on side effects and overall safety and tolerability, along with preliminary efficacy data, provide an opportunity to consider the merits of 100-minute infusions as an alternative treatment that is subjectively categorized by many psychiatrists as safer and easier to use.

Keywords: IV- Ketamine, Treatment-Refractory Depression, Mood Disorders

Disclosure: SAGE Therapeutics, Advisory Board, Clargent, Stock / Equity, Med-IQ, Inc., Consultant, Cerecor, Advisory Board

M88

Circadian Rhythms in Human Neuronal Models of Bipolar Disorder

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Background: Bipolar disorder (BD) is defined by recurrent depressive and manic episodes, as well as profound circadian rhythm abnormalities affecting sleep, energy, activity and appetite. The Risk for BD is genetically encoded and overlaps with biological systems that control circadian rhythms. However, only limited data exists describing the impact of circadian clock genetic variants on cellular phenotypes in human samples. Moreover, existing data were obtained from non-neuronal cell types that may not fully capture neuron-specific aspects of the circadian clock that are relevant to BD.

Methods: Recent advancements in reprogramming technologies have enabled the use of induced pluripotent stem cells (iPSCs) to investigate cellular neuropsychiatric disease-phenotypes in human cells. We employed iPSCs to develop neuronal precursor cells (NPC) and VGLUT2 + glutamatergic, cortical-like neurons from 6 BD patients and 3 age-matched controls. We then studied circadian rhythms in live cells over 7 days using a bioluminescent reporter gene (Per2-luc) that when inserted into cells, provides

quantitative measures of rhythmic gene expression. A specialized microscope was to study rhythms in single cells, allowing us to quantify the numbers of rhythmic cells and assess phase relationships. Quantitative, real-time PCR was used to determine gene expression rhythms for core clock genes. Rhythm parameters were measured in BD and control cells and subjected to statistical analysis using 2-way ANOVA.

Results: Compared to controls, BD patient-derived NPCs and neurons have significantly lower amplitude rhythms. Circadian period changes across the development of NPC to neurons, and this trajectory differed in BD cells. Single cell studies indicate overall weaker rhythms in gene expression. Additional time course analysis in NPCs and neurons revealed that overall expression of the negative clock regulators, CRY1 and PER2 is significantly increased.

Conclusions: Taken together, these data indicate that iPSC-derived neuronal cell lines show aberrant rhythms in BD. Our neuronal model of circadian rhythms is an ideal platform for future studies to define new targets for pharmacological modulation of cellular rhythms in NPCs and neurons from BD patients.

Keywords: Bipolar Disorder, Circadian Rhythm, iPSC

Disclosure: Janssen Pharmaceuticals, Consultant

M89

Evaluating Association of Irritability With Suicidality and its Underlying Neural Correlates in Adult Outpatients With Major Depressive Disorder: Findings From the EMBARC Study

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Background: Irritability is an important yet understudied symptom domain in patients with major depressive disorder (MDD). We recently showed that improvement in irritability with antidepressant treatment is independent of changes in depressive symptom severity. Further, early changes in irritability can be used to prognosticate longer-term clinical outcomes. This report extends the clinical utility of irritability as a symptom domain by evaluating its association with suicidality and identifying its neural correlates in adult outpatients with MDD.

Methods: Participants of Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care for Depression (EMBARC) study, randomized to sertraline or placebo, were included (n = 296). Irritability and suicidality were assessed with 5-item irritability domain of Concise Associated Symptom Tracking scale (CAST-IRR) and 3-item suicidal thoughts factor of Concise Health Risk Tracking scale (CHRT Suicidal Thoughts). Functional magnetic resonance imaging was used to compute resting state functional connectivity (FC) after parcellating cortical and sub-cortical regions in 121 parcels, and an elastic net approach was used to identify FC pairs associated with irritability. Clinical findings were replicated in two independent samples of outpatients with MDD.

Results: Sertraline was more effective than placebo in reducing irritability (effect size = 0.40). Irritability was highly associated with (r = 0.73) suicidality in EMBARC study. During the course of acute-phase antidepressant treatment, changes in irritability [standardized beta (β) = 0.68, standard error (SE) = 0.03, p < 0.0001] were stronger predictor of changes in suicidality than depressive (β = 0.08, SE = 0.03, p = 0.002) and anxiety (β = -0.04, SE = 0.03, p = 0.17) symptoms. In two separate clinical trials [Combining Medications to Enhance Depression Outcomes (CO-MED) and

Suicide Assessment and Methodology Study (SAMS)], there was a similar pattern of stronger association of suicidality with irritability (CO-MED $\beta = 0.16$, SE = 0.02, $p < 0.0001$; SAMS $\beta = 0.27$, SE = 0.04, $p < 0.0001$) than depressive (SAMS $\beta = 0.04$, SE = 0.04, $p = 0.32$; CO-MED $\beta = 0.02$, SE = 0.004, $p < 0.0001$) and anxiety (SAMS $\beta = -0.02$, SE = 0.04, $p = 0.68$; CO-MED $\beta = 0.08$, SE = 0.02, $p < 0.0001$) symptoms. Using an elastic net approach, we found that 76 FC pairs (out of 7260) were associated with irritability in the EMBARC study. Over half (43/76) of these FC pairs included either striatum or amygdala. We found that lower connectivity of limbic network (LN) with executive control network, dorsal attention network, and visual network were associated with higher severity of irritability. Stronger connectivity of default mode network (DMN) with LN was associated with higher irritability, while stronger connectivity of DMN with salience network was associated with lower severity of irritability.

Conclusions: Irritability is highly associated with suicidality in adult outpatients with MDD. This association is stronger than that of suicidality with other depressive and anxiety symptoms; thus adding to the clinical utility of irritability as a distinct symptom domain. Further, irritability is associated with changes in brain circuits. These include aberrant functional connectivity of amygdala and striatum with brain regions in default mode, executive control, and dorsal attention networks.

Keywords: irritability, Suicidality, Corticostriatal circuit, prefrontal-amygdala-connectivity, Resting State Functional Connectivity

Disclosure: Acadia Pharmaceuticals, Grant, Janssen Research & Development, Grant

M90

Major Depressive Disorder is Associated With an Aberrant Immune Response to Epstein Barr Virus

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Background: Recent studies have identified an altered immune system as a central feature of major depressive disorder. One manifestation of an altered immune system is the inability to mount an effective immune response to common infectious agents Epstein Barr Virus (EBV) is a highly prevalent human herpesvirus capable of infecting the central nervous system and establishing persistent infection. In a previous study (Dickerson et al. Schizophr Bull 2018 Nov 20; PMID 30462333) we found that individuals with schizophrenia had marked elevations in the levels of antibodies to EBV virions as compared to the control population. Further analyses indicated increased levels of reactivity to EBV Viral Capsid Antibody (VCA) but not to EBV nuclear antigen-1 (EBNA-1) or to other human herpesviruses. To our knowledge, EBV exposure has not been studied in individuals with major depression ascertained in a psychiatric treatment setting. The purpose of the current study was to measure the levels of antibodies to EBV virions and defined EBV proteins in a cohort of individuals with major depression and compare these to levels in a group of control individuals without a history of psychiatric symptoms.

Methods: We employed solid phase immunoassay techniques to measure IgG class antibodies to Epstein Barr Virus virions and defined proteins in 87 individuals with major depressive disorder and 312 individuals without a history of a psychiatric disorder. Western blot testing was performed to document reactivity to specific EBV proteins. We also measured antibodies to the other human herpesviruses HSV-1, HSV-2, CMV, VZV, and HHV6. Levels

of antibodies between the groups were compared by multivariate analyses adjusted for age, sex, and race.

Results: Individuals with major depression had reduced levels of reactivity to EBV nuclear antigen-1 (EBNA-1) (coefficient = -0.345 , 95% CI -0.570 , -0.121 , $p = .003$) but not to EBV Viral Capsid Antibody (VCA) or to the EBV virion ($p > .05$). Western blot analysis confirmed decreased reactivity to EBNA proteins in the group of individuals with depression and also documented an increase to one VCA protein. In an analysis including all three of the EBV antibody measures, the level of whole virion antibodies was significantly increased in the depression group (coefficient = $.128$, 95% CI $.059$, $.196$, $p < .001$) while the level of EBNA antibodies and of VCA antibodies were decreased (coefficient = $-.069$, 95% CI $-.116$, $-.023$, $p = .004$; coefficient = $-.095$, 95% CI $-.166$, $-.024$, $p = .009$, respectively). We also measured antibodies to the other human herpesviruses HSV-1, HSV-2, CMV, VZV, and HHV6. There were no significant differences between the depression and the control group in any of these antibody levels except for VZV which was significantly reduced in the depression group (coefficient = $-.305$, 95% CI $-.546$, $-.065$, $p = .013$, adjusted for age, sex, and race).

Conclusions: Individuals with major depression have altered levels of antibodies to EBV proteins indicating an aberrant response to Epstein Barr Virus infection. This aberrant response may contribute to the ineffective suppression of EBV infection and increased viral replication within the central nervous system. The role of EBV in the pathogenesis of major depressive disorder and other psychiatric disorders should be the subject of further investigations.

Keywords: Depression, Infection, Herpesvirus

Disclosure: Nothing to disclose.

M91

20-Years Trends in the Pharmacologic Treatment of Bipolar Disorder by Outpatient Psychiatrists

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Background: Pharmacological options for treating bipolar disorder have increased over the past 20 years. Most notably, several second-generation antipsychotics received regulatory approval in the 1990s for the treatment of bipolar disorder. Yet few studies have examined how the availability of these new medications have affected prescribing patterns for individuals with bipolar disorder in the United States.

Methods: Data from the National Ambulatory Medical Care Survey (1997-2016) were used to examine prescribing trends in US office-based psychiatric practice focusing on clinical, demographic, and prescription medication characteristics of visits with a bipolar diagnosis. Logistic regression models were developed using survey year and other relevant covariates as independent variables of interest to identify statistically significant trends over the time period examined.

Results: Second-generation antipsychotics were increasingly more commonly prescribed during the 20-year period, increasing from 12.4% (1997-2000) to 51.4% (2013-2016) of bipolar outpatient visits (adjusted odds ratio [AOR] 5.05, 95% CI 3.65-7.01). Use of traditional mood stabilizers, which included lithium, valproic acid, lamotrigine, and carbamazepine, decreased from 62.3% (1997-2000) to 30.2% (2013-2016) of bipolar visits (AOR 0.21, 95% CI 0.15-0.30). Prescription of any antidepressant

occurred in 47.0% of bipolar visits in 1997-2000 and 57.5% in 2013-2016. Prescription of an antidepressant without a mood stabilizer increased substantially from 17.9% (1997-2000) to 40.9% (2013-2016) (AOR 2.88, 95% CI 2.06-4.03).

Conclusions: Substantial changes have occurred in the treatment of bipolar disorder over the last 20 years, with second-generation antipsychotics in large measure supplanting traditional mood stabilizers and persistence of antidepressant prescriptions despite a lack of evidence. Further work is needed to determine the comparative efficacy and tolerability of newer agents with respect to traditional mood stabilizers to understand the implications for these changes in national practice patterns on public health.

Keywords: Bipolar Disorder, Mood Stabilizers, Antipsychotic Agents

Disclosure: Janssen, Grant, Janssen, Consultant, Ovi Therapeutics, Consultant, Biohaven, Consultant

M92

Alterations in Inflammatory Metabolism Related Biomarkers in Adolescence as Early Biological Predictors of Altered Behaviours and Depression Vulnerability and Novel Targets for Prevention

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Background: Early life stress, especially when experienced during the prenatal period or childhood, affects the brain developmental trajectories leading to an enhanced vulnerability for stress-related psychiatric disorders later in life. Although both clinical and preclinical studies clearly support this association, the biological pathways altered by such exposure, and the effects of early life stress in shaping the neurodevelopmental trajectories, have so far been poorly investigated. Moreover, peripheral biomarkers associated with an enhanced risk are not available.

Methods: By using the prenatal stress (PNS) model, a well-established rat model of early life stress, we performed transcriptomic analyses in the prefrontal cortex of rats exposed or not to PNS and sacrificed at different postnatal days (PNDs 21, 40, 62). We first investigated the mechanisms and pathways affected by exposures to PNS that may contribute to the long-lasting vulnerability of developing altered behaviours in adulthood (at PND62). Moreover, by focusing on transcriptomic changes, we evaluated the effects of PNS in shaping brain trajectories with the aim to identify the most critical temporal window of vulnerability, when biological alterations are already present, but clear symptoms not manifested yet.

Results: In adult rats (PND 62), PNS modulates 389 genes which resulted to be involved mainly in the stress and inflammatory system response. Moreover, when we looked at temporal trajectories in term of gene expression, we found the most significant effects of PNS during adolescence (between PND40 versus 21) with an effect on pathways related to stress, inflammation and metabolism that was then maintained until adulthood. We are also investigating whether the effect caused by PNS on these neurodevelopmental trajectories can be restored by pharmacological treatment during adolescence, by preventing the onset of behavioural and molecular alterations later in life.

Moreover, we measured the C-reactive protein (CRP) in saliva samples of adolescents which have been characterized for childhood trauma events and also for clinical features and we

found higher levels of CRP in subjects exposed to childhood trauma and also showing emotional dysregulation, depressive traits and reduced cognitive performances.

Conclusions: Interestingly, our data suggest that molecules belonging to inflammation may serve as biomarkers of risk to allow the identification of adolescents that have been exposed to adversities and are at high risk to develop mental illness later in life, and that may benefit from early preventive interventions with novel pharmacological or non-pharmacological interventions able to target these biological systems.

Keywords: Early life Stress, Vulnerability, Inflammatory Markers, Prevention

Disclosure: Nothing to disclose.

M93

NV-5138 A Novel, Direct Activator of the Mechanistic Target of Rapamycin Complex 1 (mTORC1): A Phase 1b Randomized, Double-Blind, Placebo-Controlled Single Oral Dose Study in Subjects With Treatment-Resistant Depression (TRD)

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Background: NV-5138 is a novel small molecule that activates mTORC1, a central modulator of cellular metabolism, which is suppressed in the brain of subjects suffering from severe depression (Sengupta, S. et al., (2019) *Sci. Rep.* 9:4107). Preclinical data demonstrate that NV-5138 produces rapid upregulation of key synaptic proteins, synaptic remodeling in the prefrontal cortex and hippocampus, and sustained antidepressant behavioral responses (Kato, T. et al., (2019) *J. Clin. Invest.* 130:2542). In this report, we present topline data from a cohort of subjects diagnosed with treatment resistant depression (TRD) who received a single oral 2400 mg dose of NV-5138. The primary objective was an assessment of safety and tolerability; the secondary objective was a preliminary exploration of efficacy for hypothesis generation (ClinicalTrials.gov: NCT03606395).

Methods: Eligible subjects, whose TRD diagnosis was confirmed by a site-independent review process, were hospitalized for 7 days. Central raters evaluated potential subjects during a 4-day single-blind placebo lead-in period and excluded subjects whose total MADRS score was <21 or changed $\geq 25\%$ at any time from Day -4 to baseline (Day 1). At Day 1, eligible subjects were randomized 1:1 to either a single oral dose of NV-5138 (2400 mg) or placebo and were evaluated in-clinic for 3 additional days. Safety endpoints included the Brief Psychiatric Rating Scale, positive sub-scale (BRPS+) and Clinician-Administered Dissociative States Scale (CADSS). on the exploratory efficacy measures included the MADRS, as assessed by blinded central raters, site-based HAM-D-6 and CGI-S, and subject-rated IDS-SR30. All efficacy measures were assessed at baseline, 24, 48, and 72 hours post-dose. The HAM-D-6 was also administered at 2, 4, 8, 12, and 36 hours post-dose. No formal power calculations were conducted to inform the sample size. The sample size (N = 32) was considered sufficient for exploratory purposes.

Results: 93 subjects were screened, 61 screen-failed, including 23 subjects who were excluded during the single-blind placebo lead-in period. Of the 32 randomized subjects, 31 were a-priori included in the efficacy sample. Subjects were predominantly male (62.5%). Average age was 52.5 years. The mean total MADRS score at baseline was lower in the NV-5138 group than the placebo group (34.7 vs 36.9, respectively). Eleven subjects in the NV-5138 group reported treatment-emergent adverse events

(AEs) compared to 6 subjects in the placebo group. AEs reported in ≥ 2 subjects included somnolence (4 NV-5138 vs 2 placebo), headache (2 NV-5128 vs 1 placebo), and dizziness (2 NV-5138 vs 0 placebo). All AEs were mild or moderate. There were no dissociative effects reported or observed on the BPRS + or CADSS scales, and no severe or serious AEs that led to early discontinuation. Change from baseline effect sizes (ES) on the total MADRS score, IDS-SR 30, and CGI-S at 24 hours post-dose were 0.1, 0.2 and -0.1, respectively. The HAM-D6 showed rapid and clinically meaningful benefits on the core symptoms of depression as early as 2 hours post-dose (ES 0.6; $p = 0.068$). Greater benefits were observed through 12 hours post-dose (ES 0.8; $p = 0.020$). Treatment effects persisted from 24 hours (ES 0.4; $p = 0.195$) through the 72-hour observation period (ES 0.5; $p = 0.167$). Post-hoc analyses of the MADRS-6 and MADRS-8 scores supported the observed treatment benefits on core symptoms of depression (ES 0.3 for both at 24 and 48 hours). Responder analyses were consistent with these observations. Five (31.3%) NV-5138-assigned subjects versus only 1 (6.7%) placebo-assigned subject had a $\geq 50\%$ response on the HAM-D-6 at 24 hours post-dose. In post-hoc analyses, younger subjects (≤ 55 years) and subjects who were more severely depressed at baseline (MADRS ≥ 36) showed the greatest improvement of their depressive symptoms across all scales, including the MADRS total score (ES 0.5 and 0.4, respectively, at 24 hours post-dose).

Conclusions: In this exploratory double-blind, placebo-controlled assessment, NV-5138 administered as a single oral 2400 mg dose was safe and generally well tolerated without clinical signs of dissociative effects. NV-5138 also showed clinically meaningful signals of rapid and sustained efficacy on the core symptoms of depression in subjects with TRD. These data support conducting multiple dose studies of NV-5138 in subjects with TRD.

Keywords: Treatment Resistant Depression, Selective mTORC1 Activation, Novel Antidepressant, Rapid-acting Antidepressant

Disclosure: Navitor Pharmaceuticals, Employee

M94

Esketamine Nasal Spray for Rapid Reduction of Symptoms of Major Depressive Disorder in Adult Patients at Imminent Risk for Suicide: A Post-Hoc Analysis of North American Subjects

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Background: ASPIRE-1 and ASPIRE-2 are the two global phase-3 studies in the registration program to evaluate efficacy and safety of esketamine nasal spray (ESK) vs placebo (PBO) nasal spray, given in the context of a comprehensive standard-of-care (SoC), in patients with major depressive disorder (MDD) at imminent risk for suicide. This post-hoc analysis of the combined data from these two identically-designed studies, focuses on results from North American subjects.

Methods: This is a post-hoc analysis of two double-blind, placebo-controlled studies (NCT03039192, NCT03097133), that enrolled patients (aged 18-64 years) with moderate to severe MDD (DSM-5 criteria) who had active suicidal ideation with intent and required psychiatric hospitalization. Patients were randomized (1:1) to ESK 84-mg or PBO nasal spray twice-weekly along with comprehensive SoC antidepressant treatment for 4 weeks. Comprehensive SoC included inpatient hospitalization for a recommended minimum of 5 days, initiation or optimization of oral antidepressant treatment and twice weekly clinic visits. Change in the Montgomery-Åsberg Depression Rating Scale (MADRS) total

score from baseline to 24-hours post first dose score was evaluated as the primary endpoint. Treatment differences were also examined at other time points. Treatment differences in MADRS total score were examined using the ANCOVA model. Baseline disease and demographic characteristics of North American subjects were compared to those of subjects from the rest of world (ROW). Regional differences were assessed using ANOVA models or the Cochran-Mantel-Haenszel (CMH) test without multiplicity adjustment.

Results: 122 randomized North American subjects with MDD and active suicidal ideation with intent received either ESK + SoC ($n = 65$) or PBO + SoC ($n = 55$); 121 (99%) were from U.S.-based clinical trial sites and 94 (77%) completed the double-blind phase. The clinical trial population from ROW encompassed a total of randomized 328 subjects from 19 countries from Europe, Asia and South America, 268 (82%) of whom completed the double-blind phase. With respect to subject demographics data, North American subjects were younger (mean age in years (SD) 36.6 (13.54) vs 41.4(12.56), $p = 0.0004$) and had a larger average body mass index (BMI) than patients from ROW (mean kg/m² (SD) 28.7 (7.57) vs 26.7(6.53), $p = 0.0070$). At baseline, mean MADRS total scores of North American subjects were similar to those of subjects from ROW (40.6 vs 40.3, respectively). A greater proportion of North American subjects experienced ≥ 6 episodes of MDD over the course of their lifetime (12.5% vs 30.9%) and similarly a greater proportion had a longer duration of their current episode (mean months (SD): 59.7(75.97) vs. 34.6 (59.14), $p < 0.001$). Compared to the ROW, a greater proportion of North American subjects reported thinking about suicide "very often" (50.4% vs 36.0%) and a greater proportion reported "severe" intensity of suicidal thoughts (61.8% vs 48.2%) at screening. North American patients receiving ESK + SoC showed significant improvement in MADRS total score vs PBO + SoC at 24-hours after first dose -21.3 vs -14.8; LS-mean [SE] difference: -6.9 (95% CI -10.61; -3.18) and at Day 25 (-26.7 vs -21.8; LS-mean [SE] difference: -5.9 (95% CI -9.91; -1.85).

Conclusions: North American subjects, enrolled in these clinical trials, appear to have some disease characteristics suggestive of a more chronic illness and greater frequency and intensity on some measures of suicidal thinking at screening than subjects from the ROW. ESK + SoC treatment was efficacious in rapidly reducing depressive symptoms in patients with MDD with active suicidal ideation and intent in a North American-based population. Further characterization and treatment outcomes of this patient population will be explored and reported.

Keywords: Esketamine, Major Depressive Disorder (MDD), Clinical Trial

Disclosure: Johnson and Johnson, Employee, Johnson and Johnson, Stock / Equity

M95

Parsing Neurocognitive Heterogeneity in Bipolar Disorder – Clinical Implications and Immune Biomarkers

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Background: Patients with bipolar disorder (BD) were once thought to achieve complete inter-episode recovery, particularly with regard to cognitive dysfunction. More recent data suggest persistent, trait-like cognitive impairments in many BD patients, even during periods of affective remission that contribute directly to functional disability.

At the group level, the severity of these deficits is $\frac{1}{2}$ to 1 full standard deviation below average; however, substantial cognitive heterogeneity exists. Using empirical approaches to classify individuals along cognitive dimensions into more homogenous subgroups, we previously reported three resultant cognitive subgroups with differential profiles that are relatively equally distributed as 1) cognitively-intact; 2) selectively-impaired; and 3) globally-impaired. These subgroups do not simply recapitulate existing clinical subtypes (e.g. BD I vs II; psychotic subtype) but represent a novel classification¹. This work has been replicated in several independent samples and the subgroups are predictive of level of community functioning, validating this approach.

Methods: A new, independent cohort of 285 affectively-stable men and women with BD were recruited and characterized using standard diagnostic, clinical, cognitive, and functional measures. As before, we used hierarchical clustering followed by a K-means algorithm to classify patients into cognitive subgroups and compared the derived subgroups on several relevant early life factors, illness features, and biomarkers of inflammation, using analysis of variance (ANOVA). Multivariate models were then employed to identify joint and independent predictors of cognitive subgroup and to determine their role in everyday functioning.

Results: We find the best fit clustering model is a three-group solution, where one group is cognitively intact, another is mild-moderately impaired, and a third is severely-impaired. Premorbid IQ estimates (global = 91.4 ± 15.3 ; selective = 103.4 ± 12.9 ; and intact = 111.3 ± 8.6 ; $F=43.3$; $p<.001$) differ for all pairwise comparisons and education levels are significantly lower in the globally impaired group relative to the other two subgroups ($F=7.5$; $p=.001$). The globally-impaired BD patients report more severe physical abuse ($F=5.0$; $p<.01$) and physical neglect ($F=8.4$; $p<.001$) during childhood vs. the other subgroups. The cognitively-intact group also has fewer prior hospitalizations for mania ($F=2.95$; $p<.05$), reports better sleep quality ($F=3.41$ $p=.04$), has lower lifetime rates of comorbid substance use disorders ($\chi^2=5.89$; $p<.05$), and uses more adaptive coping strategies than the other two subgroups. Biomarkers indicate that the globally-impaired subgroup shows evidence of chronic inflammation, that is not seen in the other two subgroups ($p<.002$).

Conclusions: These results suggest that both early life risk factors and illness-associated risk factors contribute to differential cognitive outcomes, supporting a role for both neurodevelopment and neuroprogression in BD. It further appears that there are identifiable predictors for poor cognitive outcome, which are modifiable and should be targeted for treatment. The interpretation of our data is limited by the cross-sectional nature of the study; longitudinal studies will be needed to address causality and cognitive change over time.

Keywords: Cognition, Bipolar Disorder, Immune Biomarkers

Disclosure: Sumitomo Dainippon Pharma, Advisory Board

M96

Translating a Non-Human Primate Behavioral Paradigm to Probe Psychopathology in Youth: A Cross-Species Study of Threat-Avoidance

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Background: Irritability and anxiety are common psychiatric symptoms in youth. Data-based developmental models posit

aberrant responses to threat as a core deficit; however, little is known on how these phenotypes are uniquely and commonly linked to threat-related approach vs. avoid responses. Non-human primates (NHPs) provide an essential translational model for understanding psychopathology, due to their similarities with humans in brain structure and function, as well as in their socio-emotional behaviors. Parallel work in humans and NHPs can inform the investigation of approach-avoidance behaviors and corresponding pathophysiology.

Previous work with NHPs developed the human intruder paradigm (HIP), which has characterized context-specific, adaptive and maladaptive threat related responses. The HIP consists of three conditions: alone (A), no eye contact (NEC) and stare (ST); the direct threat presented in the ST condition tends to elicit threat-related behavioral activation (e.g., aggression), whereas the more uncertain threat of the NEC condition tends to elicit behavioral inhibition (e.g., freezing, reduced vocalizations).

The goal of the current study was to translate the HIP to humans so that it can be used to assess responses to direct vs. uncertain threat in youth with varying degrees of psychopathology. The primary aim was to establish and validate a paradigm in youth that parallels the well-established HIP. The second aim of this study was to understand responses to this paradigm in relation to individual differences in irritability and anxiety, focusing specifically on approach-avoidance responses. Here, data is summarized to demonstrate proof of concept and feasibility of developing and using a translationally relevant task in youth.

Methods: We enrolled a transdiagnostic sample of youth with varying levels of irritability and anxiety ($N=33$, 60% females), age 8-18. In 120 trials participants are presented with face stimuli that increases in size over the course of a 3-seconds trial, as if approaching the participant. Faces are portrayed with either angry or calm emotion type and with either direct or averted eye contact (i.e., 2 within-subject conditions). During each trial, participants can choose to squeeze grip force devices to "push the face image away". When squeezed, the face retreats.

Variables of interest are: 1) Eye gaze measures of dwell time and mean of fixation number towards areas of interest (AOI) (e.g., eye region), extracted from eye-tracking data recorded using EyeLink 1000; 2) Reaction time (RT) for behavioral response; and 3) Hand grip force used to push the face image. Self-report questionnaires, completed by child and parent, measure symptoms of irritability (Affective Reactivity Index; ARI) and anxiety (Self-Report for Childhood Anxiety Related Disorders; SCARED) are collected.

Results: Preliminary results demonstrate AOI effect ($F(4,28)=8.08$, $p<.01$) showing participants spent more time gazing on the eye region and on peripheral areas of the face relative to all other AOIs (e.g., mouth, nose, background). Controlling for mean RT and age, repeated measures ANOVA revealed significant main effects for eye contact ($F(1,28)=23.01$, $p<0.01$) and emotion type ($F(1,28)=6.80$, $p=0.02$), presenting increased fixation number in the direct condition compared to the averted condition, and for angry condition compared to the calm condition. In relation to dwell time, a significant eye-contact by emotion interaction was found ($F(1,28)=6.45$, $p=.02$) with longer dwell time specifically in the direct angry condition compared to all other conditions.

Preliminary correlation analyses showed that both, dwell time and mean fixation number, were negatively associated with anxiety ($r=-.46$, $p=.02$; $r=-.42$, $p=.04$; respectively) and irritability ($r=-.50$, $p=.01$; $r=-.38$, $p=.06$, respectively) scores as reported by the parents.

RT analysis yielded a significant effect for eye contact indicating that participants squeezed the grip force device faster in the direct condition compared to the averted condition ($F(1,28)=5.22$, $p=.03$). Finally, a trend was found for the effect of emotion type on maximum grip force participants used to push the face image. Specifically, participants squeezed the grip force device harder

when presented with an angry face compared to a calm face ($F(1,28) = 3.40, p = .07$).

Conclusions: Preliminary findings indicate that the different conditions incorporated into this paradigm evoke different responses, supporting the early validity of this task. Overall, these results confirm that direct vs. indirect eye contact elicits differential responses in irritable and anxious youths, which is in line with the behavioral effects reported in NHPs. There are no phenotypic unique effects; however, this is a small sample. Data collection is ongoing and future analyses are planned to understand the dimensional nature of the reported associations. Together these results support the future feasibility of this paradigm as a tool for understanding the pathophysiology of extreme irritability and anxiety in youths. This study highlights the importance of cross-species translational research that links dimensional traits in disordered youths to behavioral responses in NHPs, providing the unique ability to explore causal interventions that elucidate the neural mechanisms underlying pathological irritability and anxiety.

Keywords: Cross-Species Translation, Approach/Avoidance, Irritability, Anxiety, Youth

Disclosure: Nothing to disclose.

M97

Combined Estradiol and Progesterone Exposure and Subsequent Withdrawal Induce Differential Cellular Responses in Women With Postpartum Depression

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Background: Postpartum depression (PPD) affects approximately 1 in 9 women and is one of the leading causes of maternal death. Given the burden of this illness, there is immense value in defining molecular markers that may confer PPD risk, symptomology, and heritability in women. Previously, we demonstrated that asymptomatic, ovarian-suppressed women with a history of PPD develop depressive symptoms during withdrawal from, and occasionally during addback of, supraphysiologic levels of estradiol and progesterone (E2 + P4), whereas women without a history of PPD showed no changes in mood despite participating in an identical hormone manipulation protocol (Bloch et al., 2000). This differential behavioral sensitivity to steroid hormone manipulation may be reflected at a cellular level, a possibility supported by the observation of several alterations in DNA methylation patterns in women with PPD (Kaminsky and Payne, 2014; Mehta et al., 2014).

Methods: To investigate possible differences in transcriptional cellular response to changes in E2 and P4 during pregnancy, we derived lymphoblastoid cell lines (LCLs) from blood samples of women with a past PPD ($n = 9$), and matched asymptomatic control women (AC) ($n = 9$), with no history of PPD or other Axis 1 psychiatric illness. These women had all participated in a hormone-manipulation protocol similar to the one employed by Bloch et al. to define their ovarian steroid-behavioral sensitivity outside of obstetrical, medical, or social issues that could also be sources of depression. These cell lines were then treated with E2 + P4 (300nM each) in three different experimental conditions: 1) 96hrs of vehicle-treated steroid-free media, to establish a baseline; 2) 96hrs of E2 + P4-treated media, to examine the effects of high levels (addback) of ovarian steroids; and 3) 72hrs E2 + P4-treated media that was then changed to vehicle-treated media for 24hrs,

to mimic parturition-induced steroid withdrawal on a cellular level. Following polyA selection of the mRNA, cDNA libraries were established and then analyzed for changes in transcriptional response in all three experimental conditions, using whole-transcriptome RNA sequencing. Quality control measures, unsupervised clustering analyses, and EDGE-R analysis of differential gene expression were all performed using an RNA-seq pipeline in R.

Results: We detected significant transcription expression changes between women with PPD and AC in all three treatment conditions. In particular, we saw a profound difference in transcriptional response in the addback (high E2 + P4 condition) phase, where over 850 genes were differentially expressed (FDR corrected $p < 0.05$) between AC and PPD. In this treatment group, the top hit was the gene IMPACT, which is a translational regulator that ensures constant elevated levels of translation under a variety of stress conditions. This gene was significantly decreased in cases [$p\text{FDRCorr} = 2.70 \times 10^{-5}$, $\log_2(\text{Fold Change}) = -2.03$]. Further, while the magnitude of this significant decrease in IMPACT was most extreme in the addback phase, the difference was also significant at baseline [$p\text{FDRCorr} = 0.0012$, $\log_2(\text{Fold Change}) = -1.73$], and trended toward significance during E2 + P4 Withdrawal [$p\text{FDRCorr} = 0.101$, $\log_2(\text{Fold Change}) = -1.33$].

Conclusions: Our results support the hypothesis that depression during pregnancy and postpartum may be linked to a differential sensitivity to ovarian steroids. Our finding that IMPACT is significantly decreased in women with PPD may suggest a dysregulated ability to translate transcript to protein in these women when they are exposed to supraphysiologic ovarian steroids. Given this preliminary finding, studies are currently underway to test the hypothesis that normal cellular homeostasis is disrupted under the stress of pregnancy or the hormonal changes of the puerperium specifically in women with PPD. Finally, the surprising finding that the most extreme transcriptional differences were during the addback, not the withdrawal, portion of the study are in line with recent work that suggests PPD may begin before parturition. Further studies are currently underway to replicate our findings, as well as identify pathways and networks that would be impacted by an altered ability to regulate transcriptional response. Future studies will also investigate the transcriptomic response of these LCLs to allopregnanolone, a neurosteroid that is currently being administered as Brexanolone as a treatment for PPD.

Keywords: Postpartum Depression, Estradiol, Progesterone, Ovarian Hormones, Bench-to-Bedside

Disclosure: Nothing to disclose.

M98

Open Board

M99

Efficacy of Cariprazine on Cognitive Symptoms in Patients With Bipolar Depression

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Background: Cognitive impairment in bipolar disorder is associated with psychosocial and workplace impairment, a worse course of illness, and functional disability. Cariprazine, a dopamine

D3-preferring D3/D2 receptor and serotonin 5-HT_{1A} receptor partial agonist, demonstrated efficacy versus placebo (PBO) for improving depressive symptoms in 3 phase II/III randomized, double-blind, PBO-controlled studies in patients with bipolar I depression. These post hoc analyses investigated the efficacy of cariprazine on cognitive symptoms in patients from these studies.

Methods: Data from 3 clinical trials in patients with bipolar I disorder and a current major depressive episode were pooled. Efficacy outcomes were assessed for the cariprazine 1.5 mg/d, 3 mg/d, and 1.5-3 mg/d groups compared with placebo. Depressive symptoms were assessed by change from baseline to Week 6 in Montgomery-Åsberg Depression Rating Scale (MADRS) total score. Cognitive symptoms were evaluated using the MADRS concentration item and the Functioning Assessment Short Test (FAST) cognitive functioning subscale (administered in one study only at Week 8). In addition, overall functioning was assessed using the FAST total score in this study. Efficacy was evaluated in the overall intent-to-treat (ITT) populations as well as in subgroups of patients with cognitive impairment, defined as baseline MADRS concentration item score ≥ 3 (mild or worse) or ≥ 4 (moderate or worse) for MADRS outcomes, and as baseline FAST cognitive subscale score of ≥ 2 on 2 or more items for FAST outcomes. All outcomes were assessed using a mixed-effects model for repeated measures.

Results: A total of 1222/1383 patients (88%) had a baseline MADRS concentration item score ≥ 3 ; 913/1383 (66%) had a baseline MADRS concentration item score ≥ 4 . Of the 388 patients in the ITT population of the study that administered the FAST, 294 (76%) had FAST-defined cognitive impairment at baseline. At Week 6, mean reductions in MADRS concentration item scores were significantly greater in the cariprazine treatment groups compared with placebo in the overall ITT population (placebo [-1.2] versus 1.5 mg [-1.6], 3 mg [-1.4], and 1.5-3 mg [-1.5]; $P < .05$ all) as well as in patients who were cognitively impaired at baseline (baseline score ≥ 3 : placebo [-1.3] versus 1.5 mg [-1.8], 3 mg [-1.5] and 1.5-3 mg [-1.7]; baseline score ≥ 4 : placebo [-1.5] versus 1.5 mg [-1.9], 3 mg [-1.7], and 1.5-3 mg [-1.8]; $P < .05$ all). Mean change from baseline to Week 6 in MADRS total score was also significantly greater for all cariprazine treatment groups compared with placebo in patients with baseline cognitive impairment (baseline concentration score ≥ 3 : placebo [-12.0], 1.5 mg [-15.1], 3 mg [-14.7], and 1.5-3 mg [-14.9]; baseline concentration score ≥ 4 : placebo [-12.3], 1.5 mg [-15.6], 3 mg [-15.2], and 1.5-3 mg [-15.4]; $P < .001$ all). Mean changes from baseline to Week 8 in FAST cognitive functioning subscale scores were significantly greater in the cariprazine 1.5 mg/d and cariprazine 1.5-3 mg/d group compared with placebo in both the ITT population (placebo [-2.4], 1.5 mg [-3.6; $P < .01$], 3 mg [-2.9; $P = 0.23$], and 1.5-3 mg [-3.3; $P < .05$]) and in patients with FAST-defined cognitive impairment (placebo [-3.0], 1.5 mg [-4.4; $P < .01$], 3 mg [-3.5; $P = 0.27$], and 1.5-3 mg [-4.0; $P < .05$]). Mean changes from baseline in FAST total score were also significantly greater for cariprazine 1.5 and cariprazine overall in the ITT population (placebo [-9.8], 1.5 mg [-15.1; $P < .01$], 3 mg [-13.0; $P = 0.055$], and 1.5-3 mg [-14.1; $P < .01$]) and in patients with cognitive impairment (placebo [-11.1], 1.5 mg [-17.8; $P < .001$], 3 mg [-14.6; $P = 0.09$], and 1.5-3 mg [-16.3; $P < .01$]).

Conclusions: In patients with bipolar depression, cariprazine demonstrated efficacy versus placebo in improving concentration and depressive symptoms in the overall population as well as in patients with baseline cognitive impairment. Additionally, cariprazine demonstrated efficacy on measures of overall and cognitive functioning, as measured by the FAST, in patients with cognitive impairment at baseline. These results collectively suggest that cariprazine may be beneficial for patients with bipolar depression and cognitive impairment.

Keywords: Cariprazine, Bipolar I Depression, Cognition

Disclosure: Allergan, Employee

M100

A Phase 2a Randomized, Double-Blind, Placebo-Controlled Study Investigating the Efficacy and Safety of Adjunct Treatment With the FAAH Inhibitor JNJ-42165279 in Subjects With Major Depressive Disorder With Anxious Distress

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

Methods: This was a multi-center, double-blind, placebo-controlled, randomized, parallel-group study in subjects with MDD with anxious distress who have had an inadequate response to SSRI/SNRI treatment. The study was conducted in Spain, United Kingdom, Moldova, Russia, Ukraine and the US. Subjects who met the inclusion and exclusion criteria and were then enrolled and were maintained on their SSRI/SNRI treatment throughout the study to determine whether adjunctive treatment with JNJ-42165279 reduced symptoms of MDD with anxious distress.

The study consisted of a screening phase of up to 4 weeks, a treatment phase of 11 weeks, and a 3-week post-treatment phase. The treatment phase of the trial consisted of 3 periods. The first period was a placebo (PBO) lead-in of variable duration (1 to 3 weeks), after which subjects were randomly assigned to receive JNJ-42165279 or to continue on placebo for 6 weeks. Subjects who completed the double-blind treatment period entered the PBO withdrawal period of variable duration (2-4 weeks, depending on the duration of the placebo lead-in) for the remaining time of the treatment phase of the study. Investigators and subjects were blind to the duration of the lead-in and withdrawal period, to the actual start of the 6-week double-blind treatment period, and to the PBO response criteria.

At the end of the PBO lead-in, response status of the subjects was based on reduction in the 17 item Hamilton Depression Rating Scale (HDRS17) relative to lead-in baseline. Both lead-in PBO responders and lead-in PBO non-responders were randomly assigned in a 1:1 ratio to receive either 25 mg JNJ-42165279 or PBO in the treatment period. The primary endpoint was the change from baseline on the HDRS17 over the 6-week treatment period in subjects who did not respond to placebo during the lead-in period. Secondary endpoints included the Hamilton anxiety scale (SIGH-A), subscales of the HDRS17 and SIGH-A, Clinical Global Impression (CGI) scale, and Medical Outcomes Scale (MOS)-Sleep. Blood samples were taken for plasma JNJ 42165279 and AEA concentrations.

Results: A total of 153 subjects were randomized, 76 to PBO and 77 to JNJ-42165279. Average age was 43.2 years, 73% were female, and median duration of antidepressant treatment prior to enrollment was approximately 12 weeks. Approximately 30% of subjects enrolled in the study ($n = 153$, 75 PBO, 76 JNJ-42165279) were identified as significantly improving on the HDRS17 from Visit 1 over the PBO lead-in, leaving 99 subjects (49 PBO, 50 JNJ-42165279) for the primary (enriched) analysis set.

Compared to PBO, treatment with JNJ-42165279 did not result in a significant change from baseline in HDRS17 total score at Week 6 of the double-blind treatment period, in the 'enriched' (PBO non-responders) intention to treat (ITT) set. Efficacy results based on secondary endpoints (HDRS17 improvement rates, HAM-D6 score, SIGH-A total score, SIGH-A improvement rates and CGI, and the MOS-Sleep) were consistent with the findings from the primary endpoint analysis and did not favor JNJ-42165279 over PBO.

The drug was well tolerated, and no notable neurological adverse events of interest or findings occurred in either treatment group. No deaths occurred and most adverse events were mild to moderate in severity. During the double-blind treatment period, 1 subject in the JNJ-42165279 group had a serious adverse event (SAE): elective hospitalization for treatment of foot pain, and 1 subject in the placebo group with acute hospital treatment for gastroenteritis. Both SAEs were reported as not related to study treatment. Mean changes from baseline in hematology, serum chemistry, and urinalysis parameters were minimal, and were similarly distributed in the PBO and JNJ-42165279 treatment groups, with none considered clinically relevant.

Post-hoc analysis of PK and biomarkers from the study indicates a high correlation between trough drug concentrations and plasma AEA levels.

Conclusions: The trial design did appear to function as intended in identifying subjects who have an early response to placebo. These subjects may have had a strong expectation bias on the value of an experimental treatment, were responding to their standard treatments in the context of frequent clinic visits, or were recovering from their MDD episode. We did not identify significant therapeutic effects of 25 mg JNJ-42165279 per day for 6 weeks as an adjunct treatment for MDD with anxious distress. The dose was chosen using inhibition of FAAH in WBCs, occupancy of FAAH in the brain (as measured by PET) and plasma and CSF measures of AEA turnover in Phase 1 studies.

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

Keywords: FAAH Inhibitor, Anxious Depression, Endocannabinoid System, Anandamide, Clinical Trial

Disclosure: Janssen, Employee, Stock / Equity

M101

Identification of an Affective Valance Switch in the Rat Medial Prefrontal Cortex

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Background: Rat 50-kHz and 20-kHz ultrasonic vocalizations (USVs) have been shown to reflect positive and negative affective states, respectively. The medial prefrontal cortex has a causal role in the generation of affective vocalizations in both rats and primates, and this region controls emotional expression in humans.

Methods: EEG recordings were made from depth electrodes in the prelimbic cortex of male rats as well as from skull screws over the prefrontal cortex during the generation of hedonic and aversive USVs. Positive affect was induced by tickling and spontaneous positive affect was measured during the lights-off to lights-on transition during 24 hr homecage recordings. Negative affect was induced by tickling and by 7 hrs of sleep deprivation induced by continuous handling. Electrical stimulation of the prelimbic cortex

was also utilized to induce USVs in which USVs, field potentials, and self-stimulation rates were evaluated as the dependent measures.

Results: Positive affect increased theta-alpha (4-8 Hz) EEG power whereas negative affect increased delta (1.5-3 Hz) EEG power, as measured from both depth and surface prefrontal cortex recordings. Electrical stimulation of the medial prefrontal cortex using theta bursts stimulation (6 trains per second, of five 100 Hz pulses per train) robustly induced hedonic 50-kHz USVs (~100 fold over baseline) and increased rates of self-stimulation. In contrast, delta train stimulation (2 trains per second, of fifteen 100 Hz pulses per train) induced aversive calls (~50 fold over baseline), decreased hedonic calls, and reduced rates of self-stimulation. High-frequency stimulation (HFS; 100 Hz) was able to induce a long-lasting potentiation (1 hr post-HFS) of both hedonic calls as well as excitatory post synaptic potentials (~1.5 fold over baseline for both measures) in the medial prefrontal cortex whereas low-frequency stimulation (2 Hz) depotentiated this response to baseline levels for both measures.

Conclusions: These studies show that positive affect is induced by theta/alpha power whereas negative affect is induced by delta power in the medial prefrontal cortex. The switch from positive to negative affect is likely controlled by an LTP / LTD synaptic plasticity-like mechanism. These findings provide a novel in vivo electrophysiological measure of affect that should be relevant for assessing both vulnerability and treatment responsivity in affective disorders. In addition, the preclinical theta / delta burst stimulation protocol reported here should inform clinical transcranial magnetic stimulation and deep brain stimulation protocols for the treatment of affective disorders.

Keywords: Emotion, EEG, Deep Brain Stimulation, Long Term Potentiation

Disclosure: Aptinyx Inc., Employee

M102

Neural Responses to Social and Monetary Incentives in Healthy Participants

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Background: Abnormal responses to reward and loss are central to psychiatric disorders including major depression and substance use disorders. Most studies examine responses to monetary incentives, however responses to social incentives (e.g., social rejection, social acceptance) are clinically relevant and may be represented differently in the brain. The goal of this study was to map neural responses to social and monetary incentives in a sample of healthy participants.

Methods: Participants (n = 59) were physically and mentally healthy young adults (31 women, 28 men, mean age $\pm$ s.d., 21.6 ± 2.1 years), as determined by a physical exam and the Structured Clinical Interview for DSM-IV. Exclusion criteria included any DSM-IV disorder, actively abusing substances including alcohol. Participants performed the Social Feedback Task (SFT) and the Monetary Feedback Task (MFT) during fMRI. The SFT and MFT were designed to examine unique effects of social vs. monetary incentives and were performed sequentially in the same scan session (order was counterbalanced between participants). The SFT examined responses to social acceptance and rejection (each vs. a neutral social condition), whereas the MFT examined response to monetary wins and losses (each vs. a neutral monetary condition).

Results: Whole-brain corrected, voxel-wise analysis (threshold: $P < 0.05$, $k > 5$) revealed several clusters during rejection (vs. neutral) including the right and left ventrolateral prefrontal cortex (vlPFC, BA47), right anterior insula, supplementary motor area (BA6), and prefrontal BA9 (P 's < 0.01). Acceptance (vs. neutral) revealed two clusters in the medial prefrontal cortex (BA8/9/10) (P 's < 0.05). Monetary loss (vs. neutral) revealed a single cluster in the supplementary motor area (BA6) ($P < 0.0001$), and monetary win (vs. neutral) revealed a single cluster in the dorsal midline thalamus ($P < 0.01$).

Conclusions: Common activation of the BA6 supplementary motor area during rejection and monetary loss may represent top-down regulation of negative affect across monetary and social domains. Specific activation of the vlPFC and anterior insula during rejection may be associated with evaluating others' intentions, consistent with prior work. Social acceptance activated prefrontal areas that are associated with monitoring reward values. In contrast, monetary wins revealed a single cluster in the dorsal midline thalamus, consistent in location to the paraventricular thalamic nucleus (PVT), involved in reward-seeking behavior in animal models. These results indicate common and distinct neural responses to monetary and social incentives in healthy participants. Future studies may evaluate abnormal function of these areas in disorders characterized by sensitivity to the social environment including major depressive, social anxiety, and borderline personality disorders.

Keywords: Social Rejection, Social Reward, Social Brain, Depression, Monetary Reward

Disclosure: Nothing to disclose.

M103

Efficacy and Safety of Vortioxetine (5, 10, and 20 mg) in Relapse Prevention: Results of a Randomized, Double-Blind, Placebo-Controlled, Phase 4 Study in Adults With Major Depressive Disorder (MDD)

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Background: Treatment leading to full remission and maintenance of efficacy are key priorities in the management of recurrent major depressive disorder (MDD). An ex-US relapse prevention study previously demonstrated the long-term efficacy of vortioxetine (VOR) at 5 and 10 mg, but included neither US patients nor the highest recommended dose of 20 mg, which accounts for 45% of US prescriptions. Furthermore, the optimal dose for maintenance, after response and stabilization to acute treatment with VOR, has not been formally evaluated.

Methods: This was a randomized withdrawal design study, which enrolled over 1100 US patients with recurrent MDD with a currently relapsed major depressive episode (Montgomery-Åsberg Depression Rating Scale [MADRS total score] ≥ 26). All patients were initially treated with a 10-mg dose of VOR in the open-label phase for 16 weeks. Those who responded and were stabilized ($\geq 50\%$ reduction from baseline in MADRS total scores at week 8, followed by a MADRS total score ≤ 12 at weeks 14 and 16) were eligible for entry into the 32-week double-blind (DB) treatment period. Eligible participants were randomized to one of three fixed doses of VOR (5, 10, or 20 mg) or placebo. The primary endpoint was time from randomization to relapse (defined as MADRS ≥ 22 or lack of efficacy or other unsatisfactory treatment response) during the first 28 weeks of the DB period. Secondary endpoints included change

from DB baseline in MADRS; Clinical Global Impressions Scale-Severity (CGI-S); CGI-Improvement (CGI-I); time from randomization to relapse during the entire 32-week DB period; and safety assessments (adverse events [AEs], laboratory values, and vital signs). Target enrollment of about 1100 subjects into the open-label period was planned in order to achieve randomization of 600 subjects (150 per group) into the DB period with 85% power at a 5% significance level. A Cox proportional hazards model, with treatment as a factor and baseline MADRS total score as a covariate, was used to analyze the primary endpoint.

Results: Of 1106 study participants enrolled in the open-label period, 580 were randomized to the DB period (placebo, $n = 151$; VOR 5 mg, $n = 140$; VOR 10 mg, $n = 145$; and VOR 20 mg, $n = 144$). Patients in all treatment groups were similar in terms of demographics and disease severity at the start of the DB treatment phase. For each dose of VOR, the relapse rate at week 28 was significantly lower than with placebo, with VOR 5, 10, and 20 mg at 19.3%, 17.9%, and 17.4% vs placebo at 32.5% (with P values of 0.006, 0.002, and 0.003, respectively). Cox regression analyses for the first 28 weeks of the DB period demonstrated an overall risk reduction of 48%–52% and a longer time to relapse of MDD for all three doses of VOR compared to placebo as follows: VOR 5 mg, with a hazard ratio (HR) = 0.517 (95% confidence interval [CI]: 0.323, 0.828); VOR 10 mg, HR = 0.476 (95% CI: 0.296, 0.767); VOR 20 mg, HR = 0.482 (95% CI: 0.297, 0.781). For the secondary endpoints of changes from DB baseline in MADRS and CGI-S scores throughout the 32-week DB period, all doses of VOR compared favorably to placebo, with statistical significance reached for the majority of the time points assessed. The Kaplan-Meier plot showed clear separation between the curves for the three VOR-treated arms and placebo, indicating a statistically significant longer time to relapse for each VOR dose compared to placebo ($P < 0.05$) over the 32-week DB period. Finally, most AEs (across all four treatment groups during the DB period) were of mild to moderate severity, and the most frequently reported events were upper respiratory infections (5.7%), nasopharyngitis (4.5%), nausea (4.1%), weight increase (4.0%), and back pain (2.1%). Of note, during the open-label period, nausea was reported at a frequency of 26.4% whereas during the DB period, rates were considerably lower for each treatment arm: 2.9% for VOR 5 mg, 3.4% for 10 mg, and 9.0% for 20 mg.

Conclusions: VOR demonstrated robust maintenance efficacy in the US study population across the entire approved dose range (5–20 mg) in patients who initially responded and were in remission on the 10-mg dose. VOR was well tolerated with a safety profile consistent with previously reported data.

Keywords: Vortioxetine, Relapse-Prevention, Antidepressant, Major Depressive Disorder (MDD), Major Depressive Episode

Disclosure: Acadia, Inc., Akili, Inc., Allergan, Inc., H. Lundbeck, A/S, Johnson & Johnson (Janssen), Otsuka Pharmaceutical Company, Ltd., Advisory Board; Acadia, Inc., Allergan, Inc., Axome Therapeutics, Inc., Intracellular, Inc., Johnson & Johnson (Janssen), Otsuka Pharmaceuticals, Inc., Patient-Centered Outcomes Research Institute (PCORI), Takeda, Grant; American Psychiatric Foundation, Guilford Publications, Herald House, W.W. Norton & Company, Inc., Royalties; Peloton Advantage, Employee (Spouse)

M104

Gene X Environment Interactions That Mediate Vulnerability to Early Life Stress

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Background: Early life adversity is a significant predictor of psychiatric illnesses, including major depression and anxiety disorders. However, not every individual responds to stressful experiences in the same way. Understanding the neurobiological mechanisms that mediate individual differences in susceptibility and resilience to early life stress may therefore have great potential to reveal new strategies to treat or prevent the development of psychiatric disorders that have their origin early in life. One potential source of differential stress vulnerability and risk for psychiatric disorders is genetic variation that impacts neurobiological function. Genetic studies in humans have indicated that a single-nucleotide polymorphism in the 5-HT1A gene promoter is associated with increased risk for psychiatric hospitalization and suicide attempts. This variant may lead to altered transcriptional regulation of 5-HT1A, which in turn alters serotonin release from serotonergic neurons in the raphe nuclei, resulting in decreased serotonin action in brain areas involved in cognition and emotion regulation. We have previously shown that the ventral hippocampus is a crucial mediator of individual differences in stress susceptibility and resilience. In particular, increased activity in the ventral dentate gyrus region of the hippocampus promotes susceptibility to stress-induced anxiety-like behavior in mice. To model gene-by-environment interactions that are relevant for individual differences in vulnerability to early life stress, we here used a transgenic mouse model with high- or low expression of 5HT1A autoreceptors in a limited bedding model of early adversity. We then assessed anxiety-like behavior, cognition, and ventral dentate gyrus activity, to elucidate the behavioral- and neurobiological consequences of GxE interactions on susceptibility to early life stress.

Methods: We used a transgenic mouse line in which expression levels of 5HT1A autoreceptors can be increased by doxycycline administration, using a doxycycline-inhibited transcriptional silencer (tTS) under the control of the Pet1 promoter (Pet1-tTS; Htr1atetO/tetO mice). We then exposed mice with high- and low 5HT1A autoreceptor expression to the limited bedding model of early adversity. Litters raised under limited bedding conditions were placed on wire mesh cage flooring with their dam and provided with one-third of standard nesting material from postnatal day (PND) 3 to 10. Litters raised under limited bedding conditions were compared to litters raised under control conditions, which were standard-housed and provided with regular nesting material. Male ($n=21$) and female ($n=22$) offspring were weaned on PND21 and tested for anxiety-like behavior (elevated plus maze) and contextual fear learning from PND35 to PND45. Brains were harvested on PND45 1h after the end of the last behavioral test. Immunohistochemistry for the immediate early gene, *cfos*, was performed in the dentate gyrus to evaluate neural activity in a subgroup of animals ($n=8$).

Results: Male and female offspring raised under limited bedding conditions showed lower body weights on PND21 and PND28 compared to control offspring ($***p=0.0001$). Offspring of both sexes reached similar body weights as controls on PND35. Male offspring with high 5HT1A autoreceptor expression spent less time in the open arms of the elevated plus maze following limited bedding (LB) than control (Ctrl) mice (LB, $n=8$; Ctrl, $n=12$; $*p=0.03$). In contrast, male offspring with low 5HT1A autoreceptor expression did not differ in the time spent in the open arms when comparing LB and control mice (LB, $n=5$; Ctrl, $n=5$; $p=0.12$). These data suggest that variations in 5HT1A autoreceptor expression levels may mediate individual differences in vulnerability to early life adversity. No effect on anxiety-like behavior in the elevated plus maze was observed in females with high- or low 5HT1A expression. Both, male and female offspring raised under limited bedding conditions showed impairments in contextual fear learning compared to controls, indicated by lower freezing levels in a fear-associated context 24h after a footshock was delivered in the same context (LB, $n=15$; Ctrl, $n=20$; $*p=$

0.04). Immunohistochemistry analysis of *cfos* expression revealed that mice exposed to early adversity in the form of limited bedding showed increased neural activity in the ventral dentate gyrus (LB, $n=8$; Ctrl, $n=8$; $*p=0.03$), but not in the dorsal dentate gyrus (LB, $n=8$; Ctrl, $n=8$; $p=0.24$). These data are consistent with the notion that increased activity of the ventral dentate gyrus promotes susceptibility to stress-induced behavioral abnormalities.

Conclusions: Here, we used a mouse model that integrates early life stress and genetic variation in 5HT1A receptor expression, to model gene-by-environment interactions that are relevant for the pathogenesis of psychiatric disorders. Our results show that adversity during early life can have long lasting and sex-specific effects on anxiety-like behavior, fear learning, and ventral hippocampus activity. In addition, the behavioral consequences of early life stress are mediated by differences in the expression of 5HT1A receptors, as high levels of 5HT1A autoreceptors may increase susceptibility to early life stress. Our data suggest that GxE interactions involving early life stress and the serotonergic system are important mediators of neurobiological- and behavioral disturbances that may contribute to psychiatric disorders.

Keywords: Early Life Stress, Serotonin 1a Receptor, Dentate Gyrus, Behavior, Neurogenesis

Disclosure: Nothing to disclose.

M105

Neural Response to Accurately Predicting Rejection but Not Monetary Loss is Associated With Depression and Anxiety in Youth: A Preliminary fMRI Study

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Background: Peer relationships become more salient during adolescence, as the brain undergoes changes in networks critical for processing reward. Although social reward, such as peer acceptance, is highly salient for adolescents, neural response to reward has largely been studied in the monetary domain. Testing reward processing in the social domain may be particularly important when considering the neural mechanisms that promote symptoms of anxiety and depression. These symptoms increase dramatically in adolescence, and are associated with alterations in reward-related brain function. Although social stressors often potentiate symptoms of anxiety and depression, direct tests of the association between symptoms and neural responses across reward domains are rare. Moreover, most research examining relations between brain function and reward processing have confounded the intrinsically rewarding experience of being correct with the receipt of positively-valenced outcomes. Yet, symptoms of adolescent anxiety and depression may be differentially associated with dysregulated processing of intrinsic (being correct) and extrinsic (receiving a positively-valenced outcome) rewards across social and non-social domains. Our prior work in late adolescents used well-matched EEG-based tasks to disentangle the brain's response to the intrinsic reward of being correct from its response to positively and negatively valenced outcomes in social (like, dislike feedback) and non-social (monetary gain, loss) domains. Regardless of domain, the reward positivity, an event related potential that indexes cortico-striatal engagement following the receipt of a reward, was blunted with more severe symptoms of depression and potentiated with more severe symptoms of anxiety. Additionally, more severe symptoms of anxiety were associated with a greater reward positivity to correctly predicting dislike feedback, suggesting a

mechanism by which negative peer feedback may be intrinsically rewarding to anxious youth. We extend this work by implementing novel fMRI-based tasks adapted from our prior methods in mid-adolescents with a range of anxiety and depression symptoms.

Methods: Adolescents ($N = 27$; 18 females; 13.32 ± 1.28 years) completed novel monetary and social outcome tasks while undergoing fMRI. For the monetary task, a pair of doors appeared on the screen. Half of the trials included blocks with a positive valence goal: correctly predict the door that will result in a monetary win (win trials). On the other blocks of trials, there was a negative valence goal: correctly predict the door that will result in a monetary loss (lose trials). Incorrect predictions resulted in a null outcome. The social task had identical attributes except doors were replaced with photos of age-matched peers. Participants were told that some peers had rated them after receiving a text of their picture. Positive and negative valence goals were to correctly predict which peer had liked (like trials) or disliked them (dislike trials), respectively. Incorrect predictions resulted in a null outcome, reflecting that the purported peer never received a text. Given the preliminary nature of the study, a priori region of interest analyses focused on the ventral striatum, a critical hub in the reward processing network. ANOVA analyses tested relations between brain function, reward processing, and symptom severity.

Results: The hypothesized Domain X Valence X Outcome X Anxiety X Depression interaction emerged ($F(1,24) = 5.04$, $p = .034$, $\eta^2 = .17$). When decomposed, correct, but not incorrect trials, resulted in a Domain (monetary, social) X Valence (monetary win/social like, monetary loss/social dislike) X Anxiety X Depression interaction ($F(1,24) = 7.19$, $p = .013$, $\eta^2 = .23$). When correct trials were further probed a Valence (monetary win/loss; social like/dislike) X Depression X Anxiety interaction emerged in the social ($F(1,24) = 8.58$, $p = .007$, $\eta^2 = .26$), but not monetary domain. For interpretation purposes, youth were split into groups based on level of depressive symptoms. Among youth with low levels of depression, more severe anxiety symptoms were associated with greater activation in the striatum when participants learned they had correctly predicted that a peer disliked (vs. liked) them ($r = 0.5$, $p = 0.056$). The opposite relation occurred in youth with high levels of depression: more severe anxiety symptoms were associated with greater activation in the striatum when participants correctly predicted that a peer liked (vs. disliked) them ($r = -0.62$, $p = 0.043$). Relations between low and high depression groups differed (Fishers r to $z = 2.78$, $p = 0.005$). No effects were observed in right ventral striatum.

Conclusions: We provide preliminary support for a novel fMRI-based approach for comparing neural response to social and monetary rewards. Additionally, consistent with our EEG work, results show a brain-based mechanism by which correctly predicting negative peer feedback may be intrinsically rewarding, and thus contribute to maintaining anxiety symptoms. Together, this work suggests a biologically based, symptom-specific target for novel therapeutic intervention, particularly in anxious youth without comorbid depression. Given a significant number of anxious youth fail to respond to treatment, identifying such markers is especially important.

Keywords: Social Reward, Monetary Reward, Adolescent Anxiety, Adolescent Depression, Ventral Striatum

Disclosure: Nothing to disclose.

M106

Convergent Findings From Genetic and Functional Studies Implicate a Vital Role of FZD6 in Depressive Symptoms

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Background: Depression is a common complex disorder with substantial heritability. However, searching for susceptibility variants has achieved only limited success.

Methods: We conducted an exome-wide association study for depressive symptoms assessed by the Center for Epidemiological Studies Depression (CES-D) score with a sample size of 4,817.

Results: We revealed three SNPs to be significantly associated with the CES-D score after Bonferroni correction: (1) rs61753730 ($p = 8.46 \times 10^{-9}$) in the frizzled class receptor 6 (FZD6) gene; (2) rs35024632 ($p = 3.08 \times 10^{-8}$) in the amphiphysin (AMPH) gene, and (3) rs117406702 ($p = 5.46 \times 10^{-7}$) in the zonadhesin (ZAN) gene. Further evidence supporting an association of FZD6 with depression was obtained through gene-based association analysis ($p = 7.8 \times 10^{-3}$). Consistently, Fzd6 knockout (KO) rats displayed depressive-like behaviors, and patients with major depressive disorder (MDD) exhibited decreased mRNA of FZD6 compared with healthy controls. Subsequently, molecular study with CRISPR/Cas9-based FZD6-KO in HEK293T cells also demonstrated an important role of FZD6 in depression through the canonical Wnt/ β -catenin signaling pathway. In silico analysis revealed that the nonsynonymous SNP rs61753730, which gives rise to a glutamine-to-glutamate substitution at position 152 in FZD6, had a significant influence on the tertiary structure and stability of the protein. Finally, we demonstrated the effect of rs61753730 on luciferase activity and mRNA decay of luciferase reporter gene.

Conclusions: Taken together, these findings from genetic and functional studies strongly demonstrate that FZD6 plays a vital role in the pathogenesis of depression.

Keywords: Human Genetics, MDD, Molecular Genetics

Disclosure: Nothing to disclose.

M107

Suicide Prediction in Major Depressive Disorder Using Connectome Based Modeling

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Background: Major depressive disorder (MDD) is a severe, often chronic, mental illness with increased risk for suicide. Recent neuroimaging studies have implicated several brain regions in the pathophysiology of suicide in MDD. Using a connectome-based predictive modeling (CPM), we aimed to investigate whether variability in the brain functional connectome will predict the severity of suicidal behavior in MDD patients.

Methods: Two hundred and sixty-one patients diagnosed with MDD completed resting-state functional magnetic resonance imaging (rs-fMRI) scans. CPM, a data-driven approach, was implemented to determine whether functional connectivity fingerprints can predict suicidal behavior among MDD patients. Using cross validation, CPM uses whole brain connectivity data to provide a generalized prediction of behavioral measures. The Columbia Suicide Severity Rating Scale (C-SSRS) was used to assess suicide severity.

Results: CPM successfully predicted the severity of suicidal behavior, showing significant correlation between predicted and actual values of the C-SSRS ($r = 0.2646$, $p = 0.000015$). Post hoc exploration implicated nodes within the salience, default mode,

and visual networks, with top degree centrality in prefrontal nodes. Significant values included edges connecting the right prefrontal area and frontoparietal network, which are critical to behavior coordination and cognitive control and commonly compromised among different stress-related psychopathologies including MDD.

Conclusions: The study provided a biological data-driven model for predicting suicidal behavior. While statistically significant, the predictive model does not fully account for the reported severity, highlighting the need for combined models and multimodal data to enhance the prediction. Most importantly, the results identified critical nodes and circuits that may be a target to better understand, to prevent or to treat suicidality.

Keywords: Major Depression Disorder, Suicide Prediction, Neuroimaging, Mood Disorders, Suicide

Disclosure: Nothing to disclose.

M108

Autoantibody Burden in Mood and Psychotic Disorders

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Background: There is a recognised epidemiological relationship between autoimmune disease and mood disorders, and psychosis. Recent work has tied autoantibodies that bind to neuronal surfaces and synaptic targets, including specific potassium channel-related proteins, to psychiatric symptoms occurring in the context of limbic encephalitis. This has led to the hypothesis that a subset of psychiatric disorders may be caused by latent autoimmune disease. However, a broad survey of autoantibody prevalence within a psychiatric patient population has not yet been performed.

Methods: In a cohort of 712 participants with major depressive disorder (MDD), bipolar disorder (BD), schizoaffective disorder (SZA), schizophrenia, and controls, we measured 9,357 serum IgG autoantibodies to natively-folded proteins using the ProtoArray™ Human Protein Microarray by Thermo Fisher Scientific. We focused a priori, on antibodies against the potassium channel-related protein, leucine-rich glioma inactivated 1 (LGI1), a common cause of limbic encephalitis with psychiatric symptoms. Second, we performed an analysis on 41 other potassium channel-associated proteins included on the array, and finally we performed an array-wide analysis of overall autoantibody burden. For each patient and probe, the presence/absence of an autoantibody was defined based on whether a patient's value fell above or below 3 standard deviations from the mean of healthy control samples. Statistical analysis of individual antibodies was performed using Fisher's exact test based on this presence/absence call. Autoantibody burden for a patient was calculated as the number of 'presence' calls across the set of N probes for a patient (50 in the case of potassium-channel antigens and 9,357 for all antigens in the array).

Results: We found that LGI1 antibodies were more frequent in participants with SZA (OR=9.7, $p=0.006$) or affective psychosis (OR=8.4, $p=0.006$) than in healthy controls or participants from other patient groups ($p<0.05$). A secondary analysis was performed on the remaining potassium-channel associated proteins. Anti-potassium channel tetramerization domain containing 18 (KCTD18) antibodies were higher in patients with BD (OR=10.0, $p<0.001$) and MDD (OR=8.6, $p<0.001$) than HC, with no significant effect among SZA or SZ patients. Given the low prevalence of autoantibodies against individual proteins in the

population and the large number of proteins tested, unsurprisingly after FDR correction none of the remaining 9,307 individual autoantibodies remained significant. However, compared to controls there was a significantly higher burden of the 9,357 autoantibodies in all patient groups compared with controls ($p<0.05$, FDR-corrected): schizophrenia > SZA ~ BD ~ MDD > HC.

Conclusions: A significant subset of psychiatric patients may have underlying, autoimmune activity, which either may be directly pathogenic or may contribute to chronic inflammation; in either case such conditions may be responsive to immunomodulating medications. Most notably, LGI1 autoantibodies have been shown to cause some cases of limbic encephalitis by altering potassium channel and AMPA receptor signaling and may be directly pathogenic in a small subset of patients with depression and/or psychosis.

Keywords: Autoimmune Encephalitis, Inflammation, Major Depressive Disorder, Schizoaffective Disorder, Bipolar Disorder

Disclosure: Nothing to disclose.

M109

Proposing a Novel Metabolomic Analysis Using Free Form of Plasma Metabolites as More Specific Biomarkers for Major Depressive Disorders

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Background: Major depressive disorder (MDD) is one of the most crucial psychiatric disorders and a major contributor to the overall global burden of disease worldwide. Researchers have long been investigating useful biomarkers in blood to discriminate MDD patients from healthy subjects, which are primarily focused on changes as a whole in protein, peptide, cytokine, and metabolite levels. Plasma metabolites are transported while interacting with abundant protein such as albumin. However, conventional metabolite biomarker analysis using LCMS has little taken into consideration of the mode of transport in the blood. We propose a highly effective preprocessing methodology for LCMS-based plasma metabolite biomarker analysis, which allows to separate plasma metabolites into free- and protein-bound form, and then verify the effectiveness of them in the free-form on biomarker investigation using clinical samples with MDD.

Methods: Blood samples of persons with MDD and healthy volunteers were collected in Department of Neuropsychiatry, Kyushu University Hospital and its related affiliations. Psychiatric diagnosis was made by trained psychiatrists, according to the Structured Clinical Interview for DSM-IV (SCID). Plasma free metabolites were prepared using Amicon Ultra-0.5 ml centrifugal filters. Twenty microliter of plasma was added with 480 μ l of ultrapure water and applied to the filter and centrifuged (14,000g, 20°C, 15 min). Collect 400 μ l of the flow-through fraction and add an equal volume of methanol (400 μ l) and 5 μ l of the solution (equivalent to 0.02 μ l of plasma) is subjected to LSMS measurement using LCMS 8040 and LCMS 8060 instrument (Shimadzu, Japan).

Results: It is generally known that hydrophobic fatty acids are mostly bound with transport proteins such as albumin, whereas hydrophilic amino acids except tryptophan are present in the free form. Interestingly, 10-20% of tryptophan and the structurally-related metabolites are found to be present as the free form in the blood. Since these compounds have been shown to be depression-related biomarkers, we addressed whether the free form of the metabolites could be useful information for characterizing MDD patients and/or severity of depression. As a

result, plasma kynurenic acid in the free form, but not whole amount of one, is identified to be the best biomarker that significantly correlated with the severity of depression and shows highest predictive performance using plasma from drug-free MDD patients ($n = 16$).

Conclusions: Our results suggest that the free form of the plasma metabolites may be a promising information for the biomarker analysis, more reflecting the pathological condition in the blood. Further investigations should be conducted to validate our pilot findings.

Keywords: Metabolomics, Peripheral Biomarker, Kynurenine Metabolism, Major Depressive Disorder (MDD)

Disclosure: Nothing to disclose.

M110

Age Moderates the Relationship Between Affective Response Control and Bipolar Disorder

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Background: Bipolar disorder (BD) patients have impairments in neurocognition, including affective processing and affective response control. While studies suggest that cognitive control may decline with age in BD, less is known about age-related changes in affective response control.

Methods: 258 BD participants and 54 healthy controls (HC), ages 18-70, completed the Cambridge Neuropsychological Test Automated Battery Affective Go/No-Go task (CANTAB AGN) to assess affective response control. We examined the relationship between BD and affective response control (number of commission errors and response time), as well as a potential moderating effect of age, using mixed effects linear regression models.

Results: BD participants made more commission errors overall than HC ($p < 0.001$), while all participants had slower reaction times on negative than positive target words. We found a 3-way group-by-age-by-valence interaction on reaction time ($p = 0.018$). For negative target words, older BD participants exhibited a slower response time than either younger BD participants or HC participants. No significant moderating effect of age was observed for positive target words.

Conclusions: These cross-sectional findings suggest an effect of emotional stimuli on response control in adults with BD and that the relationship between BD and affective response control to negative targets may be age-dependent. Longitudinal studies are needed to examine patterns of within-individual changes in affective response control with aging in BD.

Keywords: Bipolar Disorder, Affective Response Control, Ageing

Disclosure: Nothing to disclose.

M111

Effectiveness of Ketamine and (2R,6R)-Hydroxynorketamine to Reverse Anhedonia Subtypes Induced by Stress in Mice

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Background: Anhedonia, a lack of capacity to experience pleasure, afflicts individuals suffering from depression, schizophrenia, and other neuropsychiatric disorders. Recent evidence indicates that low, sub-anesthetic, doses of the anesthetic ketamine rapidly ameliorate anhedonia, distinct from its capacity to reduce other symptoms of depression. Anhedonia can be separated into different subtypes including consummatory, anticipatory and motivational. Each of these subtypes involves both overlapping, as well as distinct brain circuits. In this preclinical study we investigated the effectiveness of ketamine and (2R,6R)-hydroxynorketamine (HNK) to ameliorate distinct anhedonia subtypes.

Methods: Male Balb/c mice were trained to lever press for a sucrose pellet reward. Following stable responding, mice were placed in a progressive ratio (PR) schedule of reinforcement. Upon stable PR responding, mice underwent a 10-day chronic social defeat stress (CSDS) and concomitantly were tested with the PR to test for motivational anhedonia. Consummatory anhedonia was assessed with the sucrose preference and female urine sniffing tests. Mice were then treated with either saline ($n = 8-9$; 7.5 ml/kg, i.p.), ketamine ($n = 8$; 10 mg/kg, i.p) or (2R,6R)-HNK ($n = 9$; 10 mg/kg, i.p) and subsequently retested on the anhedonia measures. In a different cohort, male C57BL/6 mice underwent chronic CSDS, and were tested for the induction of consummatory anhedonia followed by treatment with either saline ($n = 7$; 7.5 ml/kg, i.p.) or ketamine ($n = 7$; 20 mg/kg, i.p.). Sucrose preference was measured daily until recovery of preference was observed. Then mice were re-exposed to a novel aggressive CD-1 mouse for 1 min for reinstatement of stress-induced sucrose preference deficits.

Results: CSDS induced a decrease in PR breakpoint, as well as a decrease in sucrose and female urine preference indicating induction of both motivational and consummatory anhedonia. Treatment with ketamine and (2R,6R)-HNK reversed sucrose and female urine preference deficits. However, at the dose of 10 mg/kg, (2R,6R)-HNK, but not ketamine, increased the PR breakpoint after stress. Moreover, ketamine administration reversed CSDS-induced decreases in sucrose preference and also resulted in a protective effect against sucrose preference deficits induced by a brief re-exposure to an aggressive CD-1 mouse.

Conclusions: Ketamine and (2R,6R)-HNK reverse consummatory anhedonia induced by chronic stress; however, (2R,6R)-HNK may be more effective in reversing motivational anhedonia.

Keywords: Anhedonia, Ketamine, Hydroxynorketamine

Disclosure: Nothing to disclose.

M112

Individuals With High Trait Negative Emotionality are More Sensitive to the Subjective Effects of a Microdose of LSD

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Background: Numerous anecdotal reports suggest that repeated use of very low doses of lysergic acid diethylamide (LSD), known as "microdosing," improves mood and cognitive function. Yet, the behavioral, neural, and subjective effects of low doses of LSD have only recently been tested in a controlled laboratory setting, and little is known about inter-individual variability in the drug's effects. We have examined the effects of single low doses of LSD on mood and behavior in healthy volunteers under double-blind conditions. Here we report on individual differences in responses to a single low dose LSD (13µg) in relation to personality traits.

Methods: As part of two studies, healthy young men and women (N = 35) attended laboratory sessions during which they received placebo or 13µg LSD in separate sessions at one-week intervals, under double blind conditions in mixed order. Subjects completed mood questionnaires and behavioral tasks assessing emotion processing and cognition during the sessions. They completed the Altered States of Consciousness questionnaire at the end of each session, and the Multiphasic Personality Questionnaire (MPQ) at intake.

Results: LSD produced modest subjective effects, including increases on ratings of “feel drug” and “feel high” and on the “Blissful State” domain of the Altered States of Consciousness questionnaire. These subjective effects were most pronounced in individuals who scored high on Negative Emotionality on the MPQ ($r = 0.37$ $p = 0.03$; $r = 0.37$ $p = 0.03$; $r = 0.45$ $p = 0.01$).

Conclusions: A threshold “microdose” of LSD (13µg) produced modest subjective effects in healthy volunteers and these effects were strongest among individuals with high negative emotionality scores. These findings are consistent with the idea that the drug improves mood in individuals with symptoms of depression.

Keywords: LSD Microdosing, Psychedelic Medicine, Mood

Disclosure: Nothing to disclose.

M113

Neural Flexibility Correlates of Adolescent Depression and Suicide Risk

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Background: Suicide is the 2nd leading cause of death in adolescents and young adults. Novel intervention development will require advanced understanding of the mechanisms underlying depression and suicide risk. Prior to a suicide attempt, people often report a sense of narrowed options, which may reflect an underlying neural rigidity. Novel approaches capable of estimating neural flexibility from resting-state fMRI data have recently emerged. These include drawing from information theory to measure entropy of brain signals, and from dynamic connectivity analyses to measure switching between states (where states are characterized by distinct configurations of connectivity within resting-state networks).

Methods: We examined relationships between neural flexibility measures and depression / suicide thinking in 3 adolescent studies: (1) a longitudinal study examining resting-state fMRI before and after intravenous ketamine infusions in 13 adolescents with treatment-resistant depression; (2) a study of adolescents with mood disorders (20 with unipolar depression, 10 with bipolar disorder, 20 healthy controls) examining resting-state fMRI at baseline and 6 months later; and (3) a preliminary analysis of a subset ($n = 50$) of baseline data from an ongoing longitudinal study of female adolescents (aged 12-16) with and without non-suicidal self-injury (NSSI). Resting-state fMRI data (eyes open, watching a fixation cross) were acquired using a 3T Siemens Prisma scanner using multiband acquisition protocols informed by the human connectome project (HCP). Standard HCP pipelines were used for preprocessing and artifact removal. We excluded scans with > 30% of volumes with “excessive motion”, defined by framewise displacement > 0.5 mm and/or temporal derivative of time courses root mean square variance > 8 (after initial motion correction). For all 3 studies, Shannon entropy was calculated from wavelet-transformed time-courses from fronto-limbic regions of

interest (focusing on wavelets approximating 0.1 Hz). State-switching frequency was measured in 2 studies using the dynamic functional connectivity (dFNC) toolbox. We conducted ICA, inspected components to identify neural networks (versus noise) to include in sliding window analysis, and computed correlation values between each component within 848 windows (windows 64TR; onset spacing 1TR). We then applied k-means clustering to window correlation matrices to yield clusters (“states”) representing a configuration of whole-brain resting-state connectivity; adolescents would “dwell” in or move between states in varying fashion).

Results: Entropy, Study 1. Clinical improvement following ketamine correlated with increased entropy in right nucleus accumbens ($p = 0.0007$). A similar pattern was observed in the other ROIs examined but the results were not significant after Bonferroni correction. Entropy, Study 2. Adolescents with mood disorders showed lower nucleus accumbens entropy than healthy controls ($p = .06$). Depression scores inversely correlated with entropy in left pallidum, right caudate, right hippocampus, and right accumbens (r values $-.33$ to $.45$, p 's $.007$ to $.05$). Suicide scores also inversely correlated with right caudate entropy ($r = -.60$, $p = .005$) and right nucleus accumbens ($r = -.56$, $p = .01$), and left thalamus, left putamen, and right hippocampus (r : $-.32$ to $-.42$). When reassessed 6-12 months later, decrease in depression correlated with increased entropy in left caudate, left accumbens and bilateral amygdala (r : $-.55$ to $-.76$) and with increased entropy in left pallidum, right caudate, right accumbens, left hippocampus (r 's $-.30$ to $-.49$). Decreased suicidal ideation correlated with increased entropy in right amygdala ($r = -.74$, $p = .004$) and left accumbens ($r = -.52$, $p = .07$). Entropy, Study 3. Adolescents with NSSI ($n = 30$) showed lower entropy than those without ($n = 20$) (most significant in left amygdala, $p = 0.01$). Fronto-limbic entropy negatively correlated with depression scores (right amygdala: $r = -.42$, $p = 0.03$; left BA25: $r = -.3$, $p = 0.04$; right nucleus accumbens $r = -.36$, $p = 0.07$) and suicidality scores (left subgenual cingulate $r = -.3$, $p = 0.03$). State-switching, Study 2. Among adolescents with mood disorders (but not controls), state-switching frequency correlated inversely with depression scores ($r = -.54$, $p = 0.01$) and suicidal thoughts ($R = -.41$, $p = 0.1$). State-switching, Study 3. Among adolescents with NSSI (but not controls), state-switching frequency correlated inversely with depression scores ($r = -.42$, $p = 0.03$) and suicide scores ($r = -.34$, $p = 0.08$). Finally, in Study 3, we examined the relationship between entropy measures and state-switching frequency. Positive correlations were noted for most entropy ROIs, significant ($p < 0.05$) or trend ($p < 0.1$) in about half of these analyses, most significant for right accumbens entropy ($r = 0.4$, $p < 0.001$).

Conclusions: Across 3 adolescent studies, we found consistent evidence supporting the idea that depression and suicidal thoughts in adolescence are related to reduced neural flexibility as measured by dynamic functional connectivity and entropy, that these neural flexibility correlate with each other, and that they show neuroplastic changes with recovery.

Keywords: Adolescent Depression, Resting-state fMRI, Suicide, Neural Flexibility, Entropy

Disclosure: Nothing to disclose.

M114

Pup Removal Shortly After Birth Induces Long-Lasting Alterations in Behavior and Dopamine Activity in Late Postpartum Rats: Partial Modulation by Social Context

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Background: Maternal mood during postpartum can be dependent on offspring exposure (Feldman et al. 1999). In humans, the loss of a child can lead to severe grief and depression in mothers (Badenhorst and Hughes, 2006). In rodents, repeated long duration separations from pups impair maternal behavior and alter emotionality in late postpartum rats (Boccia and Pedersen, 2001). Both repeated long separations and pup removal shortly (<24-hours) after birth increase immobility duration in the forced swim test (FST) in rat dams, which is interpreted as increased depressive-like behavior (Pawluski et al., 2009; Boccia et al., 2007). However, the use of the FST as a measure of depressive-like behavior has recently been questioned (Molendijk and de Kloet, 2019); and other measures of depressive-like behavior, as well as effects on dopamine (DA) system function, in rat dams following pup removal remain unexplored. Here we evaluated long-lasting effects of pup removal, and their possible modulation by social context on maternal affect and DA activity.

Methods: 3 groups were used: i) dams with pups, ii) dams with no pups, single-housed, iii) dams with pups removed co-housed with another dam. All underwent a behavioral test battery including: sucrose consumption, forced swim, elevated plus maze, and social approach tests during postpartum days (PD) 21-23. In vivo electrophysiological recordings of ventral tegmental area (VTA) DA neurons were performed (PD22-23) in subset of animals used for behavioral testing (EPM, social approach) to measure 3 parameters: number of spontaneously active DA neurons (i.e. population activity), firing rate, and firing pattern (i.e. burst firing).

Results: Dams that underwent pup removal exhibited no alterations in anxiety-like behavior in the EPM but exhibited disruptions in social motivation to a novel conspecific compared to dams that were housed with pups or dams that had pups removed and were co-housed (one-way ANOVA: $p < 0.05$; $n = 6-9$). Pup removal also increased FST immobility and reduced latency to immobility in the FST regardless of housing condition (one-way ANOVA: $p < 0.05$; $n = 9$). Furthermore, single-housed dams that underwent pup removal exhibited an attenuation in VTA DA activity (i.e. reduced number of active DA cells) compared with dams that were co-housed or control dams kept with pups (one-way ANOVA: $p < 0.05$; $n = 6-9$).

Conclusions: These data indicate that disruption of species-expected social relationships during the postpartum period (i.e. offspring) induces long-lasting alterations in depressive-like and social behavior as well as VTA DA activity, and that a subset of these effects that can be prevented through social experience.

Keywords: Postpartum, Dopamine, Electrophysiology, Social Behavior

Disclosure: Nothing to disclose.

M115

The Clinical Utility of Imaging-Defined Biotypes of Depression and Transcranial Magnetic Stimulation: A Decision Curve Analysis

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Background: Several studies have demonstrated that neuroimaging may provide objective patterns of functional connectivity that are predictive of antidepressant response to transcranial magnetic stimulation (TMS). In one of the largest of these studies, Drysdale et al. (Nat Med. 2017;23(1):28-38) demonstrated that functional connectivity patterns could be used to identify four "biotypes" of depression that are associated with different

patterns of response to TMS. Despite these exciting results, the translation of predictive neuroimaging models into clinical practice remains an unmet goal. Challenges to this goal include costs, relevant applications, reproducibility, and the lack of a simple method to evaluate a test's clinical utility. In this study, we apply decision curve analysis (DCA) to two theoretical treatment strategies constructed from the Drysdale et al. data to evaluate the utility of incorporating these approaches in guiding TMS treatment decisions.

Methods: TMS outcome and biotype identification data were obtained from the Drysdale et al. study. This data set included 124 individuals with depression treated with dorsomedial prefrontal TMS (utilizing repetitive or intermittent theta-burst TMS). Forty-five of these subjects (approximately 36%) achieved clinical response, defined as a 50% reduction in symptoms. As noted, this response differed by connectivity-defined biotype at baseline, with Biotypes 1 and 3 achieving higher response rates (65% and 32%, respectively) compared to Biotypes 2 and 4 (12.5% and 15%). Theoretical treatment strategies for identifying TMS responsive patients included treat only Biotype 1 or treat Biotypes 1 & 3. DCA was used to assess the clinical utility of these strategies in terms of net benefit over a clinically relevant range of threshold probabilities.

Results: Both treatment strategies were associated with a greater proportion of clinical responders receiving treatment (Fisher's exact test, odds ratio Biotype 1 = 6.24, $p < 0.001$; odds ratio Biotypes 1 & 3 = 5.65, $p < 0.001$). Treating only Biotype 1 resulted in a sensitivity of 58%, a specificity of 82% and 73% accuracy for identifying TMS responsive individuals. Conversely, treating both Biotypes 1 & 3 resulted in a sensitivity of 87%, a specificity of 47% and 61% accuracy for identifying responders. DCA analysis revealed that the net benefit of treating Biotypes 1 & 3 exceeded both the treat only Biotype 1 and the "treat all" strategies over a range of threshold probabilities between 14% and 32%. For threshold probabilities above 32%, treating just Biotype 1 achieved greater net benefit than alternative strategies. Greater net benefit translates into a higher proportion of successful TMS treatments and reduced cost per response. Assuming a TMS treatment series costs \$15,000 and takes 36 sessions, the "treat all" strategy currently used costs \$41,333 and 99 visits per clinical response. Adding the predictive neuroimaging strategies and an approximate cost of \$1,500 and a one-hour visit per MRI scan, the predictive approaches yield reduced costs per response for both strategies (Biotype 1 only: cost \$30,231, 60 visits; Biotypes 1 & 3: cost \$35,923, 78 visits).

Conclusions: This study demonstrates the feasibility of evaluating predictive neuroimaging models in a clinical decision framework using DCA. These results suggest that treatment strategies that incorporate the functional connectivity depression biotypes proposed by Drysdale et al. could be a useful and cost-effective tool in guiding clinical TMS treatment decisions, resulting in a greater proportion of successful treatments and an improved risk-benefit discussion between clinicians and patients. The strength of DCA is that it bridges the gap between neuroscience research and practical clinical decision-making. Furthermore, it demonstrates that predictive models can be clinically useful without perfect accuracy - as long as they address the right clinical questions. With recent advances in neuroimaging and computation in psychiatry, there is a clear need for a framework to evaluate the clinical utility of predictive models and thus direct future research towards applications that change clinical practice.

Keywords: Decision Curve Analysis, Transcranial Magnetic Stimulation, Biotypes, Depression Subtypes, Translational Biomarker Development

Disclosure: Nothing to disclose.

M116

Neurokinin-1 Receptors in the Nucleus Accumbens Shell Mediate Sensitivity to Social Defeat Stress**Sadie Nennig, Hannah Fulenwider, Kimberly Whiting, Jesse Schank****University of Georgia, Athens, Georgia, United States*

Background: Chronic social defeat stress (SDS) is a widely used preclinical model of depression. In this procedure, mice are exposed to a brief physical defeat by a larger, aggressive mouse for 10 consecutive days. Aggressor mice and defeated mice are then housed in the same cage, separated by a perforated divider, until the physical defeat session on the following day. Exposure to SDS induces depressive-like phenotypes in mice including anhedonia, social withdrawal, and increased drug and alcohol consumption. In our prior work, we have found that expression of the neurokinin-1 receptor (NK1R) is increased in the nucleus accumbens (NAC) of mice that are sensitive to this stressor. The NK1R is the endogenous receptor for the neuropeptide substance P, and plays a prominent role in stress, anxiety, and addiction.

Methods: In the present study, we used genetic, pharmacological, and viral vector strategies to demonstrate a functional role of the NK1R in the NAC shell in sensitivity to SDS.

Results: First, we exposed NK1R ^{-/-}, which have a genetic deletion of this receptor, to the SDS procedure. Surprisingly, we found no effect of this genetic manipulation on sensitivity to SDS. We hypothesized that this was due to developmental compensatory adaptations in the neurokinin systems in these mice. To inhibit the NK1R without affecting developmental adaptations, we delivered the NK1R antagonist L703606 prior to each physical defeat and found that this treatment was able to decrease the sensitivity to SDS exposure, providing protection from the social withdrawal inducing effects of this stressor. Conversely, we then overexpressed the NK1R in the NAC shell using viral vector strategies and found that this increased the sensitivity to SDS.

Conclusions: Together, these experiments provide evidence for a functional role of the NK1R in the NAC shell in the sensitivity to SDS.

Keywords: Depression, Social Defeat Stress, Neurokinin, Substance P, Neuropeptides

Disclosure: Nothing to disclose.

M117

Quantification of Antidepressant and Antipsychotic Exposure Increase Caused by CYP2C19 and CYP2D6 Intermediate and Poor Metabolizer Status**Filip Milosavljević, Nikola Bukvić, Vesna Pešić, Zorana Pavlović, Čedo Miljević, Espen Molden, Magnus Ingelman-Sundberg, Marin Jukic****The Karolinska Institutet, Stockholm, Sweden*

Background: Most of the psychiatric drugs are metabolized by CYP2C19 and CYP2D6 enzymes. Both CYP2D6 and CYP2C19 genes are polymorphic and metabolic capacity of the enzymes is genotype-determined. Homozygous Null allele carriers do not possess active enzyme, and they are referred to as CYP2C19 or CYP2D6 poor metabolizers (PM). Certain genotypes do not abolish the enzyme completely, but they do cause a drastic reduction of

metabolic capacity and carriers of such genotypes are referred to as intermediate metabolizers (IM). It is known that CYP2C19 and CYP2D6 PM and IM status cause an increase in exposure of certain antidepressants and antipsychotics; however, due to small sample sizes of the previously published studies, the magnitude of this effect still cannot be estimated with sufficient precision. Therefore, the aim of this meta-analysis was to pool all these studies and estimate the magnitude of drug exposure increase caused by CYP2C19 and CYP2D6 PM and IM status, compared with normal metabolizers (NM).

Methods: The inclusion of the drugs used for the literature survey for meta-analysis was based on the list of new-generation antidepressants and antipsychotics found on consensus guidelines for therapeutic drug monitoring. Initially, the studies were screened for inclusion by the PubMed search 'DrugName' AND (CYP2C19 OR CYP2D6) for all listed drugs. The studies were included in the meta-analysis if (1) the patients were appropriately genotyped for CYP2C19 or CYP2D6; (2) adequate sorting of patients into NM, IM, and PM was possible; (3) the study included at least three patients per subgroup; and (4) drug exposure was measured in a representative way as (a) dose-harmonized area under plasma level (time) curve, (b) dose-harmonized steady-state plasma levels, or (c) apparent total clearance of the drug from plasma after oral administration (CL/F, reciprocal value represented the drug exposure). Meta-analysis for a specific drug was performed if five or more studies met the inclusion criteria. Based on the outcome of the literature survey, it was possible to perform meta-analysis for escitalopram (N = 2,125), venlafaxine (N = 266), risperidone (N = 1,006), and aripiprazole (N = 824). Drug exposure head-to-head comparisons were made between PM or IM subjects and the NM subject group, which served as a reference. Heterogeneity across the studies was assessed using Cochran's Q test at a given significance level and the percentage of total variability across the studies attributable to heterogeneity was quantified by using I-square value.

Results: The magnitude of the drug exposure increase in comparison to NM is presented as Odds ratio [95% Confidence interval]. Escitalopram exposure was 1.37-fold [1.30-1.44] increased in CYP2C19 IM and 2.44-fold [2.27-2.61] increased in CYP2C19 PM. Venlafaxine exposure was not significantly changed in CYP2D6 PM, 1.10 [0.99-1.22]. Risperidone and aripiprazole exposure increase was similar for CYP2D6 IM and PM. Risperidone exposure was 1.42 [1.36-1.51] increased in CYP2D6 IM and PM admixed. Aripiprazole exposure was 1.52 [1.45-1.58] increased in CYP2D6 IM and PM admixed.

Conclusions: According to the results, (1) reducing escitalopram dose by 60% in CYP2C19 PM and by 30% in CYP2C19 IM are appropriate dosing decisions, (2) reducing risperidone and aripiprazole dose by 30% in CYP2D6 PM is appropriate dosing decision, and (3) CYP2D6 metabolizer status does not seem to be a clinically relevant feature in venlafaxine dosing.

Keywords: Antidepressant, Antipsychotic, Pharmacokinetics, Pharmacogenetics, Precision Medicine Neuropsychiatric Diseases

Disclosure: Nothing to disclose.

M118

Neurofilament Light Protein as Potential Biomarker of Treatment Resistant Major Depression**Susanne Spanier*, Hannah Kilian, Dora Meyer, Thomas Schlaepfer***Medical Center - University of Freiburg, Freiburg, Germany*

Background: Treatment resistant major depression is

accompanied with a sizable impact on quality of life with severe consequences for social integrity, individual health and socio-economic state. Lacking effective and well-established treatment strategies for these severe affected patients in- and outpatient care remains challenging for both patients and the health system. Therefore, novel research focuses are needed. Biomarkers reliably reflecting neuropathological processes could help to understand the actual mechanisms in treatment resistance leading to innovative and more effective treatment options. Due to novel imaging techniques and new ways of neuromodulatory interventions mood disorders are commonly regarded as dysfunctions of neural networks. One important substrate of neural networks is obviously the axon. There is evidence that major depressive disorder (MDD) might be associated with axonal damage. For a short time only one is able to detect correlates of axonal damage in plasma via the specific biomarker neurofilament light protein. Neurofilament light protein is an abundant part of the axonal cytoskeleton and can be released into the cerebrospinal fluid (CSF) following axonal damage. Recent technological advancements enable its determination in very low concentrations not only in cerebrospinal fluid but also in plasma. Plasma levels of neurofilament light protein have already been discussed as a reliable biomarker of neuroaxonal damage in neurological and neurodegenerative diseases. Due to accumulating evidence that major depression is associated with axonal damage, treatment resistance in major depression may be correlated with lasting axonal damage within circuits processing affective responses. This axonal damage could be reflected by increased plasma levels of neurofilament light protein.

Methods: To evaluate our hypothesis we obtain plasma samples of 32 patients with severe treatment resistant major depression participating in an efficacy study of deep brain stimulation of the superolateral branch of the medial forebrain bundle (slMFB) - FORESEE III (Controlled Randomized Clinical Trial to assess Efficacy of Deep Brain Stimulation (DBS) of the slMFB in Patients with Treatment Resistant Major Depression). The FORESEE III study is a randomized, sham-controlled, double blind (patient and observer blinded) clinical trial to assess the antidepressant effect of DBS compared to sham.

Plasma samples are obtained before neurosurgery as well as at several time points during DBS and sham condition intervals. The plasma samples will be analyzed with the single molecule array (SiMoA) technology to determine plasma concentrations of neurofilament light protein. The results will be correlated with clinical response parameters of the FORESEE III study and will be compared to plasma levels of neurofilament light protein of healthy controls.

Results: Results of baseline samples of the first 10 patients will be presented in December 2019.

Conclusions: There is a significant need for sensitive biomarkers detecting and reflecting well defined neuropathological mechanisms in treatment resistant mood disorders. Measuring plasma levels of neurofilament light protein in patients with treatment resistant major depression could not only shed more light on neuropathological mechanisms of treatment resistance but also helps to establish objective criteria for neurobiological based distinctions between treatment resistant depression and the larger disease category of depression it contains. The strong correlation between CSF and plasma concentrations make neurofilament light protein superior to other biomarkers like proinflammatory cytokines. Establishing the characteristics of a sensitive and low invasive biomarker neurofilament light protein could be not only a promising biomarker for a distinct diagnostic use in treatment resistant major depression but also for the purpose of monitoring disease process and treatment response.

Keywords: Treatment-Resistant Depression, Neurodegeneration, Biomarker

Disclosure: Nothing to disclose.

M119

Inflammation Associated Tryptophan Metabolites and Memory Performance in Depression

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Background: The presence of abnormal immune activity has frequently been implicated in depressed mood and especially major depressive disorder. One theory in particular suggests that inflammatory cytokines such as interleukin-6 can activate a set of neurobehavioral changes referred to as "sickness behavior" but that the depression represents the activation of sickness with a specific downstream pathway metabolizing tryptophan. In this model inflammation increases the activity of indoleamine-2,3-dioxygenase (IDO) which increases the conversion of tryptophan (TRP) to kynurenine (KYN). When kynurenine enters the brain, it is further metabolized down two arms, producing kynurenic acid (KYNA) on the one hand, and quinolinic acid (QUIN) on the other. Activated microglia produce quinolinic acid so under inflammatory conditions the relative amount of quinolinic acid increases. This is thought to produce neurotoxicity leading to depression, and in certain relatively narrow contexts (i.e. cytokine therapies) it has been shown convincingly that tryptophan metabolism plays a role in the progression from fatigue and other sickness symptoms to a full depression syndrome. Previous studies have also shown that this pathway may predict alterations in cognition, and in one case that increased quinolinic/kynurenic acid ratios correlated inversely with hippocampal volume. Based on this data, we hypothesized that KYN/TRP and KYNA/QUIN ratios would predict cognitive performance in depression. Specifically, we predicted that associative memory, mediated by the hippocampus, should be affected, and that performance would decrease as the inflammation associated ratios (KYN/TRP and QUIN/KYNA) increased.

Methods: 80 subjects, adults age 18-60 with unmedicated, SCID validated, major depressive episodes underwent memory testing with a face-name associative recognition task in which pairs of faces and names were shown to them either 1 or 3 times in a teaching phase, and then were tested on their recall of the correct pairings by showing three types of test items. 1) intact pairings in which the face and name were correctly matched, 2) rearranged pairings in which both the face and name were studied but incorrectly matched, and 3) new pairings in which both the face and name were not studied. Performance data was processed to calculate the metric D' reflecting the ability of each subject to discriminate between correctly paired and rearranged face-name pairs for stimuli shown 1 and 3 times in the teaching phase of the task. In healthy adults memory performance for items with three teaching exposures is superior to that for a single exposure and this difference declines with age. Subjects underwent phlebotomy for plasma collection on the morning of the same day, and were fasting. Plasma was analyzed for TRP and its metabolites KYN, KYNA and QUIN. Ratios of KYN/TRP and QUIN/KYNA were calculated. Linear regression was applied to KYN/TRP and QUIN/KYNA as predictors, with D' for each class of item used as the response variables.

Results: In our sample subjects performed overall as expected with better recall of face-name pairs presented 3x as compared to 1x. KYN/TRP ratio was not associated with task performance but QUIN/KYNA ratio predicted performance on trials of face-name pairs with 3x exposure in the study phase. QUIN/KYNA correlated with d' for rearranged 3x items with $\rho = -0.27$ and $p = 0.015$,

and d' for intact 3x items with $\rho = 0.074$. No correlations for items presented 1x were significant.

Conclusions: Our results supported our hypothesis that inflammation induced imbalances in the tryptophan metabolic pathway may play a role in the symptomatology of MDD and specifically in cognitive function. Our results were analogous to findings in healthy aging (although our sample had an upper age cut off of 60 to minimize age related memory declines) that show that inflammation impairs the normal learning process in which repetitive exposure to stimuli improves associative. More detailed cognitive assessment in larger samples of patients may improve our understanding of the role of tryptophan metabolism in the biology of depression.

Keywords: Depression, Inflammation, Memory

Disclosure: Nothing to disclose.

M120

Molecular, Cellular, and Bioenergetic Actions of Ketamine and Lithium Combinatorial Treatment in Antidepressant Resistant Rats

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Background: Whether co-treatments can improve ketamine's efficacy in treatment-resistant depression is still relatively unexplored. Lithium is a common adjunctive treatment for refractory depression, as well as a mood stabilizer for bipolar disorder. Its molecular mechanisms overlap with those of ketamine and we have previously shown both are moderated by insulin signaling in treatment resistant rat models and human subjects. Here, we aimed to quantify the molecular, cellular, bioenergetic and behavioral effects of ketamine and/or lithium in a rodent model of antidepressant treatment resistance.

Methods: To establish an antidepressant-resistant phenotype, male Wistar rats were administered adrenocorticotrophic hormone (ACTH; 100ug/day, 14 days). Rats were subsequently treated with ketamine (10mg/kg; 2 days; $n = 12$), lithium (37mg/kg; 2 days; $n = 12$), ketamine + lithium ($n = 12$), or control vehicle saline (0.9%; $n = 12$) and underwent open field (6 minutes) and forced swim (6 minutes) tests. Thirty minutes after behavioral testing, rats were euthanized, and cardiac blood and brain tissue were immediately collected. Peripheral blood and prefrontal cortical tissues were isolated for molecular and bioenergetic analyses. Western blot and enzyme-linked immunosorbent assays (ELISAs) were performed to detect mammalian target of rapamycin (mTOR), glycogen synthase kinase-3 β (GSK3 β), and glutamate ionotropic receptor AMPA type subunit 1 (GRIA1) concentrations. Oxygen consumption (OCR) and extracellular acidification (ECAR) rates, a proxy of glycolytic rate, were assessed before and after exposure of tissues samples to brain-derived neurotrophic factor (BDNF), Wnt3a, and tumor necrosis factor α (TNF α) using a Seahorse XF analyzer.

Results: ACTH-pretreated rats receiving ketamine and lithium had significantly reduced immobility in the forced swim test relative to all other groups ($p < 0.05$). Open-field testing did not reveal significant pre-existing differences in locomotor activity between groups. Rats treated with both ketamine and lithium had higher plasma insulin than all other groups, together with associated expression of insulin signaling pathway proteins in brain and blood (peripheral mTOR and central mTOR and GSK3 β). Prefrontal GRIA1 expression was also increased in animals treated with ketamine and lit-

hium. Prefrontal tissue bioenergetics analyses revealed that respiratory response to exogenous glucose, mitochondrial substrates, and adenosine diphosphate were reduced in animals receiving ketamine or lithium. BDNF-stimulated ECAR and TNF α -stimulated OCR were increased in animals pretreated with lithium, while BDNF-stimulated OCR was reduced in ketamine pretreated animals. The OCR response to Wnt3a was reduced in animals pretreated with lithium alone or in combination with ketamine.

Conclusions: The combination of ketamine with lithium was effective in promoting insulin release, insulin signaling and GRIA1 expression in ACTH-pretreated antidepressant resistant rats compared to either ketamine or lithium alone. Each treatment variably impacted tissue bioenergetics, suggesting the antidepressant-like effects by ketamine-lithium co-treatment are not mediated through direct facilitation of cellular metabolism. These results suggest lithium augmentation will functionally enhance ketamine's promotion of insulin signaling, cellular plasticity, and its antidepressant effects in antidepressant-resistant individuals.

Keywords: Ketamine, Lithium, Treatment Resistant Depression, Antidepressant, Insulin

Disclosure: Nothing to disclose.

M121

Long-Lasting Kappa Opioid Receptor Desensitization Following the Administration of Ketamine

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Background: Patients suffering from intractable major depressive disorder (MDD) exhibit rapid and sustained alleviation of their symptoms following ketamine administration. Recent clinical studies suggest that the effects of ketamine may be mediated by the endogenous opioid system. Kappa opioid receptor (KOR) agonists cause negative behavioral affect and dysphoria in animal models and human volunteers. In these studies, we investigated the hypothesis that ketamine administration counteracts these effects, in part by producing a protracted desensitization of KORs that lasts beyond the acute effects of the drug. We examined whether (1) exposure to ketamine produces internalization of KORs in cell culture, and (2) exposure to ketamine leads to desensitized behavioral and electrophysiological responses caused by the activation of KORs 24 h after ketamine administration.

Methods: We assessed KOR internalization in HEK293 cells transfected with yPET-OPRK1 plasmids following stimulation for 6 h with the endogenous opioid peptide dynorphin, the selective KOR agonist U50,488 (U50), or ketamine. To test whether ketamine desensitizes responses induced by KOR activation, 8–12-week old male C57BL/6J mice were treated with ketamine (0, 10 or 20 mg/kg, i.p.) 24 h prior to assessment with U50 (0, 10 or 20 mg/kg, i.p.) on three different behavioral assays. Behavioral tests included nest building, prepulse inhibition (PPI), and the hot plate test. We also tested whether ketamine desensitizes KORs in the mouse lateral habenula (LHb) by utilizing ex-vivo electrophysiology in slices. Mice were treated with ketamine (10 mg/kg, i.p.) or vehicle 24 h prior to dissection. The number of action potentials produced following depolarizing current steps at baseline and following bath application of U50 (10 μ M) were then assessed in LHb neurons.

Results: Significant internalization of yPET-OPRK1 transfected into HEK293 cells was measured following incubation with dynorphin, U50, or ketamine. Levels of internalization were similar in magnitude across all three compounds. In the nest building test, exposure to U50 (10 mg/kg) suppressed final nest score values at 5 h ($p < 0.01$), but pretreatment with ketamine 24 h prior to U50 administration blocked U50-induced suppression of nest building ($p < 0.05$). U50 (20 mg/kg) administration impaired sensorimotor gating and pre-attentive sensory filtering on the PPI test when assessed after 15 min ($p < 0.05$). Pretreatment with ketamine 24 h prior to the U50 injection normalized U50-induced PPI impairment ($p < 0.01$). On the hot plate, an injection of U50 (20 mg/kg) induced antinociception when tested 15 min later ($p < 0.0001$), and ketamine given 24 h prior to testing blunted the antinociceptive effects of U50 ($p < 0.01$). KOR activation by U50 bath application significantly increased neuronal excitability in the lateral habenula, and this effect was abolished by ketamine pretreatment ($p < 0.0001$).

Conclusions: Our studies in cell culture confirm that acute ketamine acts like a KOR agonist and suggested that reduced KOR signaling after ketamine may influence behavioral responses associated with KOR activation. A pattern of desensitized KOR-mediated responses, on suppression of nesting behavior, reduction of PPI, and thermal nociception, emerged when mice were tested with U50 24 h after ketamine administration. Ketamine also produced long-term desensitization of KORs in the lateral habenula. This was observed by the ability of ketamine to block U50-induced increased LHB excitability. The LHB is an epithalamic nucleus associated with aversion, and increased activity of the LHB is associated with stress-related disorders. Furthermore, ketamine has been shown to reverse the hyperexcitability of the LHB in numerous early life and chronic stress paradigms. Because KORs have been associated with negative affect and aversion, a long-lasting reduction of KOR signaling following ketamine may partially explain the persistent clinical effects following ketamine administration.

Keywords: Depression, Ketamine, Kappa Opioid Receptors, Antidepressants, Lateral Habenula

Disclosure: Nothing to disclose.

M122

Lack of CASP1, IFNGR and Nos2 Genes Alter Behavior and Gut Microbiota

Abstract not included.

M123

Initial Human Pharmacokinetics and Positron-Emission Tomography (PET) Occupancy of BTRX-335140, a Selective Kappa Opioid Receptor (KOR) Antagonist

Abstract not included.

M124

Modulation of Amygdala Activity for Emotional Faces Due to Botulinum Toxin Type A (OnabotulinumtoxinA) Injections That Prevent Scowling

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Background: The “facial feedback hypothesis” suggests that creating emotional facial expressions influences the processing of emotional faces. Here, we sought to investigate the facial feedback hypothesis by using botulinum toxin type A (onabotulinumtoxinA; onabotA) injections to induce temporary paralysis in the muscles of the glabellar region (responsible for depressing and bringing the eyebrows together) and measure functional activity in the brain during the processing of emotional faces. Based on predictions from the facial feedback hypothesis, we hypothesized that the inability to draw the eyebrows down into a scowl may alter the emotional processing in the amygdala.

Methods: 10 females (ages 33–40 years old) participated a pre- and post-design: the first MRI scan session was collected 4–14 days prior (Pre) to the onabotA injection and the second was collected 13–23 days post-injection (Active). Participants received 20 units of botulinum toxin in the glabellar region from a trained physician. During scanning, participants viewed pictures of happy and angry faces during two functional magnetic resonance imaging (fMRI) scan sessions: one prior to onabotA injections and one following onabotA injections after the corrugator muscles were paralyzed.

Results: For our a priori hypotheses, we entered the average fMRI activity from the left and right amygdala for each subject into 2x2 repeated-measures ANOVA with Session (Pre vs Active) by Emotion (Happy vs Angry) as variables. The left amygdala showed a main effect of Session with greater fMRI activity in the onabotA-Active than onabotA-Pre condition ($F(1,9) = 6.8$, $p < .05$). We also found some evidence for a main effect of Emotion (Happy > Angry, $F(1,9) = 4.4$, $p = .07$), but no reliable evidence for an interaction ($F(1,9) = 2.7$, $p = .14$). In contrast, while the right amygdala showed a main effect of emotion (Happy > Angry, $F(1,9) = 5.5$, $p < .05$), it showed no effect of Session ($F(1,9) = 0.16$; $p = .70$) or interaction ($F(1,9) = 1.5$; $p = .24$). Thus, we found evidence consistent that the amygdala’s responsiveness to emotion can be modulated by inactivation of the corrugator muscles using onabotA. In an exploratory, whole-brain analysis, we found greater fMRI activity in onabotA-Active than onabotA-Pre ($F(1,9) = 63.2$, $p < .01$) in the right fusiform gyrus, a brain region involved in the processing of human faces.

Conclusions: In this small study, we found an increase in amygdala activity for both happy and angry faces following onabotA injections, suggesting that paralysis of the glabellar region affects central processing of viewing both happy and angry facial expressions. According to the facial feedback hypothesis, when we see an angry or happy face, we contract or flex the relevant muscles to recreate the expression to assist in identifying and experiencing the emotion reflected. Based on our results, the feedback from these muscle movements appear to contribute to processing the relevant emotion by the amygdala. Consistent with our predictions, preventing scowling through paralysis of the glabellar region altered amygdala processing for angry faces. Notably, we also found an effect for happy faces, suggesting that paralysis of this region impacted smiling or happy expressions, resulting in alterations in amygdala activity for happy faces as well. The modulation of amygdala and fusiform gyrus activity from the administration of onabotA may reflect compensatory processes engaged in a network of brain regions involved in emotional processing when facial feedback is impaired. While there remains much more to explore regarding the role of facial feedback on amygdala and fusiform gyrus activity for emotional faces, these data contribute to a growing body of literature suggesting that paralysis of facial muscles alters neural activity for emotional processing.

Keywords: Amygdala, Botulinum Toxin, fMRI, Emotion

Disclosure: Nothing to disclose.

M125

Acute Restraint Stress Disrupts Reward Seeking by Reorganizing VTA-NAc Communication

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Background: Stressful experiences frequently precede depressive episodes, yet the mechanism by which stress leads to depression remains unknown. Anhedonia, or disrupted reward seeking, is a core symptom of depression that is present in most depressed patients. In rodents, stress also disrupts reward seeking. However, the neural basis by which this occurs remains unclear. Here, we tested if stress-induced neural activity in the ventral tegmental area (VTA) and nucleus accumbens (NAc) disrupts subsequent reward processing.

Methods: We trained mice to associate a tone with water reward availability (CS+) and another tone with no water reward (CS-) to a criterion of 70 percent correct responses to the CS+ for two consecutive days. After reaching criterion, mice were either stressed (30 minutes of restraint) or unstressed (30 minutes in a familiar environment). To determine the effect of acute stress on the VTA-NAc circuit, we simultaneously recorded single unit and local field potential (LFP) activity in the VTA and LFP activity in the NAc of mice during restraint, familiar environment, and subsequent reward processing. We expressed opsins (channelrhodopsin and archaerhodopsin) by injecting AAV vectors into the VTA of VGAT-cre, VGLUT-cre and DAT-cre mice. We analyzed the data using custom MATLAB scripts.

Results: We found that stress reduced lick rates during the anticipation phase of a cue-reward task ($F=7.417$, $p < 0.05$, ANOVA, $n = 11$ mice). During restraint, a prominent low-frequency oscillation emerged in the NAc that was not seen in the familiar condition (Wilcoxon sign rank test, $p < 0.001$; $n = 21$ mice). Lag analysis of VTA multi-unit activity revealed that VTA activity precedes this oscillation, and pharmacological inhibition of the VTA with muscimol prevents this oscillation. Optogenetic inhibition of neuronal subtypes within the VTA further revealed that VTA GABA neurons contribute to this stress-induced oscillation. We therefore hypothesized that optogenetic stimulation of VTA GABA neurons at the oscillation frequency would produce similar effects to restraint stress. Indeed, we found that this manipulation increased NAc LFP power at the stress-induced oscillation frequency and produced reward processing deficits similar to those observed after restraint stress. Finally, we find that stress caused a persistent disruption of VTA neural activity underlying reward anticipation.

Conclusions: These data suggest that stress alters VTA-NAc communication, impairing subsequent reward processing.

Keywords: Acute Stress, Reward, Circuit, Synchrony

Disclosure: Nothing to disclose.

M126

Behavioral Response to Fluoxetine is Mediated by Dentate Gyrus Inhibition

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Background: Depression is a complex psychiatric disorder that is a major burden on society, with 33% of depressed patients attaining remission upon initial monotherapy with a selective serotonin reuptake inhibitor (SSRI). In preclinical studies using mice, chronic stress paradigms, such as chronic corticosterone and chronic social defeat stress, are used to induce negative valence behaviors. Chronic fluoxetine (FLX; an SSRI) treatment reverses these stress-induced behavioral changes in some, but not all mice, permitting stratification of mice into behavioral responders and non-responders to FLX. Recently, we reported that 5-HT_{1A} receptors, which are Gi-coupled inhibitory receptors, on mature granule cells (GCs) in the dentate gyrus (DG), are necessary and sufficient for the behavioral, neurogenic, and neuroendocrine response to chronic SSRI treatment.

Methods: Since inhibition of mature DG GCs through cell autonomous Gi-coupled receptors is critical for mounting an antidepressant response, we predicted that behavioral response to FLX would correlate with a decrease in DG GC activation compared to FLX non-responders and stress controls. Additionally, we wanted to assess whether chronic functional manipulation of DG GC activity via the usage of DREADDs could mimic antidepressant effects.

Results: Intriguingly, male and female behavioral responders show decreased DG GC activation (as measured by cFos immunostaining) relative to non-responders and stress controls. We show in both sexes that chronic inhibition of ventral DG GCs (through usage of Gi-DREADDs) results in a decrease in negative valence behaviors compared to stress controls. Furthermore, following stratification into FLX responders and non-responders, using the Novelty Suppressed Feeding, we converted FLX non-responders into responders via activation of Gi-DREADDs in the ventral DG GCs. Activation of ventral DG GCs via Gq-DREADD converted FLX responders into non-responders.

Conclusions: Taken together, these results illustrate that inhibition of DG GCs is a critical component of the response to antidepressants.

Keywords: Treatment Resistant Depression, Dentate Gyrus, DREADDs

Disclosure: Nothing to disclose.

M127

Insulin Resistance and Structural Change in the Anterior Cingulate Cortex in Youth With Depression and Obesity

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Background: Insulin resistance is an inflammatory metabolic state commonly associated with excess adiposity that can have deleterious mental and physical health consequences. Although emerging research suggests a bidirectional biobehavioral link between insulin resistance and depression, we have limited understanding of the underlying neurobiological mechanism. One way in which insulin resistance and depression may be linked is through shared or cumulative effects on brain structure, and in particular the anterior cingulate cortex (ACC). The ACC may be important for understanding the compounding effects of insulin resistance and depression, as research indicates aberrant reward processing in both conditions. Indeed, previous research documented a robust association between depression and ACC structural abnormalities in youth. Moreover, preliminary research

suggested a cross-sectional association of insulin resistance with ACC structural abnormalities in youth with depression. However, the prospective effects of insulin resistance combined with depressive symptoms, on the structure of the ACC in the developing brain, are unknown. To extend upon prior cross-sectional findings, the goal of the present study was to test the prospective associations of markers of insulin sensitivity after an oral glucose challenge on ACC volume in youth with depression and obesity.

Methods: 62 youth aged 9 to 17 (mean = 14.70, SD = 2.18 years) with currently untreated moderate-to-severe depression (Children's Depression Rating Scale-Revised [CDRS-R] score > 35) and overweight or obesity (mean BMI-z = 1.77, SD = .55) completed baseline and 6-month follow-up visits as part of a longitudinal study of brain and behavioral correlates of insulin resistance in depression. Participants completed T1-weighted structural MRI scans, tests of insulin resistance (fasting and post-glucose challenge [Matsuda index]), and clinical assessments (e.g., CDRS-R, BMI-z score) at both timepoints. Repeated measures ANOVAs were used to test changes in ACC volume from baseline to 6-month follow-up, with level of insulin resistance (high versus low) entered as a between-subjects factor to explore whether ACC volume changed as a function of insulin resistance. Baseline age, sex, BMI-z score, depressive symptoms (CDRS-R T-score), and total intracranial volume were entered as covariates. Two separate repeated measures ANOVAs were conducted, given that we tested how two different measures of baseline insulin resistance (fasting and Matsuda index) impacted ACC volume. Alpha level was set to $p = 0.025$ ($0.05/2$) to adjust for two statistical tests.

Results: With insulin levels during fasting state and prior to glucose challenge, there was a nonsignificant, trend-level group by time interaction for ACC volume ($F(1, 36) = 3.582$, $p = 0.066$), such that ACC volume decreased with time in those with high levels of fasting insulin resistance. For post-glucose challenge insulin levels, there was a significant group by time interaction for ACC volume ($F(1, 36) = 6.743$, $p = 0.014$), such that ACC volume decreased over time in those with high levels of post-glucose challenge insulin resistance.

Conclusions: Our results demonstrate that high post-glucose challenge insulin resistance showed significant associations with decreased ACC volume at 6-month follow-up in youth with depression and overweight or obesity. However, our results should be interpreted with caution due to yet limited longitudinal follow up to demonstrate structural plasticity effects of insulin resistance in youth. Given that brain structure changes slowly over time, longitudinal follow-up studies investigating the impact of insulin resistance on the developing brain over longer durations of time are needed.

Keywords: Insulin Resistance, Depression, Obesity, Structural MRI, Anterior Cingulate Cortex (ACC)

Disclosure: Nothing to disclose.

M128

Genetic vs Non-Genetic Determinants of Sleep in the Amish

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Background: Sleep is essential and fundamental to the biology of the human brain with well-established neural circuits; thus, it is likely tightly regulated by genetics. Large genetics studies on sleep have reported exciting findings using phenotyping methods

ranging from actigraphy recording, self-reports, insomnia and other sleep disorder diagnoses, to chronotypes, although there is a lack of replications in many findings (Jones et al 2019; Jansen et al 2019; Dashti et al 2019; Stein et al 2018; Kalmbach et al 2017). Sleep is a complex behavior when measured in modern society and also strongly influenced by stress levels and mental health. Adequate controls for these factors may elucidate the genetics of sleep biology. Previous studies attempted to control these influences by studying sleep in the Amish, a rural population with no or limited modern electrical devices (Evans et al 2011), and have found that wake time, self-reported morningness-eveningness preference, and daytime sleepiness to have significant but relatively low heritability (h^2) around 0.2. Building on these initial findings, the current study attempted to estimate the heritability of sleep parameters using the Pittsburgh Sleep Quality Index (PSQI), a self-report that more comprehensively captures sleep quality using measures of duration, efficiency, latency, disturbance, sleep medication, and daytime dysfunction. Further, given the relatively low expected heritability, we sought to capture non-genetic factors in cumulative life stressors, current stress, and mood disorders, and tested whether they independently contributed to sleep quality and its heritability.

Methods: The sample included 335 Old Order Amish/Old Order Mennonite (OOA/M) individuals (224 controls, 90 patients with mood disorders including 20 with bipolar disorder, 70 with major depression) recruited from Pennsylvania and Maryland areas as part of the ongoing Amish Connectome Project. Structured Clinical Interview for DSM-IV was completed to verify lifetime and current psychiatric diagnoses. Current subjective stress level was assessed by the Perceived Stress Scale (PSS); past lifetime traumatic events were accounted by a Lifetime Stressor Inventory (LSI). We examined the effect on PSQI of cumulative life stressors, current stress levels, mood diagnosis, age and sex using linear regression, and we estimated the h^2 of PSQI.

Results: Patients with mood disorders ($n = 90$) had significantly higher cumulative life stressors and current stress levels compared to healthy controls ($n = 224$) (both $p < 0.001$). Cumulative life stressors, current stress levels, and mood disorders all significantly and independently contributed to PSQI score in linear regression (all $p < 0.05$). H^2 of PSQI total and subcomponent scores were low, ranging from 0 to 0.21 after covarying for age and sex, which was consistent with previous heritability estimates using different sleep phenotyping approaches in this population.

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

Keywords: Human Genetics, Sleep, Acute and Chronic Stress

Disclosure: Nothing to disclose.

M129

Activation of Excitatory Ventral Tegmental Area Projections to the Locus Coeruleus Drives Affective Behaviors

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Background: More than 40,000 Americans commit suicide each year. Many of these victims suffered from stress-induced Major

Depressive Disorder (MDD) and anxiety disorders. Many individuals of this susceptible population are particularly at risk, as current monoamine treatments for depression do not alleviate symptoms in one third of MDD patients. Further, many MDD patients have increased expression of N-methyl-D-aspartate (NMDA) glutamate receptors in the locus coeruleus (LC), the main source of norepinephrine (NE) for the mammalian forebrain. Importantly, the LC is known to modulate stress-induced anxiety and resilience to chronic stress and is positioned as an important modulator of stress-induced MDD. While glutamatergic modulation of LC-NE neurons is often associated with behavioral flexibility and attention, data from suicide victims points to an important role of glutamate signaling in the LC-NE system in modulating affective disorders. Since previous studies have shown that blocking NMDA receptors has rapid antidepressant-like effects in humans and animal models, we investigated the neurocircuitry of traditional monoamine pathways and the role glutamate has in coordinating depressive-like phenotypes in mice. Specifically, we hypothesized that excitatory projections to the LC may modulate behaviors related to stress, depression, and anxiety while changes in NMDA receptor expression following chronic mild stress may exacerbate these behaviors in mice.

Methods: To examine functional connectivity of glutamatergic projections we used anterograde tracing and optogenetic stimulation of afferent LC inputs. Adult male and female C57BL/6J mice were injected with AAV5-CAMKII α -ChR2-eYFP ($n = 7$) and vGLUT2-Cre mice with AAV5-ef1a-DIO-ChR2-eYFP ($n = 8$) or AAV5-ef1a-DIO-eYFP ($n = 7$) into the VTA (AP -3.1, ML + 0.5, DV -4.9) and four weeks later implanted with a fiber optic into the LC (AP -5.45, ML + 1.0, DV -3.35). Following recovery, animals were tested in a series of behavioral paradigms including a Real-Time Place Test (RTPT), Open Field Test (OFT), and Elevated Plus Maze (EPM). For RTPT, mice were placed in a rectangular arena consisting of two adjacent square compartments in which one side gave optogenetic stimulation upon entry and remained constant until the animal exited. Different frequencies (0, 1, 5, 10 or 20 Hz, 10ms, 470nm) were examined over five counter-balanced trials. The time spent in the stimulation-paired side was used to determine place preference. For OFT, mice were placed in an open, square arena and allowed to freely explore for 21 mins. Optogenetic stimulation (5Hz, 10ms, 470nm) alternated over 3-min intervals. For EPM, mice were allowed to freely explore a plus maze containing two open arms and two closed arms for 15 mins. Animals received optogenetic stimulation (20Hz, 10ms, 470nm) in alternating 5-min intervals. For chronic mild stress (CMS) experiments, mice ($n = 10$) were treated to a series of seven stressful events (removal of nesting for 24 h, 5 min forced swim stress at 15°C, 8 hr food and water deprivation, damp bedding overnight, white noise, cage tilt, and disrupted home cage lighting) were randomly counter-balanced and repeated over a 3-week period. Control mice ($n = 10$) were housed and maintained in separate cages alongside CMS treated mice. Following CMS, all mice were euthanized and *in situ* hybridization was performed to examine LC expression of NMDA receptor subunit genes *Grin2b* and *Grin2c* and metabotropic glutamate receptor genes *Grm4* and *Grm5*.

Results: For optogenetic experiments, stimulation of VTA glutamatergic LC inputs at higher frequencies (10 and 20Hz) distinctly drove real-time place avoidance in CAMKII-ChR2 injected wildtype mice ($p < 0.05$, One-way ANOVA) and vGlut2-ChR2 mice ($p < 0.05$, two-way ANOVA). EPM tests revealed that animals spent less time exploring open arms during optogenetic stimulation as compared to no stimulation intervals ($p < 0.01$, one-way repeated measures ANOVA). In contrast, open field locomotion tests revealed increased exploratory behavior in the center zone of the arena during optogenetic stimulation ($p < 0.05$, paired t-test). Additionally, CMS did not alter expression of NMDA receptor subunit genes *Grin2b* and *Grin2c* and metabotropic glutamate

receptor genes *Grm4* and *Grm5* in the LC as compared to control animals (ns, Student's t-test).

Conclusions: Here we unveiled a prominent VTA glutamatergic LC afferent projection and examined this projection using *in vivo* optogenetics. By implementing behavioral assays including real-time place testing, elevated plus maze, and open field locomotion testing, we determined that this pathway plays a prominent role in mediating the LC's coordination of negative affective behaviors. Despite no change in specific mRNA expression of specific genes investigated here following CMS, further examination of this population of LC projecting neurons via *in vivo* fiber photometry and novel behavioral assays are warranted. These data could provide more precise observation of this projection in real time within more clinically relevant models of depression.

Keywords: Ventral Tegmental Area (VTA), Locus Coeruleus (LC), Glutamate, Chronic Mild Stress, Negative Affect

Disclosure: Nothing to disclose.

M130

Extreme Weather Events and the Onset of Psychotic Depression

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Background: Weather parameters are known to influence mood, behavior, and symptom severity in various disease states. Seasonal Affective Disorder is a well-established phenomenon. Additionally, a small number of investigators have found that admission rates for bipolar disorder (Shapira et al., 2004) and suicide frequency (Maes et al., 1994) both positively correlate with ambient temperature, while violent acts and emergency psychiatric visits both correlate with lowered barometric pressure as seen in advancing storm fronts (Schory et al., 2003.) Weather parameters are also observed to influence onset and symptom severity in various neurological conditions. For example, increased ambient temperature is a common trigger for many ailments, including those psychiatric, in multiple sclerosis and other neuroinflammatory conditions. This is thought to be due to Uhthoff's Phenomenon, the observation that heat decreases the speed of nerve conduction, especially in areas of demyelination (Frohman et al, 2013.) Furthermore, low barometric pressure likely induces migraines in susceptible individuals (Kimoto et al, 2011.) Given a single European investigator found an association between lowered barometric pressure and the incidence of major depression with psychotic features (Radua et al, 2007,) as well as the above evidence for the influence of weather on both psychiatric and neurological conditions, the purpose of this study is to evaluate further the effects of various changing weather parameters on the onset of psychotic depression.

Methods: The study sample consisted of 259 subjects (both male and female) with psychotic depression who participated in the National Institute of Mental Health STOP-PD (The Study of Pharmacotherapy of Psychotic Depression) between December 2002 and June 2007. Weather events were examined in relation to these subjects' onset dates. Weather data was obtained from the National Oceanic and Atmospheric Administration (NOAA). Daily mean weather variables (temperature, dew point, barometric pressure, wind speed, precipitation, cloud cover) were calculated for each of the 60 days prior to a patient's onset. Extreme weather

events (defined as 2 to 4 standard deviations from the daily historical mean (from 1980-1999) to control for seasonality and patient acclimatization) were then tabulated for each patient's pre-onset period. After examination of the distribution of the extreme weather events, we created 8 risk periods of 7 days each (total of 56 days) prior to the onset of the psychotic episode. We then fit a longitudinal logistic regression model with the psychotic episode as the binary (yes/no) outcome as predicted by a single term for risk periods (1-8 with 1 being the risk period containing the psychotic episode), an indicator for an extreme weather event, and an interaction term for risk period and extreme weather event. The single term for risk period allowed us to calculate a consistent change in odds ratio of a psychotic episode over risk periods.

Results: All of the extreme weather events that were 3 or 4 standard deviations away from the 20-year average indicated a significant relationship with the occurrence of a psychotic episode. The weather events that were 4 SD away from the average (with odds ratio [OR] for interaction term as described above) included: temperature (OR=1.37), wind speed (OR=1.42), dew point (OR=1.41), atmospheric pressure (OR=1.32), precipitation (OR=1.36), and cloud cover (OR=1.39). All ORs were significantly different from 1.0 (all $p < 0.0001$). Thus, extreme weather events close to the psychotic episode were highly predictive of the outcome episode.

Conclusions: While the mechanisms at this point are largely speculative and the implications for the treatment of psychiatric illness require further research, the present results demonstrate extreme weather events as a factor in the onset of psychotic depression. Given the known role of temperature in nerve conduction and the neurological symptoms including headache seen with pressure changes, these results also support a physiological, neurological and neuroinflammatory basis for psychotic depression.

Keywords: Weather, Depression, Psychotic

Disclosure: Nothing to disclose.

M131

The Effects of High Frequency Repetitive Transcranial Magnetic Stimulation (HF-rTMS) on Emotion Regulation in Major Depressive Disorder

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Background: Repetitive transcranial magnetic stimulation (rTMS) has demonstrated efficacy in the treatment of depressive symptoms. However, the effects of rTMS on transdiagnostic domains of dysfunction relevant to the development and severity of depressive symptoms are not well characterized. Emotion regulation deficits are a core feature of mood and related disorders and are associated with greater mood symptom severity and worse treatment outcomes. In the current study, we examined the effects of rTMS treatment on emotion dysregulation symptoms, behavior and relevant neurocircuitry using resting state fMRI, psychometric measures and a behavioral task, in order to better understand the effects of rTMS on this core disorder dimension.

Methods: Resting state fMRI, task and self-report questionnaire data were collected from 37 patients (20 female, mean age 44) receiving a course of high frequency (HF) rTMS (36 sessions, 10Hz to left DLPFC). Depressive symptoms were assessed using the Hamilton Depression Scale (HAM-D-17). Self-reported affective

control and emotional lability were measured using the Affective Control Scale (ACS) and NEO Five Factor Personality Inventory Neuroticism scale (NEO-N). To measure implicit emotion regulation, we used a modified version of the Multisource Interference Task overlaid on affective images from the International Affective Picture System (MSIT-IAPS). During each trial, a three-digit number is presented for 1.7 seconds on the screen. Each set contains two identical distractor numbers and a target number that differs from the distractors. Participants identify with a button press the target number that differs from the distractors. During Noninterference (control) trials, distractor numbers are always zeros, and the identity of the target number always corresponds to its position on the button response pad (100, 020, 003). By contrast, during Interference trials, distractor numbers are always numbers other than 0, and the identity of the target number is always incongruent with its position on the button response pad (e.g. 211, 232, 331, etc.). Each trial of the MSIT is overlaid on a negative, positive, or neutral image from the International Affective Picture System (IAPS), counterbalanced between the control and interference conditions. Thus, participants are required to regulate their attention away from distracting emotion-provoking stimuli in order to perform task goals, a proxy for implicit emotion regulation. Reaction time (RT) during each trial was modeled using a generalized mixed effects linear model (GLMM) with a Gamma distribution. Resting state fMRI data were analyzed using FreeSurfer.

Results: At baseline, patients endorsed moderate to severe levels of depression (HAM-D-17=17.62(5.57)) clinically significant levels of emotion dysregulation (Mean ACS = 3.88(.79)), and emotion lability (mean NEO-N = 34.46(8.01)), and showed significantly slower response time (RT) on the MSIT-IAPS during interference trials overlaid on negative images (Negative Interference) relative to neutral images ($t = 3.25$, $p = .001$). Slower RT to Negative Interference trials was significantly correlated with weaker dorsal anterior insula (dAI) – ventrolateral prefrontal cortex (VLPFC) functional connectivity ($r = -.55$, $p = .009$). Treatment with HF-rTMS resulted in significant reductions in depressive symptoms (HAM-D-17: $t = 5.62$, $p < .00001$), and improvements in perceived affective control (ACS: $t = 3.58$, $p = .0002$), and emotion lability (NEO-N $t = 3.01$, $p = .0006$). TMS-related changes in affective control significantly predicted changes in depressive symptoms ($\beta = .52$, $p = .008$). Patients showed significant improvements in RT to Negative Interference trials pre- to post TMS ($t = -22.51$, $p = 2.0E-16$). TMS-related changes in RT to Negative Interference trials was significantly correlated with changes in functional connectivity between the DLPFC TMS target site and the Yeo et al (2011) defined Limbic Network ($r = -.72$, $p = .001$). Post-TMS dorsal AI-VLPFC functional connectivity significantly predicted RT to Negative Interference ($\beta = -.47$, $p = .03$).

Conclusions: Treatment with 36 sessions of HF-rTMS to the left DLPFC resulted in significant improvement in emotion regulation-related task behavior and self-reported affective control. These changes were significantly related to pre- to post-TMS changes in functional connectivity between the DLPFC target site and limbic networks relevant to emotion regulation. This suggests one mechanism by which HF-rTMS may lead to symptom improvement in depression is through improvements in the neurocircuitry supporting emotion regulation, with observable effects on behavior and self-report. Results from the current study also evidenced a significant relationship between anterior insula-VLPFC functional connectivity and performance on the affective interference task both at baseline and post-treatment, confirming previous evidence that has shown a significant role for the VLPFC in emotion regulation. Future studies will examine the relative effects of DLPFC versus VLPFC stimulation on emotion regulation improvements in mood disorders.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Emotion Regulation, Major Depressive Disorder
Disclosure: Nothing to disclose.

M132

Indexing Cortical Reactivity With TMS in Major Depressive Disorder and Response to Therapeutic Brain Stimulation

Abstract not included.

M133

Lateral Habenula Circuits: Using Intersectional Expression of DREADDS and RiboTag to Define Their Roles in Stress

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Background: The lateral habenula (LHb) processes information about aversive experiences that contributes to the symptoms of stress disorders. There are three major outputs from LHb that are entirely segregated: to dorsal raphe nucleus (DRN), ventral tegmental area (VTA) or rostromedial tegmentum (RMTg). The molecular phenotype and function of the neurons comprising these discrete pathways is not known.

Methods: We used an intersectional viral vector strategy in rats by selectively transducing the three different output pathways from LHb by injecting AAV8-DIO-hM4Di, AAV8-DIO rM3Dq (coding for Gi- or Gq-coupled DREADDS) or AAV8-DIO-RiboTag (allowing immunoprecipitation and deep sequencing of ribosome-associated RNAs) into LHb and CAV2-Cre (a retrograde viral vector) into DRN, RMTg, or VTA. We used a combination of repeated forced swim stress and several behavioral outcome measures to analyze the behavioral effects of activating or inhibiting each pathway. We used DESeq2, WGCNA, and clustering algorithms to analyze differential expression of RNAs in the individual pathways in unstressed and stressed rats.

Results: Using the forced swim test, we found that chemogenetic inhibition of DRN-projecting LHb neurons reduced passive coping (immobility), whereas inhibition of the other pathways did not. Chemogenetic activation of DRN-projecting neurons using hM3Dq in another cohort did not further exacerbate immobility. We next examined the impact of inhibiting DRN-projecting LHb neurons on reward sensitivity, perseverative behavior, and anxiety-like behavior using saccharin preference testing, reward omission testing, and open field testing, respectively. There was no effect of inhibiting any of these pathways on reward sensitivity, locomotion or anxiety-like behavior, but inhibiting DRN-projecting LHb neurons reduced perseverative licking during reward omission testing whereas activating these neurons increased perseverative licking. These results support the idea that inhibiting LHb projections to DRN provides animals with resilience during highly stressful or frustrating conditions but not under low-stress circumstances, and that inhibiting these neurons may promote persistence in active coping strategies.

Using RNA-seq of the “translatome” from each pathway, we found that the differences between pathways was modest, but the neurons projecting to DRN and VTA were most divergent, with those projecting to RMTg being more intermediate in their molecular phenotype. We are currently validating the RNA differences that were detected between pathways in unstressed and stressed rats.

Conclusions: The DRN-projecting LHb neurons seem to be particularly involved in producing behavioral changes after stress. Our future experiments will attempt to characterize the impact of these pathways on cognition and decision making under low and high stress contexts.

Keywords: Acute Stress, RNA-Sequencing, Dual Viral Approach, Lateral Habenula

Disclosure: Nothing to disclose.

M134

Data-Driven Subgroups of Patients With Major Depressive Disorder Have Distinct Neural Correlates and Respond Differentially to Antidepressant Treatment: Findings From EMBARC Study

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Background: The heterogeneous syndrome of major depressive disorder (MDD) likely includes subgroups of patients with distinct pathophysiological mechanisms and neural circuit dysfunction. Yet, the current practice of identifying subgroups of patients relies heavily on depressive symptom severity or presence of distinct symptom clusters such as anxiety or anhedonia. However, the current approaches of subgrouping patients have been unsuccessful in predicting differential treatment outcomes to one medication versus another. In this report, we aimed to identify clinically homogeneous MDD subgroups using data-driven statistical methods, evaluate their utility in predicting response to antidepressant versus placebo, and elucidate the underlying neural correlates.

Methods: We included participants of Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care for Depression (EMBARC) study with MDD (n = 241) and healthy controls (HC; n = 40) in this report. MDD participants were randomized to 8-week double-blind course of sertraline or placebo. Both MDD and HC participants underwent detailed clinical assessment and magnetic resonance imaging (MRI). We used principal component analysis to identify shared dimensions across 27 clinical measures (depression-associated symptom domains, childhood trauma, personality traits, psychiatric and medical comorbidities, and functional outcomes). We decided to exclude measures of depression severity (17-item Hamilton Rating Scale for Depression, [HAM-D-17] and 16-item Quick Inventory of Depressive Symptomatology Self-Report [QIDS-SR]) as they were used as either outcome measures or eligibility criterion. We then grouped individual participants based on the principal component (PC) to which they contributed the most to (defined as loading score > median). As loading scores for each PC could be positive (+) or negative (-), this resulted in two sub-groups per PC. We compared the clinical outcomes (change in HAM-D-17) among these groups based on treatment arm. We used functional MRI to compute resting state functional connectivity (FC) after parcellating cortical and subcortical regions in 121 parcels with a total of n = 7260 FC pairs. To identify the neural dysfunction, we compared each subgroup of participants with MDD to HC with two-sample t-test. We used p < 0.05 after False Discovery Rate (FDR) adjustment as threshold of statistical significance.

Results: We found that 4 principal components explained the most variance in data (.). Thus, participants were grouped into

9 subgroups; two per PC and an additional. Thus, we grouped participants in the following groups (two per PC): PC1+, PC1-, PC2+, PC2-, PC3+, PC3-, PC4+, PC4-, and PC5 (additional subgroup of those who did not load on first 4 PC). We found three subgroup of participants [those with higher levels of manic symptoms (PC2-), lower levels of childhood trauma (PC3+), and higher scores on personality traits of extroversion, agreeableness, and openness (PC4-)] who had markedly greater improvement with placebo than sertraline (>3 points higher reduction on HAM-D-17). While the individual connectivity patterns of these subgroups differed among each other and from other sub-groups, increased connectivity of striatum with other cortical and subcortical regions emerged as a distinct shared trait. Participants in these subgroups also had reduced connectivity within default mode and dorsal attention networks as compared to HCs.

Conclusions: Clinical, psychological, and symptom features can be used to identify particular homogeneous subgroup(s) of depressed patients that may better inform treatment selection, prognostication and underlying neurobiology.

Keywords: Major Depression Disorder, Clinical Trial, Computational Psychiatry, Neuroimaging Analysis

Disclosure: Nothing to disclose.

M135

Quinolinic Acid is Associated With Increased Basal Ganglia Glutamate and Anhedonia in Patients With Major Depression

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Background: Chronic glutamate (Glu) dysregulation is a risk factor for the development of neurotoxicity. The precise etiology of glutamate dysregulation in neuropsychiatric disorders is unclear. In this regard, activation of the kynurenine pathway (KP) - a tryptophan-catabolizing pathway initiated by stimulation of its primary, rate-limiting enzyme i.e., indoleamine- and tryptophan 2-dioxygenase (IDO and TDO) by stress, inflammation and/or metabolic dysregulation - leading to the generation of glutamate-altering molecules such as quinolinic acid (Quin) and kynurenic acid (Kyna) may be involved. We thus examined if Quin and Kyna would be associated with basal ganglia Glu/creatine (GluCr) signals on MRS and whether their interaction would impact depressive symptoms notably anhedonia based on previous results.

Methods: Forty subjects aged 21-65 diagnosed with DSM-IV + major depression underwent MRS scans on Day1 and blood sampling and behavioral assessments on Day2. Anhedonia was assessed using a previously validated, subscale of the IDS-SR consisting of 3 items. Single-slice, multivoxel, chemical-shift Magnetic Resonance Spectroscopy (MRS) data were acquired using settings of TR/TE=1590/30ms, voxel=10.3x10.3x15 mm³, acquisition matrix = 16x16, data points=1024, and averages=7. A 2x3 voxel area in the bilateral subcortical region at the level of basal ganglia was used to obtain metabolite concentrations, and post-processing was conducted using LCModel. Omnibus MANOVA models were used to screen for associations between MRS and all kynurenine metabolites followed by linear models adjusting for covariates to test specific associations between GluCr, Quin, and Kyna. Generalized elastic net models (to control for collinearity, variable selection) were used to examine the impact of full-factorial 2-way interactions between GluCr*Quin or Kyna interactions upon anhedonia. A 'balanced auto-validation'

technique was used to recode the original data into two copies - one a training set and the other a validation set - that differed in row weightings. The weights were Dirichlet-distributed and balanced to contribute more training and less to validation set (and vice versa). Serial Monte-Carlo-based resampling (1000-repetitions) was used to test the stability of estimates and test sample power.

Results: A total of 40 patients were available for analysis as they had both MRS and plasma kynurenine measures. Mean $\pm$ SD values for the estimation sample included age=39 $\pm$ 11, BMI=31.1 $\pm$ 7.69 with females and African-Americans comprising 70% and 67% of the sample, respectively. The omnibus MANOVA was significant (Roy's Largest Root(R) = 1.49, F(8,28) = 5.20, p = 0.0005) and the association between plasma Quin and right basal ganglia GluCr (RGluCr, PE=0.09, t = 3.20, p = 0.003) primarily contributed to this effect. None of the other kynurenine markers, including Kyna, reached significance within the MANOVA model. Linear models comparing both markers indicated that Quin and Kyna had opposing but significant effects on RGluCr, although Quin effects were stronger (diff=0.15, t = 3.50, p < 0.002). Following adjustment for covariates (age, sex, BMI, race), the association between Quin*RGluCr (but not Kyna) remained significant [beta=0.61, t = 3.33, p = 0.002, d = 1.17(0.41-1.86)]. RGluCr*-Plasma QUIN interaction was significantly associated with anhedonia severity after adjustment for covariate in the Elastic Net models (Wald ChiSq=19.7, p < 0.001). This finding was replicated >90% times on 1000-fold resampling, and the magnitude of this effect size enabled the small sample size with > 90% power. There were no differences between effect sizes for right and left basal ganglia - although RGluCr effects alone reached significance.

Conclusions: Activation of the kynurenine pathway and Quin may contribute to glutamate-induced neurotoxic and neuroprogressive pathologies and behavior in mood disorders. Targeting this pathway may offer new therapeutic alternatives to treat depression and other diseases associated with kynurenine pathway activation.

Keywords: Disorders of Glutamate, Kynurenine Metabolism, 1H MRS, Anhedonia, Mood Disorder

Disclosure: Nothing to disclose.

M136

Adolescent Depression Symptom Clustering: Machine Learning Driven Hypothesis Generation

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Background: Although there has been a consensus about the heterogeneity of major depression, clinical models of depression subtypes have not converged for adolescent depression. While machine learning approaches such as hierarchical clustering have been used to study adult depression subtypes, they have not yet been applied to the pediatric population. Given the differences in symptoms of depression between adults and adolescents, we investigated symptom clusters of adolescent major depressive disorder using a variety of unsupervised machine learning techniques.

Methods: Patient and family reported data from the Treatment for Adolescents with Depression Study (TADS) (n = 439), one of the largest randomized control trials for treating moderate-to-severe adolescent depression, were used to study symptom clusters in depressive symptom checklists. Clusters of symptoms in the Children's Depression Rating Scale, Revised (CDRS), one of the

primary endpoints in the TADS trial, were identified with hierarchical clustering, a data-driven machine learning technique that does not require assumptions about the number of clusters in the data. Agglomerative hierarchical clustering was applied to the CDRS symptom scores from the baseline visit in TADS. To examine internal validity, these results were compared against hierarchical clusters of CDRS symptom scores over two subsequent checkpoints and with varying hyperparameters, clusters established by other unsupervised learning techniques (k-means clustering and DBSCAN [density based spatial clustering of applications with noise]), and hierarchical clusters of comparable features between the CDRS checklist and secondary outcome Beck Depression Inventory (BDI).

Results: Agglomerative hierarchical clustering of CDRS symptom scores revealed clusters of symptoms that persisted across 3 checkpoints (0 weeks, 6 weeks, and 12 weeks) in the TADS study. Five clusters of symptoms appeared to be robust across hyperparameter changes; higher levels of clustering were not consistent across hyperparameter changes. These five clusters were: 1. Depressed feelings, difficulty having fun, low self-esteem, irritability, excessive fatigue, and impaired schoolwork; 2. Suicidal ideation, morbid ideation, and excessive guilt; 3. Hypoactivity, listless speech, and depressed facial affect; 4. Appetite disturbance and physical complaints; 5. Social withdrawal and sleep disturbance. The symptom measure Excessive weeping was the most inconsistent in its placement amongst clusters across permutations of clustering techniques and hyperparameters.

Clusters determined by k-means clustering and DBSCAN were moderately comparable to the equivalent ones produced by agglomerative clustering. A comparison hierarchical clusters of overlapping, comparable features in the CDRS and BDI also revealed moderate similarities in clustering.

Conclusions: Hierarchical clustering techniques may reveal clinical subtypes of adolescent depression. While some of the symptom clusters were more robust than others across clustering hyperparameters and different clustering techniques, the variability in clustering may be related to the relatively small sample size in this adolescent study (as compared to that of adult studies). Although there are similarities with clusters of adult symptoms of depression, comparisons with adult studies are limited by the difference in questionnaire items. Internal validation revealed some similarity in clusters, but further external validation via comparisons to external datasets would be required to draw further conclusions.

Keywords: Machine Learning Clustering, Adolescent Depression, Clinical Subtypes

Disclosure: Nothing to disclose.

M137

Prevention of the Behavioral Effects of Ketamine in the Mouse by Naltrexone and Kappa Opioid Receptor Blockade

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Background: Ketamine has recently been shown to produce rapid and sustained behavioral effects in patients suffering from intractable major depressive disorder (MDD) and other disorders. Recent clinical studies suggest that the effects of ketamine may be mediated by the endogenous opioid system. Specifically, Williams & colleagues (2018) have shown that pretreatment with the opioid receptor antagonist naltrexone attenuated the antidepressant effects of ketamine in treatment-resistant MDD patients. However, because

naltrexone is an antagonist at kappa opioid receptors (KORs) and mu opioid receptors (MORs), the specific role of each opioid receptor in modulating the effects of ketamine effects is not known. In these studies, we specifically aimed to examine the role of KORs in regulating the behavioral effects of ketamine in the mouse forced swim test (FST). The FST is an appropriate model to study the pharmacology underlying the behavioral effects of ketamine because it reduces immobility in the FST for days after a single injection, similar to the protracted duration of effects following infusion in MDD patients. We investigated whether (1) the antidepressant-like effects of ketamine are blocked in mice with genetic deletion of KORs (KOR knockout (KO) mice) and (2) whether the antidepressant-like effects of ketamine are blocked by prior administration of the mixed opioid antagonist naltrexone or selective opioid receptor antagonists. We hypothesized that the behavioral effects of ketamine measured 24 h following administration would be absent by prior deletion or blockade of KORs.

Methods: The behavioral effects of ketamine were measured using the FST conducted 24 h post ketamine injection in 8–12-week old male C57BL/6J mice. KOR KO mice were compared to wildtype (WT) mice. Mice were pretreated with either the mixed opioid receptor antagonist naltrexone (1 mg/kg, i.p.) or the selective short-acting KOR antagonist LY2444296 (3 mg/kg, i.p.) 30 min prior to ketamine (10 mg/kg, i.p.).

Results: Low doses of ketamine exerted a behavioral response when tested in mice 24 h after injection as measured by a reduction of immobility in the FST that is similar to other antidepressant drugs. This effect was absent in KOR KO mice relative to WT. Furthermore, reduction of immobility induced by ketamine (10 mg/kg) was blocked by pretreatment with either naltrexone or LY2444296, thereby implicating KORs in mediating the behavioral effects of ketamine in the FST.

Conclusions: Activation of KORs is likely necessary for the antidepressant-like activity of ketamine observed in the FST. This was illustrated in KOR knockout mice absent the receptor and by pretreating mice with the mixed antagonist naltrexone or the selective KOR antagonist LY2444296 prior to ketamine administration to block the receptor. Acute administration of ketamine has previously been shown to activate KORs. However, the behavioral effects of ketamine were tested at 24 h after ketamine administration, a period coincident with antidepressant effects in humans and when ketamine is no longer present. At 24 h post-treatment, ketamine has caused a reduction of KOR signaling. The blockade of KORs at the time of ketamine treatment may counteract ketamine-induced suppression of immobility in the FST by preventing the subsequent KOR desensitization 24 h later. These results suggest that activation of KORs by acute ketamine injection is necessary for the emergence of behavioral effects in the FST 24 h later and possibly clinical antidepressant effects.

Keywords: Ketamine, Kappa Opioid Receptors, Treatment Resistant Depression, Antidepressants

Disclosure: Nothing to disclose.

M138

Longitudinal Changes of Nucleus Accumbens (NAc) Volume May Influence the Onset of Depressive Symptoms in Youth

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Background: Our aim was to evaluate whether NAc volumetric changes predict the onset of depressive symptoms in the transition from childhood to adolescence.

Methods: We used data from two waves of the multi-site neurodevelopmental study Brazilian High Risk Cohort (HRC): baseline (age range 6-14; mean 9.29 [s.d.1.81]; female, n = 209 [55.3%]) and 3-year follow-up (age range 9-17; mean 12.62 [s.d.1.82]). The Symmetrized Percent Change (SPC) from FreeSurfer longitudinal pipeline assessed NAc volume change over time. First, we tested NAc SPC as a predictor of depressive symptoms at follow-up. Second, we divided the sample in two groups according to NAc volume change (positive vs negative SPC values) and tested depressive symptoms as predictors of pertaining to the positive SPC change group. All models were adjusted for study site, gender, and age.

Results: Left NAc SPC predicted depressive symptoms at follow-up ($B=0.186$; $p<.001$; Partial Eta Squared = .027). In group analyses, higher left NAc SPC predicted pertaining to a group with high levels of depressive symptoms when compared to subjects with low or no symptoms (OR 3.87; 95%CI 1.36-11.02; $p=.011$). We found similar findings after excluding subjects with depressive symptoms at baseline (OR 5.18; 95%CI 1.31-20.41; $p=.019$). Higher depressive symptoms at follow-up increased the likelihood of a positive NAc SPC value (OR 1.08; 95%CI 1.01-1.14; $p=.020$).

Conclusions: Our findings suggest that volumetric changes of the NAc may affect the emergence of depressive symptoms in youth, although low effect sizes require caution when interpreting these associations.

Keywords: Structural Neuroimaging, Adolescence, Adolescent Depression, Mood

Disclosure: Nothing to disclose.

M139

Improving Brain Bioenergetics May Improve Altitude-Related Depression: Animal Model Studies

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Background: Our studies show that depression-like behavior (DLB) increases in female rats after a week of housing at moderate altitudes of 4,500ft or 10,000ft vs. at sea level, while male behavior does not change. However, both male and female rats housed at altitude fail to respond to most antidepressants of the selective serotonin reuptake inhibitor (SSRI) class, the most widely prescribed of antidepressants. People living at altitude are exposed to hypobaric hypoxia, which may cause brain hypometabolism, and a bioenergetic deficit is also linked to major depressive disorder (MDD). Our previous studies show an altitude-related deficit in the brain cellular bioenergetic marker, creatine (CR), in the prefrontal cortex of healthy people living at altitude vs. those living at sea level. A similar deficit is seen in rats after housing for a week at 10,000ft. Our next goal was thus to test the potential of the bioenergetic compounds creatine monohydrate (CrMH) and cyclocreatine (cyCR) to improve depression status in this animal model. CrMH is a bioenergetic compound widely used as a supplement by athletes to enhance energy production and usage in skeletal muscle. Dietary CrMH can improve cerebral energy reservoirs in healthy volunteers, and may improve SSRI efficacy in adult women and treatment-resistant adolescent women. CyCR is a lipophilic analog of CR, which also shows the

potential to improve brain bioenergetics, with the added advantage of crossing the blood brain barrier without the need of a transporter, thus exhibiting higher brain bioavailability vs. CrMH.

Methods: Female and male rats housed at a moderate altitude of 4,500ft were provided with 5wks of dietary augmentation with 4%w/w CrMH in powdered rat chow. Another set of rats housed at 4,500ft were given 3wks of dietary cCR supplementation (1%w/w). Control animals were fed powdered rat chow for 3 or 5wks (Food controls). After the dietary treatment period, rats were tested for depression-like behavior (DLB) in the forced swim test (FST). Rats were then sacrificed, and blood and brain regions (prefrontal cortex, striatum) were harvested for tissue analyses. Tissue was assayed for CR levels with an assay kit from BioVision (Milpitas, CA). The TCA desipramine (Des) works as an effective antidepressant in rats housed at 4,500ft, therefore was used as a positive control for dietary treatment studies. FST data were analyzed by one-way ANOVA of 3 groups: CrMH, Des and control group. The control group combined saline controls from the Des study and Food controls, which did not significantly differ in FST behavior. Student's t-test was used to analyze tissue CR levels as well as FST behavior of cyCR.

Results: A. Dietary Creatine: (1) Blood CR Levels: In pilot data, dietary CrMH improved blood CR levels vs. food controls in female (Student's t-test, $p=0.01$) and male rats ($p=0.0002$). (2) Prefrontal Cortex CR Levels: Dietary CrMH improved CR levels in the prefrontal cortex of females ($p=0.04$), but not males ($p=0.99$). (3) Depression-like Behavior: (a) Females: CrMH had an antidepressant effect comparable to that seen with Des, with over 20% decrease in immobility and over 20% increase in latency to immobility (LTI) from controls. One-way ANOVA showed a significant effect of treatment on swimming ($F_{2,78}=7$, $p=0.001$), climbing ($F_{2,81}=8$, $p=0.0007$), immobility ($F_{2,81}=11.8$, $p<0.0001$) and LTI ($F_{2,78}=20$, $p<0.0001$). In post-hoc tests, CrMH significantly decreased immobility ($p=0.05$), and increased both swimming ($p=0.001$) and LTI ($p<0.0001$) vs. controls, but didn't change climbing. (b) Males: In males, a significant effect was seen on swimming ($F_{2,63}=4.9$, $p=0.001$), climbing ($F_{2,63}=16.9$, $p<0.0001$), immobility ($F_{2,66}=7$, $p=0.002$) and LTI ($F_{2,78}=3$, $p=0.03$). In post-hoc tests, dietary CrMH significantly decreased swimming and increased climbing ($p=0.05$), but did not improve immobility or LTI. Des significantly improved climbing, immobility and LTI in the male FST. B. Dietary Cyclocreatine: In pilot studies, dietary cyCR shows a trend towards antidepressant efficacy in rats at 4,500ft. (a) Females: Dietary cyCR significantly decreases immobility and increases LTI (Student's t-test, $p=0.02$), but does not improve swimming or climbing. (b) Males: Dietary cyCR increased climbing ($p=0.03$) and LTI ($p=0.04$) vs. controls, and shows a trend to improving immobility ($p=0.05$), but did not alter swimming.

Conclusions: Rates of MDD and of suicide increase demographically with altitude of residence. Our animal studies imply that chronic hypoxia exposure via living at altitude may alter brain physiology to worsen both depression and antidepressant-resistance rates, which could aggravate suicidal ideation and behavior. Mood and vulnerability to suicide may also be affected in people with chronic hypoxic disorders (cardiovascular diseases, COPD, asthma, smoking). In our model, the SSRIs Prozac, Paxil and Lexapro do not work in rats housed at 4,500ft or 10,000ft, suggesting that SSRIs may not work in people exposed to chronic hypoxia. In the current study, we show that improving brain bioenergetics may be an effective way to provide more effective antidepressant options for those exposed to chronic hypoxic conditions. Previous CrMH studies, conducted at sea level (Allen 2010), showed a similar sex-based impact on rodent FST behavior: CrMH showed AD potential in females, but not males. This is the first study on cyCR treatment in depression, and our studies

suggest that cyCR may be a more effective antidepressant than CrMH.

Keywords: Depression, Bioenergetics, Animal Models, Antidepressant

Disclosure: Nothing to disclose.

M140

Experience Enhances Olfactory Representations in the Dentate Gyrus

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Background: In order to acquire necessities and avoid danger, animals discern important stimuli and place them onto their cognitive map of their environment. The neocortex conveys general representations of experienced sensory events (stimuli) to the hippocampus, where relationships between events are catalogued to generate this cognitive map. It remains unclear whether populations of neurons in the hippocampus inherit these general representations or actively participate in classifying them. It is also unknown if the hippocampus further sharpens the classification of stimuli that are important (i.e., are associated with the acquisition of necessities).

Methods: We used chronic in vivo 2-photon imaging in mice of dentate gyrus granule cells (DG GCs) and one of its cortical inputs (lateral entorhinal cortex, LEC) across days to understand how sensory representations within cortico-hippocampal networks are modified by experience.

Results: We found distinct neural representations of olfactory stimuli within the activity patterns of DG GCs that were much sparser than those in LEC, and that odor identity could be more accurately classified from DG GC activity than LEC activity. This difference was not accounted for by models of random connectivity. In addition, the degree to which odorants were decodable from patterns of activity in DG GCs was directly related to future olfactory learning across animals. By tracking the same neurons in DG and LEC across multiple days, we found that after learning, DG GCs, but not LEC neurons, flexibly changed their responses to odor stimuli, increasing the distance in neural representations between stimuli, becoming more responsive to the conditioned odorant and less responsive to the unconditioned odorant.

Conclusions: These data reveal that, with learning, DG GCs amplify the decodability of cortical representations of important stimuli, which may facilitate storage and recall by downstream areas to guide appropriate behaviors.

Keywords: Dentate Gyrus, Memory and Learning, Entorhinal Cortex

Disclosure: Nothing to disclose.

M141

Transcriptomic Investigation of Mechanisms Underlying Analgesic and Psychiatric Effects of Ketamine Infusion

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Background: Ketamine is an NMDA-receptor antagonist that is

increasingly used for the treatment of depression and pain. Ketamine potentially expands the clinical repertoire of antidepressive and analgesic medications to include NMDA receptor antagonists. Despite the acceleration in clinical applications, the molecular and circuit level mechanisms remain incompletely defined. Further understanding of ketamine and related compounds may clarify drug development efforts to improve their therapeutic actions, reduce side effects, and help define on-target vs. off-target actions.

Methods: Using a chronically-implanted femoral vein catheter, we gave 1hr, and 10hr infusions of ketamine. Samples also were obtained from animals that were sham operated and those with a 10hr infusion plus a 24hr recovery period. Doses used mimicked those in clinical usage. We analyzed three brain regions: frontal cortex, hippocampus and amygdala by RNA-Seq transcriptomic and in situ hybridization analyses from both male and female rats. Samples also were obtained for sham operated and 10hr infusion with a 24hr recovery period. This analysis is comprehensive in that it examines a detailed time course, three brain regions subserving different cognitive functions, and both sexes. The study was performed in duplicate with the first study performed in males (N = 7) for all time points (28 rats total, 3 brain regions each), and a second replication study performed in the females (N = 5, Control v 10hr). Cellular level neuroanatomical localization of transcripts of interest was performed using 4-plex fluorescent in situ hybridization to examine distribution in coronal sections including thalamus, hippocampus and somatosensory, cingulate, and motor cortex. Alignment was performed using MAGIC with statistics performed using limma voom (B-H adjusted p-value < 0.01).

Results: These analyses revealed contrasting increases in a subclass of immediate early genes suggesting activation of some subsets of cortical neurons, with other transcription factors indicating inhibition. Based on this we hypothesized a disinhibition and glutamate surge model, where some brain regions are activated in response to ketamine infusion. Downstream of this activity, we observed activation of the Nrf2 pathway, a canonical signaling pathway in the antioxidant cascade associated with NMDA receptor hypofunction. In general, a substantial proportion of these gene changes exhibited a rebound effect, where the gene change at 10hr was opposite that occurring at 24hr after withdrawal of the drug. The ketamine-induced transcriptional changes were largely correlated between male and female animals with a small population of genes in the amygdala being sexually dimorphic in their response to ketamine. These genes were largely related to vascular endothelial function. We additionally examined cell types in which the gene changes occur by cross-referencing our dataset to single-cell databases of frontal cortex and hippocampal neurons and non-neural supportive cells such as microglia, astrocytes and endothelial cells. Based on this analysis, we identified widespread changes in genes specific for several subclasses of interneurons, cortical pyramidal neurons, and other neuronal populations. Additionally, we also attributed much of the gene changes to non-neural support cells such as microglia, which appeared to be strongly affected by ketamine infusion. Subsequently, we localized key genes of interest using in situ hybridization, identifying a population of neurons in medial retrosplenial cortex strongly induced by ketamine, as evidenced by upregulation of Fos, Bdnf, and Nr4a1. These changes were located throughout cortical pyramidal neurons, but were most pronounced in medial retrosplenial cortex.

Conclusions: The pathways identified in our study have previously been associated with psychiatric conditions and pain, but have not been associated with ketamine directly. While, several recent detailed studies have examined ketamine effects at the systems and molecular levels, exploring transcriptional (Bagot et al., 2017), and synaptic (Moda-Sava et al., 2019) effects that greater explain its mechanism (Duman et al., 2016; Zanos and

Gould, 2018), the present study extends these findings to include a comprehensive analysis of transcriptional responses. These observations potentially connect ketamine directly with downstream effectors responsible for its psychiatric and analgesic effects and form a comprehensive foundational knowledge base from which to draw on in future studies of this burgeoning drug class. In aggregate our data corroborate that ketamine likely induces an excitatory state, as supported by increased gamma band power on quantitative electroencephalography (Sanacora et al., 2014), increased glutamate neurotransmission (Abdallah et al., 2018), and increased hippocampal synaptic potentiation in response to ketamine exposure (Nosyreva et al., 2013). These findings also corroborate several recent clinical reports looking at one or more pathways identified in this study. In general, these experiments provide a methodology to perform such studies in an animal model, as well as consolidating these findings into a systems-level framework for understanding the effects of ketamine in humans.

Keywords: IV- Ketamine, Animal Models, Transcriptomics, RNA-Seq, Sex Differences

Disclosure: Nothing to disclose.

M142

Lateralized Functional Connectivity Changes are Associated With 6 Ketamine Infusions Compared to 1 Ketamine Infusion

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Background: Major depressive disorder (MDD) has been linked to imbalanced communication among large-scale brain networks, as reflected by abnormal resting-state functional connectivity (FC). Specifically, MDD has been associated with hypoconnectivity within the frontoparietal network (FN; a set of brain regions involved with cognitive control and emotion regulation), hypoconnectivity of the dorsal attention network (DAN; a network encompassing frontoparietal systems and parietal regions involved in attending to the external environment), and hyperconnectivity within the default mode network (DMN; a network hypothesized to be involved with self-referential thought).

Abnormalities in glutamatergic signaling have been implicated in the pathophysiology of chronic stress and depression. Specifically, preclinical models have shown that chronic stress causes neuronal atrophy and decreases in the number of synapses within corticolimbic brain circuits. Recently, ketamine—an N-methyl-D-aspartate (NMDA) type glutamate receptor antagonist—has gained widespread interest for its rapid antidepressant properties and putative correction of stress-induced neuronal atrophy.

In this randomized, double-blind placebo-controlled trial comparing 6 to 1 ketamine infusions, we investigated resting-state functional connectivity changes in large-scale networks as a biomarker of ketamine response.

Methods: Eight individuals who participated in a randomized controlled trial comparing 6 ketamine infusions to 5 placebo infusions plus 1 ketamine infusions underwent resting fMRI. Depression was assessed using the Montgomery-Asberg Depression Rating Scale (MADRS) at baseline and with 1-week post-infusion series. rs-fMRI data (3T Siemens Prisma, MB=8, TR=710 ms, 680 volumes) were collected before and between 1-7 days after the intervention. Data were denoised using FSL tools, including removal of artifactual components using FSL-FIX. Time-courses were extracted using CONN for cortical and subcortical

regions of interest (ROIs) of the Harvard-Oxford atlas and bandpass filtered between 0.08-0.12 Hz. Using a priori seed regions corresponding to the FN, DAN, and DMN networks, we determined significant differences in mean correlation coefficients between participants receiving 6 ketamine infusions vs. 5 placebo plus 1 ketamine infusion.

Results: Compared to participants who received 1 ketamine infusion, participants who received 6 ketamine infusions showed increased FC between the right hippocampus and DMN ($p = 0.026$) and between the FP and DA networks ($p = 0.035$) as well as decreased FC between the right hippocampus, FP ($p < 0.005$) and DA ($p = 0.028$) networks. For participants who remitted from depression (MADRS < 9; $n = 6$), 6 ketamine infusions were significantly associated with decreased FC between the right FP network and DMN ($p = 0.030$).

Conclusions: Our findings demonstrate lateralized FC changes associated with 6 ketamine infusions compared to 1 infusion. Remission following 6 ketamine infusions was uniquely associated with correction of FC between the FP network and the DMN, which aligns with previous models of network imbalance in depression. These findings provide empirical support for a lateralized model of depression in which correction of network dysfunction underlies improvements in depression symptoms.

Keywords: Depression, IV- Ketamine, Resting-state fMRI

Disclosure: Nothing to disclose.

M143

Cognitive Performance After Repeated Administration of the NMDA Positive Allosteric Modulator SAGE-718 in Healthy Volunteers

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Background: NMDA receptor hypoactivity has been linked to a range of clinical phenomena, including cognitive dysfunction and alterations in social behavior. Potential treatments for conditions characterized by NMDA hypofunction may involve enhancement of NMDA receptor-related neurotransmission. In a recent suite of target-engagement studies, SAGE-718, an investigational positive allosteric modulator of the NMDA receptor, demonstrated effects on electrophysiological and functional imaging biomarkers in healthy volunteers after a low dose ketamine challenge. These findings are consistent with CNS penetrance and functional engagement of the NMDA receptor. Here, the effects of 10-day repeated exposure of SAGE-718 on a core cognitive battery were investigated in healthy volunteers.

Methods: Forty subjects were randomized (1:1) to either multiple oral doses of SAGE-718 ($n = 19$) plus ketamine or placebo ($n = 21$) plus ketamine, which was delivered in a double-blinded manner [SAGE-718: $n = 19$, mean (SD) age 42.7 (12.00) years, 15.8% female; Placebo: $n = 21$, mean (SD) age 42.6 (11.56) years, 15.4% female]. SAGE-718 oral solution or matching placebo solution was administered as a 1.0-mg dose in a fasted condition once daily (between 7 and 10 AM) over the course of 10 days. Blood samples were taken and processed for pharmacokinetic analysis of concentrations of SAGE-718. Plasma samples were used to calculate C_{max} as well as other PK parameters.

Computerized testing was used to measure performance on key cognitive domains, including attention, working memory, processing speed, executive function, and motor reaction time. Assessments included computerized versions of the International Shopping List Test (verbal learning), International Shopping List

Test – Delayed Recall (memory), Groton Maze Learning Test (executive function), Social Emotional Cognition Test (emotional cognition), Detection Test (psychomotor function), Identification Test (attention), One Back Test (working memory), Two Back Test (higher-order working memory), Stop Signal Reaction Test (inhibition), and the Continuous Paired Associate Learning Test (paired associated learning). A mixed-effect regression model analysis (MMRM) was applied, with change from baseline in each cognitive test score as the response variable, and treatment, visit, and visit-by-treatment interaction as fixed effects, baseline as a covariate, and measurements within the same subject as repeated measure. Unstructured covariance structure was applied for the repeated measure.

Safety was assessed by adverse event reporting, standard clinical assessments, the Brief Psychiatric Rating Scale, the Clinician Administered Dissociative State Scale, the Observers Assessment of Alertness/Sedation Scale, and the Columbia Suicide Severity Rating Scale.

Results: As compared to placebo, statistically significant improvements were observed in the Two-Back Test at days 2, 4 and 8 of testing ($p < 0.05$). Compared to placebo, statistically significant improvement was observed on the Groton Maze Test at day 6 of testing ($p < 0.05$). A dose-response relationship was observed for the Two-Back Test, with Cmax positively correlated with an increase in change from baseline performance on the Two-Back Test ($p = 0.02$). A similar trend was observed for the Groton Maze Test (Cmax vs change from baseline, $p = 0.06$).

SAGE-718 was generally well tolerated with no serious adverse events or deaths observed in the trial subjects. The most frequent treatment emergent adverse events (TEAEs) reported after SAGE-718 administration were mild and numerically similar to those seen with placebo (TEAE: SAGE-718, $n = 6$ (31.6%); placebo, $n = 7$ (33.3%)). No TEAEs resulted in discontinuation or dose reduction of SAGE-718. No significant changes in clinical chemistry, hematology, urinalysis, or ECGs were observed.

Conclusions: Healthy volunteers dosed with SAGE-718 exhibited greater improvement on tests of higher-order working memory (Two-Back Test) and complex problem solving (Groton Maze Test), which at times reached the threshold for statistical significance as compared to subjects receiving placebo. SAGE-718 was generally well tolerated by the trial subjects over 10 days of exposure. Improvements in executive performance, as reflected by significant improvements on the Two-Back and Groton Maze Tests, suggest that further investigation of SAGE-718 is warranted for the treatment of conditions characterized by states of relative NMDA hypofunction, particularly those manifesting with executive deficits.

Keywords: NMDA Receptor, positive allosteric modulators, SAGE-718, Cognition

Disclosure: Sage Therapeutics, Inc., Employee, Sage Therapeutics, Inc., Stock / Equity

M144

Using a Multimodal Biomarker Approach to Identify Functional Target Engagement of the Novel NMDA Positive Allosteric Modulator SAGE-718

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Background: NMDA receptor hypofunction has been linked to a

range of clinical phenomena, including cognitive dysfunction, motivational deficits, and alterations in social behavior. Potential treatments for conditions characterized by these symptoms may require enhancement of NMDA receptor-related neurotransmission. A novel class of compounds—positive allosteric modulators of the NMDA receptor (NMDA-PAMs)—are being developed for this purpose. The clinical development path for compounds with novel mechanisms of action is substantially de-risked by characterizing functional CNS target engagement. Ketamine is a glutamate antagonist that has been shown to induce changes in brain activity via the NMDA receptor. Given this, a suite of Phase 1 target engagement studies was designed to evaluate CNS-target engagement of SAGE-718, an investigational NMDA-PAM, by electrophysiology and magnetic resonance imaging (MRI), using a low-dose ketamine challenge paradigm.

Methods: Based on the hypothesis that an NMDA-PAM would attenuate ketamine-induced changes in healthy volunteers, a low-dose ketamine challenge paradigm was employed. Two paradigms were selected based on a single administration of SAGE-718 (3mg oral solution) vs. placebo in a randomized cross-over design, with a wash-out of approximately 10 days.

In the first study, subjects underwent MRI approximately 6.5 hours after the SAGE-718 or placebo dose to obtain a baseline assessment. Ketamine (0.25mg/kg) was then administered as an intravenous infusion over 60 minutes starting approximately 7 hours after each dose of SAGE-718 or placebo. During the infusion, a second MRI assessment was completed. Functional magnetic resonance imaging (fMRI) derived changes of the blood oxygenation level (BOLD) response and functional connectivity between regions of interest were recorded ($n = 13$, completed subjects with valid data).

In the second study to determine the effects of SAGE-718 vs placebo, electrophysiological parameters were assessed starting approximately 6 hours after the administration of SAGE-718 or placebo and approximately one hour before the administration of ketamine. A second assessment was done during the 60 min ketamine infusion. The following parameters were measured; ketamine-induced changes in single click auditory evoked potential (AEP, N100-P200), mismatch negativity (MMN), and auditory steady state response (ASSR) ($n = 18$ completed subjects with valid data).

Safety was assessed by adverse event reporting, standard clinical assessments, the Brief Psychiatric Rating Scale, the Clinician Administered Dissociative State Scale, Observer's Assessment of Alertness and Sedation (OAAAS), and the Columbia Suicide Severity Rating Scale.

Results: Ketamine-induced BOLD changes were broad, including robust increases in posterior brain regions and decreases in anterior brain regions, in particular the pre-specified dorsal anterior cingulate cortex (dACC). SAGE-718 attenuated ketamine-induced BOLD alterations independent of their directionality.

N100-P200 was significantly reduced by ketamine under placebo conditions ($p < 0.05$), but not after administration of SAGE-718. No ketamine-induced significant changes were observed for mismatch negativity (MMN) and auditory steady state response (ASSR) under any condition.

SAGE-718 was generally well tolerated with no serious adverse events or deaths. Treatment emergent adverse events (TEAEs) reported after SAGE-718 administration (but before ketamine administration) were mild and infrequent, including headache ($n = 1$ in each of the studies) and fatigue ($n = 1$) in the evoked potential study. No TEAEs resulted in discontinuation or dose reduction of SAGE-718, and no significant changes in clinical chemistry, hematology, urinalysis, or ECGs were observed.

Conclusions: Results from these studies demonstrate that SAGE-718 had effects on functional imaging in healthy volunteers. SAGE-718 also modulated the effects of ketamine on regional and global measures of resting brain activity. These effects are in line

with the presumed mechanism of action of SAGE-718 as an NMDA-PAM, which supports the hypothesis of functional engagement of the NMDA receptor. These data, in addition to safety data collected to date, support further investigation of SAGE-718 in disorders characterized by hypoactivity at the NMDA receptor.

Keywords: SAGE-718, NMDA Receptor, Positive Allosteric Modulators, Biomarker

Disclosure: Sage Therapeutics, Consultant, PTC Therapeutics, Consultant, Perception Neuroscience, Consultant

M145

US-Latin American Initiative for Genetic-Neural-Behavioral Interactions in Human Neurodegenerative Research

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Background: This 5-years proposal fosters a Consortium with support from multiple partners aimed to combine genomic, neuroimaging and behavioral (clinical, cognitive, socioeconomic) data to improve dementia characterization in Latin American Countries (LACs) and identify novel inroads to treat neurodegeneration in diverse populations. We propose an innovative, harmonized, and cross-regional approach on two of their most prevalent neurodegenerative disorders: Alzheimer's disease (AD) and frontotemporal dementia (FTD). We are slotted to secure R01 funding (US-South American initiative for genetic-neural-behavioral interactions in human neurodegenerative research) that will support a basic platform anchored in Argentina, Brazil, Colombia, and Peru, that is supplemented with clinical research expertise from the University of California, San Francisco (UCSF), genomics expertise from the University of California, Santa Barbara, and bioinformatics expertise from HudsonAlpha. We now seek to extend this proposal to collaborators in Mexico and Chile. We also wish to assess novel families across LAC via the Latin America and Caribbean consortium on Dementia (LAC-CD). In addition to the R01 strategy based on patients with familial and sporadic presentations tested for genetic risks (risk scores), it would also support recruitment of AD and FTD families with an autosomal dominant-like presentation from the LAC-CD. In this expanded framework, we would first screen all patients for known AD/FTD/ALS genes and then, for those who screen negative for known genetic causes of disease, we will perform whole-genome sequencing (WGS) for gene discovery. We will establish a network of AD and FTD families and clinicians/researchers, enabling large-scale research to identify novel genetic and SES contributions to AD and FTD in diverse populations. Our long-term goal is to identify the unique genetic and SES factors that drive AD and FTD presentation in LAC relative to the US, including risk factors, cognitive profiles and brain imaging.

Methods: To this end, we will establish a first-in-class cohort anchored in six LAC (Argentina, Chile, Colombia, Brazil, Mexico, and Peru), compared to US samples (totaling > 4200 participants, including 2100 controls, 1050 AD patients, and 1050 FTD patients). We will couple standardized clinical assessments with innovative analytical techniques to account for heterogeneity in these diverse populations. By combining standardized genetic, neuroimaging, and behavioral (cognitive and SES) measures, we will test the underlying hypothesis that there are unique risk factors for AD and FTD in LAC (e.g., genetic risk factors enriched in LAC

populations; underlying cognitive and neural vulnerability due to SES) compared to US populations.

Results: In this context, we will pursue the following Specific Aims:

Aim 1: To establish genetic contributions to AD and FTD in diverse LAC cohorts (Tier 1 study, with larger sample size than Tier 2). By elucidating the genetic substructure and familial contributions to AD and FTD in LAC relative to the US, we will be able to identify proper populations for replication of our genetic findings. By assembling this large cohort, we will also be well positioned to establish a LAC-specific polygenic risk score (PRS) for predicting AD and FTD risk in future samples.

Aim 2: To elucidate the impact of SES on clinical, cognitive, and brain imaging signatures in LAC and the US. (Tier 2 study-comprehensive imaging and cognitive evaluation in a subset of Tier 1). To compare patients across regions, we need to establish standardized neurocognitive measures and understand how SES impacts the manifestations of dementia in LAC.

Aim 3: To determine whether genetic risk and SES yield better discrimination between LAC and US patients as compared with other cognitive, neuroimaging, and clinical variables (Tier 1 & 2). To date, no study has sought to establish which potential predictors prove more sensitive to discriminate between LAC and US patients. In particular, although genetic risk and SES (Aims 1 and 2) have the potential to robustly differentiate between such samples, no study has explored their role, let alone as compared to other multimodal factors. To address this issue, we will apply data-driven machine-learning analysis to determine top factors that best discriminate patients in LAC from those in the US. Multimodal measures from controls of each country will be used for population-specific normalization of patient data. We hypothesize that the top features, better discriminating LAC from US patients will be related to SES and genetic risk (e.g., standardized PRS) in comparison to other variables (clinical, cognitive, and imaging measures).

Conclusions: The expected outcome of this study is a large Latin American cohort of harmonized, well-characterized AD and FTD patients and controls (Fig 1). Positive impacts of this work include a better understanding of genetic and SES contributions to neurocognitive manifestations of dementia and identification of novel targets for risk reduction and disease prevention in LAC. Our large multimodal, cross-sectional study will enable clinical assessment of understudied patient groups, extend and harmonize existing data sets, prompt the development of novel measures, and inform future work on the clinical value of combined multimodal profiles to predict disease presentation and progression in longitudinal studies of diverse populations.

Keywords: Neurodegenerative Disease, Genetics, Multimodal Imaging, Cognition, Machine Learning

Disclosure: Nothing to disclose.

M146

Irritability and Aggression in Huntington's Disease: A Phase 2 Exploratory Clinical Trial With a Novel Vasopressin 1a Antagonist, SRX246

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Background: Irritability and aggression are common in Huntington's Disease (HD) patients. These symptoms are highly distressing, adversely impact daily life, and often result in institutionalization. Effective treatments are unavailable and we need well-validated scales for measuring changes in these symptoms to develop these drugs. This Phase II clinical trial in individuals with HD (n = 106), Safety and Tolerability of SRX246 In Irritable/Aggressive Subjects with Huntington's Disease (STAIR; NCT02507284), rigorously evaluates the tolerability of a new drug, SRX246; provides additional safety data; and explores rating scales for the assessment of changes in these symptoms. The test drug, SRX246, is a first-in-class vasopressin 1a receptor antagonist. It exhibits high affinity and selectivity for its target, has a strong safety profile in healthy volunteers and other clinical trials, and excellent pharmacokinetics. Preclinical pharmacology and an experimental medicine fMRI study showed that SRX246 has CNS effects after oral administration and modulates brain circuits involved in responses to stimuli that elicit aggression/fear. In a Phase 2 Exploratory trial for the treatment of Intermittent Explosive Disorder, SRX246 was well tolerated, no serious adverse events were reported, and exploratory analyses revealed statistically significant differences favoring SRX246 in key clinical outcome measures. These findings suggested that SRX246 might have beneficial effects on the irritability/aggression seen in HD patients.

Methods: STAIR was a 3 arm, multicenter, randomized, placebo-controlled, double-blind, 12-week dose escalation study (22 sites in the NINDS NeuroNext network, total n = 106). Following eligibility determination, female and male participants were randomized to receive placebo or escalate from 80 mg (two weeks) to 120 mg (4 weeks), to a maximum of 160 mg twice daily for an additional 6 weeks. Participants had a Study Partner to assist with visits, taking study medication, and providing feedback about the subject's mood and behavior. Visits were either "in-person" or by "telephone". An eDiary (smart phone or tablet) prompted participants to take their capsules. The participants and study partners were also asked to answer daily questions about irritability, aggression, and other behaviors (electronic Patient Reported Outcomes, also recorded in the eDiary). Visits occurred at week 0 (baseline), 2, 4, 6, 8, 10, and 12. The primary objective was to evaluate the tolerability of SRX246. This was met through a non-inferiority test of the proportion of completers among the placebo group and each of the treatment groups. The study was powered to 80% with $\alpha=0.025$. The secondary objective was to evaluate the safety of SRX246. The objective was met through a non-inferiority test of the proportion of participants with AEs or SAEs among the placebo group and each of the treatment groups. Exploratory analyses sought changes in irritability and aggression on various rating scales, including the Aberrant Behavior Checklist, Irritability Subscale; Cohen-Mansfield Agitation Inventory; Clinical Global Impression – Improvement; Problem Behaviors Assessment – short form; Irritability Scale; Caregiver Burden Questionnaire; Huntington's Disease Quality of Life Measure, and eDiary Responses. The objective was to obtain critical data that can be used to plan future Phase 2b or 3 clinical trials.

Results: Participants in each group had similar demographics, features of HD, and baseline irritability measures. Eighty-two of the 106 participants randomized completed the trial on their assigned dose of drug. A one-sided exact-method confidence interval was used to reject the null hypothesis of inferior tolerability for each SRX246 dose group versus the placebo. Similar analyses were used to test for differences in Adverse Events (AEs) and Serious Adverse Events (SAEs); these also showed no significant differences between the active and placebo arms. Most of the adverse events in the active arms of the trial were considered unlikely to be related to SRX246. A total of 200 AEs were reported (in 85 study participants) after receiving study drug or placebo. Of the 200 AEs, nine (5%) were classified as SAEs – in 9 participants. Of these, none

were both related to study treatment and unexpected. Exploratory analyses of the scales and behavioral results are in progress.

Conclusions: SRX246 was well tolerated and safe in HD participants. The tolerability and safety profiles in this study are consistent with prior results obtained in a Phase 1 multiple ascending dose trial, an experimental medicine fMRI study, and a Phase 2 study in participants with Intermittent Explosive Disorder that also showed good tolerability and safety. These data indicate that SRX246 can move forward as a candidate to treat irritability and aggression in HD if the exploratory analyses suggest efficacy.

Financial support: NINDS U44NS090616 to Azevan Pharmaceuticals, Inc.; NINDS U01NS077179 and U01NS077352 to the NeuroNEXT Network; and Azevan Pharmaceuticals, Inc.

Keywords: Huntington's Disease, Phase II Clinical Trial, Irritability/Aggression, Vasopressin 1a Receptor Antagonist

Disclosure: Azevan Pharmaceuticals, Inc., Stock / Equity, Azevan Pharmaceuticals, Inc, Consultant, Azevan Pharmaceuticals, Inc, Board Member

M147

24(S)-Hydroxycholesterol Levels are Decreased in Early Huntington's Disease and are Associated With Deficits in Several Cognitive Domains

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Background: 24(S)-hydroxycholesterol (24(S)-HC) is an endogenous, brain specific, cholesterol metabolite that acts as a positive allosteric modulator of the N-methyl-D-aspartate (NMDA) receptor. Alterations in plasma and/or brain levels of 24(S)-HC have been identified in several diseases, including Smith-Lemli-Opitz syndrome, Niemann Pick, Huntington's disease (HD), and some forms of dementia. Although a broad range of pathology and core symptomology is observed across these different disorders, all manifest some degree of behavioral, cognitive, and psychiatric symptoms. One important question to be addressed is whether 24(S)-HC is associated with these symptoms and which features are most directly associated with decreased glutamatergic tone.

Cognitive deficits are a hallmark of HD and precede the onset of motor impairments by decades. Previous work has established that levels of 24(S)-HC are decreased in plasma and brain in HD patients, suggestive of decreased NMDA receptor function. Here, we investigated the relationship between 24(S)-HC and cognitive performance in samples from TRACK-HD, a longitudinal biomarker study of pre-manifest and early stage HD.

Methods: Plasma samples from the TRACK-HD study (60 control; 60 Pre-HD; 60 HD) were analyzed for 24(S)-HC via liquid-liquid extraction and analyzed with LC-MS/MS. Regression analysis was then performed between oxysterol levels and performance on a number of cognitive and motor endpoints.

Results: We found that levels of 24(S)-HC positively correlated with several cognitive tasks including the Stroop test, Trails A and B, symbol digit modality and processing of negative emotion in the Eckman faces task across all years of the TRACK-HD study. Interestingly, 24(S)-HC levels were not correlated with motor performance tasks and associations were not found for other oxysterols (25 and 27 hydroxycholesterol) supporting a specific role for 24(S)-HC/ NMDA dysfunction in non-motor aspects of HD.

Conclusions: These data support a critical role for NMDA receptor function in cognitive performance in Huntington's disease. We are currently evaluating the safety and tolerability

of a positive allosteric modulator of the NMDA receptor (SAGE-718) in early HD patients.

Keywords: NMDA Receptor, Huntington's Disease, 24(S)-hydroxycholesterol, Cognition

Disclosure: Sage Therapeutics, Inc., Employee, Sage Therapeutics, Inc., Stock / Equity

M148

A mRNA-Seq Study Using Post-Mortem Brain Tissue Samples in Patients With Alzheimer's Disease Compared to Cognitively Normal Control Subjects

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Background: Alzheimer's disease (AD), the leading form of dementia, is associated with abnormal tau and β -amyloid accumulation in the brain, leading to neurofibril tangle and amyloid plaque formation. However, the efficacious treatment option remains limited to non-existing.

Methods: In this study, we conducted a mRNA-seq study to identify RNAs associated with AD in post-mortem brain samples from the inferior frontal gyrus (IFG), middle temporal gyrus (MTG), and superior temporal gyrus (STG). Adapters from the sequence data were trimmed and aligned using STAR and quantified using RSEM. Differentially expressed genes were identified using limma correcting for surrogate variables that was supposed to capture hidden technical variation and gender. The results from this study was compared to the results generated from Accelerated Medicine Partnership – Alzheimer's Disease (AMP-AD) initiative. Gene set enrichment analysis (GSEA) was used to identify pathways enriched from the differentially expressed genes.

Results: We observed several hundred mRNAs that were differentially expressed between AD cases and cognitively normal controls in STG and MTG but no transcript meets the same criteria in IFG (adjusted p less than 0.05 and fold change greater than 1.2). 198 and 98 transcripts were up- and down-regulated, respectively, and shared between STG and MTG brain regions. At the gene level, the within study consistency between two temporal gyrus regions is far greater than the between study consistency between STG analyzed in this study and STG analyzed by MSSM. However, there is still largely consistency at the gene set level. When up- and down-regulated genes from AMP-AD were created as customized gene sets (same adjusted p less than 0.05 and fold change greater than 1.2 criteria) and used in GSEA, both STG and MTG DGE results shared consistent directionality of enrichment with AMP-AD derived gene sets except three gene sets (Cerebellum (CBE) up- and down-regulated gene set and temporal cortex (CTX) up-regulated gene set from Mayo cohort). Among the 23 KEGG gene sets (Supplemental Table S3) with enrichment q-value less than 0.1 in the GSEA analysis using STG differential gene expression analysis results (AD vs. CN), 13 of them were immune-related gene sets including cytokine-cytokine receptor interaction ($p = 0.002$, $q\text{-value} = 0.02$), cytosolic DNA sensing pathway ($p = 0.002$, $q\text{-value} = 0.02$), and toll like receptor signaling pathway ($p = 0.004$, $q\text{-value} = 0.04$). Other gene sets of interest include KEGG spliceosome ($p = 0.002$, $q\text{-value} = 0.02$), KEGG neuroactive ligand receptor interaction ($p = 0.002$, $q\text{-value} = 0.03$), KEGG proteasome ($p = 0.004$, $q\text{-value} = 0.04$)/REACTOME ER phagosome pathway ($p = 0.002$, $q\text{-value} = 0.05$), KEGG calcium signaling pathway ($p = 0.004$, $q\text{-value} = 0.04$), and KEGG cell adhesion molecules (CAMs)/gap junction/tight junction. Likewise, GSEA of the differential gene analysis results from MTG

also suggested the enrichment of KEGG neuroactive ligand receptor interaction ($p = 0.001$, $q\text{-value} = 0.08$).

Conclusions: This study identified largely consistent gene sets as two out of three AMP-AD cohorts (ROSMAP and MSSM) although the consistency at the gene level was relatively low. Future meta-analysis and causal inferential analysis will be helpful in pinpointing the most relevant pathway and genes to intervene in the disease process.

Keywords: Alzheimer's Disease, Transcriptome, Immune

Disclosure: Johnson & Johnson, Employee, Johnson & Johnson, Stock / Equity

M149

EEG Multifractal Analysis in Mild Cognitive Impairment/Alzheimer's Disease

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Background: Standard techniques of EEG analysis have not led to clinically useful markers of cognitive impairment in Alzheimer's Disease (AD) or its precursor, mild cognitive impairment (MCI).

Methods: We tested a novel algorithm (multifractal detrended fluctuation analysis; MF-DFA) that interprets electroencephalography (EEG) signals, with the ultimate goal to develop a biomarker that can assess a dementia patient's neurocognitive impairment and functional capacity. We tested twenty subjects' worth of EEG data (along with quantitative cognitive and clinical staging information) from an existing database, as a definitive test of this algorithm. The resting state EEG data from these 20 cases was split into two different 30 second segments, one for training, one for testing. MF-DFA was done on these segments, and parameters were extracted into a database. With cognitive test scores (total Folstein Mini-mental status examination (MMSE) score) as the variable, a classification and regression trees (CART) statistical model was trained on the training EEG dataset, to establish the best fit with MF-DFA parameters. This statistical model was then tested on the test EEG-derived MF-DFA parameter dataset, to give an estimated MMSE score from the out-of-sample EEG segments.

Results: The test parameter estimated MMSE scores were then tested against the actual MMSE scores. EEG-derived CART models were able to predict actual MMSE score based upon the out-of-sample test EEG segment, and showed excellent specificity and sensitivity.

Conclusions: These results demonstrate great promise for the potential for this algorithm as an EEG-based quantitative biomarker for clinically meaningful impairment in cognition in AD/MCI.

Keywords: Alzheimer's Disease, Quantitative EEG, Mild Cognitive Impairment due to AD

Disclosure: Nothing to disclose.

M150

Cell-Specific Release of Extracellular Vesicles From the Choroid Plexus and Dopaminergic Neurons in the Brain

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Background: Circulating extracellular vesicles (EVs) in the cerebrospinal fluid (CSF) contain a variety of signaling factors,

such as proteins, enzymes, and RNA transcripts. While EVs have been implicated in many cell-to-cell signaling contexts, the vast majority of these studies are based on findings derived from cell culture conditions. Thus, the ability to identify cell type-specific EV release from cellular subpopulations within the brain represents a critical barrier in the field. To address this knowledge gap, we utilized a novel transgenic mouse model to determine the release of cell-type specific EVs.

Methods: The recently developed transgenic ExoMap mice contain a cre-dependent floxed gene fused to a mNeonGreen fluorescent tag. For our first study, we focused on EVs produced by the choroid plexus, a tissue that has been demonstrated to release numerous signaling factors into the CSF of the brain. Adult ExoMap mice received intracranial ventricular injections of the viral vector AAV-TTR-Cre, which allowed for cre expression under the choroid plexus specific protein promoter transthyretin. For our second study, ExoMap mice were bred with the DAT-Cre mouse line to allow for dopamine cell-specific expression under the dopamine transporter promoter.

Results: In the AAV-TTR-Cre injected ExoMap mice, we found specific mNeonGreen expression in the choroid plexus and further identified mNeonGreen-positive EVs in the CSF. Interestingly, a more diffuse expression pattern of mNeonGreen was found in the medial habenula, indicating that this site may be the integration location for choroid plexus derived EVs. For the ExoMap:Dat-Cre mouse cross, we identified mNeonGreen expression in dopaminergic cells of the ventral tegmental area and substantia nigra, indicating the EV-specific marker is transcribed in dopaminergic cells. Furthermore, dopaminergic neuron-derived EVs were localized within the CSF, which surprisingly demonstrated that dopaminergic cells release EVs into the interstitial fluid.

Conclusions: Taken together, these findings reveal that both choroid plexus epithelial cells and dopaminergic neurons release EVs into the extracellular environment in vivo. Further, the EVs that are released from these cell types differ in their membrane markers. Thus, these data support the contention that EV signaling occurs between multiple cells types in the brain, which has important implications for both normal and pathological disease states.

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Keywords: Dopamine, Choroid Plexus, Extracellular Vesicles, Exosome, Transgenic Mice

Disclosure: Nothing to disclose.

M151

Peripheral Blood Gene Expression and DNA Methylation Associated With Alzheimer' Disease Progression

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Background: Identifying biomarkers associated with the rate of Alzheimer's Disease (AD) progression could improve clinical trial efficacy by reducing study duration and sample size. Whole transcriptome analysis and epigenome-wide association study (EWAS) revealed correlations between the gene expression and CpG probe methylation levels and AD diagnosis and progression as measured by pathology burden in brain samples. We explore here the association of gene expression and DNA methylation in peripheral blood with these endpoints in participants from the longitudinal ADNI study for translational application.

Methods: Whole-genome gene expression and DNA methylation profiling was performed in baseline blood samples from ~800 and

~600 ADNI participants using the Affymetrix Human Genome U219 Array and Illumina Infinium HumanMethylationEPIC BeadChip, respectively. The timing for RNA sampling and DNA sampling may differ and they may also differ from the clinical study baseline. Rates of decline in cognitive function from initial RNA/DNA sampling visit to subsequent visits were estimated by the slope of robust linear regression of the preclinical Alzheimer cognitive composite (PACC) and clinical dementia rating scale sum of boxes (CDR-SB) for cognitively normal (CN) and MCI patients, respectively. Age, sex, and estimated cell compositions were included as covariates in regression analysis using limma to identify differentially expressed and methylated CpG positions (DMPs) associated with the rate of cognitive decline and disease status conversion (i.e. from cognitively normal to mild cognitive impairment (MCI) and from MCI to AD). DMPs associated with CN to MCI or MCI to AD conversion were also examined. Enrichment in biological process of DMPs were analyzed using weighted gene set enrichment analysis. Genes located close to top ranking DMPs were analyzed for physical and functional associations using STRING protein-protein interaction (PPI) database. Predictive modeling using baseline gene expression data and different tiers of clinical predictors were also used to predict disease stage conversion.

Results: We did not identify any transcripts associated with disease conversion status but identified 15 DMPs significantly associated with the rate of decline in PACC in CN subjects (FDR < 0.1). Genes close to the top 1,050 DMPs ($p < 0.0005$) were highly connected in PPI network ($p = 0.002$). These genes contained 101 AD disease genes and members of glutamatergic and GABAergic synapses. They were also enriched in nervous system development and cognition functions. Our study revealed candidate transcripts and epigenetic markers associated with the rate of cognitive decline and disease stage conversion. Predictive models for MCI to AD (AUC ~0.78) outperform those for CN to MCI disease conversion (AUC ~0.65-0.7) using clinical predictors or clinical and gene expression predictors.

Conclusions: We identified candidate CpG probes and gene sets from peripheral blood whose methylation level correlated with rate of cognitive decline. Additional predictive modeling work is needed to expand the predictor space to methylation probes and to perform cross-modality predictive modeling on overlapping samples.

Keywords: Alzheimer's, Disease Progression, Multi-Omics, DNA Methylation, Disease Modeling

Disclosure: Janssen Pharmaceuticals, Employee, Janssen Pharmaceuticals, Stock / Equity

M152

NEGR1, an IgLON Implicated in Human Obesity, Shares a Conserved 3D Structure and Interaction Mode With Other IgLONs, but Uniquely Impacts Feeding

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Background: IgLONs modulate neural circuits and impact very diverse processes such as feeding, emotions, social behavior, and cognition, but their underlying molecular mechanisms are unknown. The IgLON family is composed of five members. They are abundant GPI-anchored cell surface proteins found in brain and they shape synaptic connections. IgLONs interact homophilically and heterophilically with each other as 'synaptic organizers', acting in trans (spanning cellular junctions) and/or in cis on the same cell surface. The IgLON, neuronal growth regulator

1 (NEGR1), is implicated in obesity in humans via SNPs and CNVs, and it is also linked to major depressive disorder, schizophrenia, dyslexia, and autism spectrum disorder. Another IgLON, neurotrimin (NTM), is linked to intelligence and cognitive function. These IgLONs promote neurite outgrowth, migration of neurons to their target brain region, spine formation, dendritic tree formation and adult neurogenesis, but little is known about how they interact with themselves, other IgLONs, and/or non-IgLON partners. It is also not known how they are accommodated in the synaptic cleft of different synapse types and how they carry out their diverse physiological functions.

Methods: We used x-ray crystallography to determine the 3D structures of NEGR1 and NTM homodimers to a resolution of 3.0 Å and 3.3 Å, respectively. We used site-directed mutagenesis and biophysical techniques to confirm key residues that mediate the interaction of IgLONs with themselves in solution. Finally, we administered soluble NEGR1 ectodomains, as well as those of other IgLONs, in vivo into the paraventricular nucleus of the hypothalamus (PVN) in rats, a brain region that regulates feeding, to assess their impact on food intake. For these studies, 7-8 male rats were used per group (with validated sites of cannulation), and statistical comparisons were a repeated measures one-way ANOVA with Dunnett's multiple comparisons test.

Results: We show that the extracellular regions of NEGR1 and NTM are composed of a linear concatenation of three Ig domains, and that they form extended V-shaped homodimers that are mediated solely through the interaction of their Ig1 domains. The Ig1 domains dock onto each other by inserting a prominent tryptophan residue that is part of a hydrophobic ridge into a hydrophobic pocket on the opposing molecule. The interaction interface between IgLONs is highly conserved in terms of both sequence and 3D-structure, as would be expected for a family of proteins that can form both homodimeric as well as heterodimeric synaptic macromolecular complexes. However, strikingly, only NEGR1 regulates food intake in rats when administered to the PVN in vivo, while other IgLONs do not; this is consistent with the fact that lesions in the NEGR1 gene reveal a unique role for this protein in mediating obesity in humans. Furthermore, in vivo administration of NEGR1 does not alter locomotor activity, suggesting that the feeding effect is not due to a general suppression of mobility.

Conclusions: Our data suggest that even though the 3D structures of IgLONs are conserved and their interaction mechanism is as well, the different IgLONs have unique functions and do not readily compensate for each other. Furthermore, we demonstrate that food intake can be directly regulated in vivo by administration of the extracellular domain of a select synaptic organizer, NEGR1, into specific brain regions. There, NEGR1 likely remodels the synaptic connections in neural circuits that underlying feeding. Because NEGR1 undergoes ectodomain shedding in vivo (releasing its extracellular domain from the cell surface), our results suggest that NEGR1 may have long range impacts in remodeling neural circuits as well. By elucidating structure-function relationships of key molecules that selectively guide synapse development and uniquely impact specific neural circuits, we hope to identify novel therapeutic targets that could be leveraged to design better treatments for brain disorders in future.

Keywords: IgLONs, Synaptic Organizers, Synaptic Protein Interaction Networks, Neural Circuits, Obesity

Disclosure: Nothing to disclose.

M153

Open Board

M154

Structural and Resting State Neural Correlates of Pediatric Obsessive-Compulsive Symptoms in the Adolescent Brain and Cognitive Development Study

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Background: Subclinical Obsessive-Compulsive symptoms (OCS) in childhood increase risk for later onset of Obsessive-Compulsive Disorder (OCD) and related impairment. Studying the neural circuits underlying subclinical OCS may facilitate the identification of neural markers of risk for later OCD as well as potential targets for novel mechanism-based interventions and prevention strategies. Yet, the neural mechanisms underlying OCS and their trajectories over development are poorly understood at present, though are hypothesized to involve differential engagement of task control circuits that underlie attentional and cognitive control processes (e.g. Maia et al., 2008). Dysfunction in these circuits and processes likely contributes to the repetitive thoughts and inappropriate actions that characterize OCD. While a growing literature has probed the neural underpinnings of OCD in children, including ENIGMA mega-analytic findings suggesting larger thalamic volumes in pediatric OCD (Boedhoe et al., 2017), few studies have examined subclinical OCS. One relatively larger study noted associations between OCS and altered gray and white matter volume in healthy children (Suñol et al., 2018). The Adolescent Brain and Cognitive Development (ABCD) provides an opportunity to examine associations between OCS and brain structure in the largest sample of children to date as well as to provide novel insight into associations with resting state connectivity of task control circuits.

Methods: Data from the 2.0.1 release (July 2019) of baseline data from the ABCD Study were examined. These data include clinical interviews, cognitive testing, questionnaires, and MRI assessments from a nationally representative sample of N = 11,876 9-10-year-old children. An 8-item subscale for OCS severity (Hudziak et al., 2006) was ascertained from parent report on the Child Behavior Checklist (CBCL). Diagnosis of OCD was based on parent report on the Kiddie-Schedule for Affective Disorders and Schizophrenia for School-Age Children (KSADS). Cognitive performance was assessed using the NIH Toolbox. Of these children, n = 10,585 successfully completed T1 structural imaging that were analyzed using FreeSurfer and that passed ABCD quality control procedures. Resting state data was also collected and analyzed with the ABCD pipelines; n = 8,341 children had >5 minutes of data retained after quality control. Within and between network connectivity was extracted from regions/networks defined in the Gordon et al., 2016 atlas. Linear mixed effects models were used to examine whether CBCL OCS related to cognitive performance, subcortical volumes, cortical thickness, or resting state connectivity of default mode and task control circuits.

Results: N = 5,257 children (44.30%) exhibited non-zero CBCL OCS scores and, as expected, scores were elevated among the N = 898 children who met KSADS criteria for current OCD (b = 2.30, t = 36.82, p < .001, d = 1.35). CBCL OCS associated with worse performance on NIH Toolbox measures of inhibitory control, executive function, and working memory (all t < -2.2, p < .05). No associations between CBCL OCS and brain structure passed correction for multiple comparisons. CBCL OCS associated positively with resting state connectivity between the dorsal attention and default mode networks, the dorsal and ventral attention networks, and ventral attention and cingulo-parietal networks (all t > -2.79, p < .005). CBCL OCS associated

negatively with connectivity within the dorsal attention network ($t = -2.95$, $p = .003$).

Conclusions: We use the nationally representative ABCD Study to examine effects of OCS in the largest MRI dataset to date of 9-10-year-old children. OCS were associated with slight decrements in performance on several standardized measures of cognitive performance. While no differences in brain structure were found to pass correction for multiple comparisons, our findings highlight novel associations between OCS in children and patterns of resting state connectivity within the dorsal attention network and between this and other task-positive and task-negative networks. These findings build on prior work highlighting the dorsal attention network as a key predictor of OCD symptom severity in adults (Brennan et al., 2019). Our ongoing work with this sample will examine the specificity of these effects to OCS vs. other comorbid symptoms and longitudinal associations with changes in symptoms over development.

Keywords: Obsessive-Compulsive Disorder (OCD), Children, MRI, Resting State Functional Connectivity, ABCD Study

Disclosure: Nothing to disclose.

M155

Neurocognitive Correlates of Insight in Hoarding Disorder

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Background: Clinical insight – broadly defined to include awareness of illness, attribution of symptoms to illness, and recognition of need for treatment – is a transdiagnostic aspect of neuropsychiatric functioning with prima facie clinical relevance. A growing body of evidence from diverse conditions implicates disturbance of frontoparietal function in insight impairment. In DSM-V, obsessive compulsive and related disorders are specified by level of insight. However, in hoarding disorder (HD), the degree to which affected individuals manifest insight impairment is controversial, and accurate assessment is confounded by reliance on self-report measures. In this study, we explored whether individuals with HD underreport their clutter, and whether the degree of underreporting correlates with demographic, clinical, or neurocognitive behavioral measures.

Methods: Data were obtained from $n = 68$ individuals (average age 57.2 (range 24-75), 76% female) who screened voluntarily for participation in a study of HD. Individuals underwent clinical diagnostic interviews and self- and clinician-rated assessments including the Clutter Image Rating (CIR; an established pictorial rating scale of clutter), Saving Inventory-Revised (SI-R), Saving Cognitions Inventory (SCI), Hamilton Depression Rating Scale-17 (HDRS), Depression Anxiety Stress Scales (DASS), and Intolerance of Uncertainty Scale (IUS). A home visit followed (average interval 25 days, range 0 to 100) during which a trained, independent evaluator completed the CIR. Self-rated (SR-) and independent evaluator (IE-) CIR scores for dually-scored individual rooms were averaged for each subject. SR- and IE- CIR ratings were compared via a two-tailed paired t test. A CIR 'error' score (CIR-error) was generated by subtracting the SR-CIR average from the IE-CIR average and dividing the difference by the IE-CIR average. CIR-error was used as a dependent variable for linear regression using clinical and demographic variables as predictors. A subset of participants ($n = 42$, average age 57, 81% female) completed a validated computer-administered battery of neurocognitive tests (Brain Resource WebNeuro). The relationship between CIR-error scores and reference-population normalized scores for

performance on individual neurocognitive measures was similarly modeled using linear regression.

Results: Average CIR scores generated by self-report were lower than those generated by independent evaluators (3.83 vs 4.27, $t = 3.76$, $p < 0.001$). Linear regression showed that CIR-error could be predicted by severity of IE-assessed clutter (IE-CIR; $\beta = 0.11$, $R^2 = 0.37$, $p < 0.001$), though not by self-reported clutter score (SR-CIR; $\beta = -0.00$, $R^2 < 0.001$, $p = 0.87$). Lower scores on the 'difficulty discarding' subscale of the SI-R (SI-R-DD) predicted greater CIR-error ($\beta = -0.02$, $R^2 = 0.10$, $p < 0.008$), but no other relationship was observed between CIR-error and other established measures of hoarding severity (SI-R, SCI), depression (HDRS) or anxious distress (DASS, IUS). There was similarly no significant relationship between CIR-error and age, gender, handedness, estimated IQ, or medication use. Using a multivariate linear model, severity of clutter remained a significant predictor of CIR-error when controlling for age, gender, handedness, estimated IQ, depression, and use of psychotropic medication ($\beta = 0.13$, $R^2 = 0.46$, $p = 0.002$). In the sample that underwent neurocognitive testing, CIR-error was predicted by total errors on a Go/NoGo task ($\beta = 0.15$, $R^2 = 0.25$, $p < 0.001$), differential reaction time on a Stroop color/word interference test ($\beta = -0.15$, $R^2 = 0.16$, $p = 0.010$), and completion time for an attention switching task ($\beta = 0.11$, $R^2 = 0.14$, $p = 0.018$). Considering all objective measures predictive of CIR-error, a multivariate linear model was tested incorporating Go/NoGo errors, Stroop interference, attention switching performance and objective clutter assessment (IE-CIR). This model revealed IE-CIR ($\beta = 0.09$, $p < 0.001$), Go/NoGo errors ($\beta = 0.08$, $p = 0.02$), and Stroop interference ($\beta = 0.09$, $p = 0.04$) to be significant independent predictors of CIR-error, and explained 59% of the variance of CIR-error measurements.

Conclusions: Clutter underreporting increases with objective severity of clutter, the cardinal symptom of HD, suggesting insight impairment may reflect core pathophysiology of HD. The neurocognitive predictors of clutter underreporting implicate impairment in frontoparietal circuits underlying response inhibition and selective attention/interference control. Clutter underreporting in HD and clinical insight deficits in other disorders may share a conserved neural basis.

Keywords: Insight, Neurocognitive Assessment, Cognitive Control, Obsessive-Compulsive and Related Disorders

Disclosure: Nothing to disclose.

M156

Disrupted Amygdala Subregional Functional Connectivity in Obsessive-Compulsive Disorder

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Background: The amygdala, which is composed of the basolateral amygdala (BLA), centromedial (CM), superficial (SF) amygdala and amygdalostratial transition area (AStr), has long been associated with emotional and motivation, playing an essential part in processing both fearful and rewarding environmental stimuli and is perhaps the most strongly implicated brain structure in the pathophysiology of OCD. Imaging studies have shown significant alterations in the volume and activity of the amygdala in OCD patients. Animal studies have demonstrated more specifically that basolateral amygdala input to the medial prefrontal cortex controls obsessive-compulsive disorder-like checking behavior. Mouse functional magnetic resonance imaging (fMRI) study also verified the BLA-mPFC functional connectivity was increased in

OCD mice. These studies point to specialized roles of subregional amygdala functional connectivity associated with OCD symptom. However, this level of investigation has been largely absent in human studies, despite the critical role of amygdala in OCD pathology.

Therefore, in the current study, we aimed to examine the alteration of amygdala subregional networks in adult patients with OCD to explore whether different parts of the amygdala that are functionally connected to different regions contribute differently to the mechanism of OCD.

Methods: The study was approved by the Ethics Committee of the West China Hospital, Sichuan University, and all participants provided written informed consent to participate in the study. A total of 88 OCD patients and 88 sex- and age- matched HCs were recruited and diagnosed were based on the Structured Clinical Interview for DSM-IV Axis I disorders (SCID) by two experienced psychiatrists. All participants were right-handed and native Chinese speakers. Resting-state fMRI scans were obtained via a 3-Tesla GE MRI system with an 8-channel phase-array head coil using a gradient-echo echo-planar imaging sequence in addition to the high resolution T1-weighted 3D Spoiled Gradient Recall (SPGR) sequence. The rs-fMRI data were preprocessed with slice timing and head motion correction and an explicit motion correction strategy were applied as suggested by previous study.

Functional connectivity maps of four distinct regions of the amygdala, including the amygdalostratial transition area (AStr) and the basolateral (BLA), centromedial (CM) and superficial (SF) amygdala, were generated and compared between the two groups with a 2-by-2-by-4 full factorial analyses of variance (ANOVA), with subregion (AStr vs. BLA vs. CM vs. SF) and hemisphere (left vs. right) as within-group factors and group (OCD vs. HC) as a between-group factor. The significance threshold was set to $p < 0.005$ at the voxel level and corrected for multiple comparisons using cluster-level family-wise error (FWE) thresholding $p < 0.05$. Correlation with amygdala volume, symptom severity and illness duration were examined by extracting FC z scores from regions showing group differences and correlating these with the volumes of bilateral amygdala and clinical scores including YBOCS, HAMA and HAM-D.

Results: Relative to healthy control group, OCD patients showed increased FC between left CM and the right precentral gyrus and cuneus which negatively correlated with obsession ($r = -0.25$, $p = 0.019$), and decreased FC between the left CM and the left caudate which correlated with duration ($r = -0.293$, $p = 0.006$). Referring to the right CM, we found decreased FC between the right CM and bilateral striatum which positively correlated with obsession ($r = 0.22$, $p = 0.042$).

Increased FC was observed between the left BLA and the precuneus extend to the PCC in OCD patients compared to HC and it is negatively correlated with compulsion ($r = -0.30$, $p = 0.005$). Decreased FC between the left BLA and dACC, bilateral striatum and SCC were also detected in OCD. Increased FC was also observed between the right BLA and the precuneus extend to the PCC and the bilateral striatum in patients compared to HC. FC between the right BLA and the precuneus extend to the PCC was negatively correlated with YBOC scores ($r = -0.22$, $p = 0.037$).

Increased FC between the left AStr and the postcentral gyrus was observed in OCD patients with a positive associated with obsession ($r = 0.33$, $p = 0.002$). Decreased FC was observed between the left AStr and the caudate in addition to the dACC extend to the SMA which negatively correlated with HAMA scores. Finally, OCD patients exhibited decreased FC between the left SF and the SCC which was negatively associated with duration, as well as between the left SF and the ventromedial prefrontal cortex (VMPFC).

Conclusions: The present results suggest that subregional functional Connectivity of amygdala can reflect the network dysfunction in OCD in a more comprehensive way and it is related

to different symptom and clinical characteristics. We verify the animal study that demonstrate specific contribution of CM and BLA in OCD pathology from a network point of view.

Keywords: Obsessive-Compulsive Disorder (OCD), Amygdala, Functional Connectivity

Disclosure: Nothing to disclose.

M157

Examining Perinatal Adverse Events as Risk Factors for Pediatric OCD

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Background: Environmental factors play a role in the development of psychiatric disorders, including Obsessive Compulsive Disorder. Limited research exists on the relationship between perinatal risk factors and the development of early-onset OCD, although several studies have found significant associations between them (Brander, Rydell & Kuja-Halkola, 2016; Geller et al., 2008). In this analysis, we examined data from a family genetic study of OCD, a contemporaneous case-control study of ADHD, and a research patient data registry to compare perinatal risk factors between pediatric patients with and without OCD. We hypothesized that children and adolescents with OCD experienced higher rates of perinatal adverse events than healthy controls.

Methods: Participants with OCD were recruited as part of the OCD Collaborative Genetics Association Study (OC GAS) (NIMH R01MH 079489) at Massachusetts General Hospital (MGH) in Boston, MA, which took place between 2007 and 2011. Healthy controls were recruited from a contemporaneous family case-control study of ADHD. In addition, a second set of healthy controls was obtained from the Partners HealthCare Research Patient Data Registry (RPDR). Healthy controls were obtained from RPDR by querying for patients who did not have an OCD diagnosis, were born at a Partners hospital, and had been seen at MGH in 2008.

OCD participants' perinatal history was assessed using the MGH Psychiatric Neurodevelopmental Genetics Unit (PNGU) Medical Questionnaire—Parent on Child Version (Appendix 1). This questionnaire was completed by participants' parents and contained items relating to personal and family medical history. Healthy controls' perinatal risk history was assessed using standardized questions added to the Kiddie-Schedule for Affective Disorders and Schizophrenia-Epidemiological version (K-SADS-E) (Orvaschel and Puig-Antich, 1987). The K-SADS-E is a semi-structured, DSM-IV based diagnostic interview conducted with the parent and child to obtain a past and current history of psychiatric disorders. Perinatal data for healthy controls from RPDR was obtained from data captured during patient hospital visits.

Equivalent variables from the PNGU Medical Questionnaire, the K-SADS-E, and RPDR were extracted to compare perinatal history between children with OCD and healthy controls. Chi-square analyses were used to compare frequencies of each perinatal risk factor in the OCD and healthy control groups. Fisher's exact test was used for cases where there were fewer than 5 events. In addition, a binomial logistic regression was run to predict the probability of perinatal risk factor clusters among the groups. An exact logistic regression was used for variables with fewer than 5 events.

Results: Participants were 94 pediatric subjects with early-onset OCD ($M = 14$ years-old, $SD = 2.7$; range 8-18 years old) and 50 healthy controls from case-control ADHD study ($M = 12$ years-old, $SD = 3$,

range 6-18 years old). 123 healthy controls were obtained from RPDR (M = 21 years-old, SD = 1.3, range 19-24 years old). All participants with OCD had OCD diagnoses per DSM-IV criteria and had their first onset of obsessions and/or compulsions before the age of 18.

When compared to the first set of healthy controls on individual risk factors, children with OCD had mothers with significantly higher rates of illness during pregnancy requiring medical care ($\chi^2 = 5.23$, $p = 0.02$) and higher rates of alcohol use during pregnancy ($\chi^2 = 6.47$, $p = 0.01$). Children with OCD also had significantly higher rates of formula-switches during infancy ($\chi^2 = 4.50$, $p = 0.00$). When comparing perinatal risk factor clusters, children with OCD had mothers with significantly higher rates of prenatal complications not directly related to pregnancy, including illnesses and/or accidents requiring medical care ($\chi^2 = 4.50$, $p = 0.03$). Children with OCD also experienced a significantly higher rate of postnatal complications (incubation in the ICU, surgery during the first month, formula type switch, and low birth weight) than healthy controls ($\chi^2 = 4.57$, $p = 0.03$). Participants with OCD were 2.82 times as likely to experience postnatal complications as healthy controls (OR = 2.82, 95% CI [1.062, 7.483]; $p = 0.04$). Similar findings were demonstrated when comparing children with OCD to healthy controls from RPDR. Children with OCD had mothers with significantly higher rates of illness requiring medical care ($\chi^2 = 15.57$, $p = 0.00$), alcohol use during pregnancy ($\chi^2 = 18.34$, $p = 0.00$), and breech delivery ($\chi^2 = 4.55$, $p = 0.04$).

Conclusions: Overall, children with OCD had higher rates of postnatal complications than healthy controls and they had mothers with higher rates of prenatal complications not directly related to pregnancy. Our findings are consistent with previous studies and suggest there may be an association between perinatal factors and risk for early-onset OCD. Further research with a larger sample size is needed to assess the relationship between specific perinatal risk factors and early-onset OCD. Future studies may also examine how the prevalence of perinatal adverse events in children with OCD compares to children with other psychiatric diagnoses, to investigate the specificity of these risk factors to OCD.

Keywords: Obsessive Compulsive Disorder, Pediatric, Clinical Trial, Perinatal

Disclosure: Nothing to disclose.

M158

Cathodal Transcranial Direct Current Stimulation Targeting the Pre-Supplementary Motor Area on Resting State Functional Connectivity in OCD

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Background: Background: Obsessive-compulsive disorder affects 1-2 percent of the population. Its intrusive, anxiety-provoking obsessions and ritualized compulsions are distressing and can be disabling. A third of OCD patients are poorly responsive to medication or behavioral therapies. Some alternatives, including deep brain stimulation and stereotactic ablative procedures, invasive and not without risk, are suitable for only a small subset of the most seriously affected. Noninvasive methods that are effective are urgently needed for the wider proportion of those treatment-refractory individuals who are less severely affected but still impaired. This has motivated studies using repetitive transcranial magnetic stimulation (rTMS) and, more recently,

transcranial direct current stimulation (tDCS) in OCD. tDCS is attractive as a low cost, easily clinically deployable technique. However, few such tDCS studies have been done, particularly in using neuroimaging measures of brain function before and after stimulation. Here, we tested whether a course of tDCS had effects on brain circuitry plausibly implicated in OCD using fMRI resting-state functional connectivity (RSFC).

Methods: Methods: Twelve OCD participants and 16 healthy controls (HC) were included in the final analysis. Participants underwent open-label 1.5mA cathodal tDCS to the pSMA for one week, 2 sessions per day. All underwent MRI scans within one week prior to tDCS initiation, and 3-4 days after the last tDCS session. tDCS sessions were 20 minutes in duration, with one hour between each tDCS session. We placed a 5 x 5cm cathodal electrode at FCz, and a 5 x 7cm anode on the right cheek. Follow-up clinical interviews were carried out with the OCD group at 1 month and 4 months post-stimulation. Basic MRI preprocessing, functional connectivity preprocessing, and first-level connectivity models were carried out using the CONN Toolbox. A priori regions of interest (ROIs) or "seeds" for functional connectivity were based on coordinates reported in prior OCD studies, or on an exploratory multivariate pattern analysis. Analyses included group-level seed-to-voxel analyses between groups (HC v OCD) as well as post-tDCS clinical change. Results were corrected for multiple comparisons using the uncorrected voxel threshold of $p < .005$ and the false discovery rate-corrected cluster threshold of $p < .05$. The primary behavioral measure was the Yale-Brown OCD scale (YBOCS).

Results: Results: After covarying for age, RSFC between left dorsomedial thalamus and left IFG was lower in patients with OCD compared to controls ($t(25) = -3.74$, $p\text{-FDR} < .05$). In participants with OCD, decreases in left lateral OFC functional connectivity to the left IFG correlated with reductions in YBOCS OCD symptom severity ($t(10) = -3.31$, $p < .05$). In contrast, reductions in left lateral OFC-to-right dorsomedial thalamic anti-correlations correlated with increases in symptom severity ($t(10) = 3.45$, $p < .05$). MVPA identified six voxel clusters where functional connectivity differed between imaging sessions, located along the CSTC circuits and including bilateral caudate, right pars triangularis, and right superior frontal gyrus (lateral to SMA). An additional cluster was also found in the right middle temporal gyrus (all $p\text{-FDR} < .05$). Our post hoc analysis of MVPA-defined seeds revealed that functional connectivity of left caudate to the cortex proximal to the stimulation site (pre-SMA) differed post-tDCS. Functional connectivity to the insula, as well as a number of regions associated with the default mode network and higher visual processing, also changed across treatment.

Conclusions: Conclusion: This preliminary study examined changes in resting state functional connectivity after a 10-session course of tDCS over pSMA in individuals with OCD. Results indicated that changes in left lateral OFC RSFC to CSTC targets in left IFG and dorsomedial thalamus across treatment were correlated with changes in OCD symptom severity. MVPA demonstrated that functional connectivity in regions along the CSTC loops including bilateral caudate, right IFG, and lateral superior frontal gyrus changes after tDCS. This open-label study demonstrated changes in the circuitry related to OCD symptomatology after 10 sessions of tDCS. This initial work supports continued research into the use of non-invasive stimulation for treatment of OCD symptoms.

Keywords: Neurostimulation, Obsessive-Compulsive Disorder (OCD), tDCS

Disclosure: Nothing to disclose.

M159

Memantine Enhances Measures of Auditory Fidelity and Learning in Schizophrenia

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Background: Neurocognitive deficits and global function in schizophrenia (SZ) are mediated by deficits in early auditory information processing (EAIP). In both antipsychotic-medicated SZ patients and healthy comparison subjects (HCS), acute treatment with the NMDA antagonist, memantine (20 mg), enhances EEG- and EMG-based measures of EAIP, including mismatch negativity (MMN), auditory steady state response (ASSR) power and coherence and prepulse inhibition (PPI) of startle. Studies in progress are moving beyond EEG and EMG, to test the impact of memantine on functional measures of auditory processing in SZ patients and HCS, focusing on auditory discrimination (ability to identify words embedded in noise) and learning ("sound sweeps" frequency modulation task).

Methods: To date, 18 well-characterized SZ subjects (M:F = 8:10; mean age (range, y) = 40.83 (26-54)) and 13 HCS (M:F = 6:7; mean age (range, y) = 27.00 (18-49)) have been tested on two days about a week apart, after ingesting either placebo or 20 mg memantine po, in a double-blind, within-subject cross-over random order design. Laboratory testing included measures of neurocognition (MATRICS Comprehensive Cognitive Battery (MCCB)), audiometric screening, EAIP (MMN, ASSR, PPI), auditory fidelity ("Words-in-Noise" (WIN), "Speech-in-Noise" (QuickSIN) and "Gaps-in-Noise" (GIN)) and frequency modulation learning ("Sound Sweeps"). Present analyses focused on auditory fidelity and learning.

Results: Memantine enhanced some measures of auditory fidelity. ANOVA of WIN performance revealed the expected degrading effects of reducing signal-to-noise level (dB difference between words and background noise) on word recognition ($p < 0.0001$), no significant effect of diagnosis, and a significant interaction of dB x pill ($p < 0.013$). The "threshold" for degraded performance was a signal-to-noise difference of 4 dB, and at this level, ANOVA revealed significantly greater performance after memantine vs. placebo ($p < 0.033$), with no dose x diagnosis interaction. When subjects were divided based on baseline audiometry (threshold at 1000 Hz), memantine's beneficial effects on WIN performance were evident only among subjects with detection thresholds above the median normal levels. Memantine effects on QuickSIN performance were more subtle, reaching trend levels ($p < 0.09$) across all sound levels vs. the focused threshold impact for WIN. No effects of memantine were detected on GIN performance. Sound Sweeps "learning" was also enhanced by memantine, specifically in SZ patients; ANOVA of gains in auditory processing speed revealed a significant interaction of diagnosis x pill ($p < 0.019$); among SZ patients, memantine significantly enhanced TCT learning ($p < 0.036$). This effect in patients was most evident among younger patients, whose age approximated that of the HCS group. Exploratory analyses will examine the relationship between memantine-enhanced auditory fidelity and learning, and subject performance in measures of neurocognition (MCCB) and EAIP.

Conclusions: These preliminary findings suggest that memantine-enhancement of EEG- and EMG-based measures of EAIP may reflect, or result in, gains in processes that contribute to auditory discrimination and frequency modulation learning. Ongoing studies will assess the "pathway" responsible for these memantine-induced changes in auditory performance measures. Perhaps more importantly, memantine-induced gains in auditory learning may provide a basis for pharmacologic augmentation of the pro-cognitive effects of auditory-based targeted cognitive training (TCT).

Keywords: Schizophrenia, Memantine, Early Auditory Information Processing

Disclosure: Nothing to disclose.

M160

Treatment of Schizophrenia With L-Tetrahydropalmatine (L-THP) for Positive and Negative Symptoms of Schizophrenia: Results of a 4-Week Double Blind Trial

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Background: Schizophrenia is a devastating illness that is largely managed through antipsychotic medications which modulate the dopamine system; yet, many patients only partially respond to this treatment. There is growing evidence that schizophrenia may be partially due to an inflammatory process. Thus, treatments that have anti-inflammatory properties but also possess dopamine modulation may prove to be beneficial in treating people with schizophrenia. Once such potential treatment includes L-tetrahydropalmatine (L-THP), which has been clinically used for over 40 years in China, has robust anti-inflammatory properties, particularly tumor necrosis factor (TNF- α) and intercellular adhesion molecule 1 (ICAM-1) inhibition and possesses a pharmacological profile of D1, D2 and D3 receptor antagonism with similar profiles to that of the superior antipsychotic, clozapine.

Purpose: To determine if adjunctive L-THP is superior to placebo for the treatment of positive and negative symptoms of schizophrenia and to examine the tolerability and safety profile in L-THP relative to placebo in people with schizophrenia.

Methods: L-THP was tested (30 mg BID) as an adjunct treatment in a randomized, double-blind, 4-week trial, in which we assessed treatment efficacy, and collected peripheral venous blood to secondarily measure changes in peripheral cytokine concentrations and ICAM-1. Psychiatric symptoms were assessed via multiple scales including the Clinical Global Impressions (CGI) Scale, the Brief Psychiatric Rating Scale (BPRS) and the Schedule for Assessment of Negative Symptoms (SANS). Differences of psychiatric symptoms were examined using repeated measures ANCOVA, controlling for baseline and Cohen's D effect size (ES).

Results: Results did not show a significant improvement in psychiatric symptoms with L-THP; however, it was well tolerated. There was a trend for improvement on the CGI ($F=3.54$, $df=1,55$, $p=0.07$, $ES=-0.28$). There were 7/29 (24%) who had a robust improvement ($>25\%$ on total BPRS) compared to 3/32 (9%) on placebo. The adjunctive treatment did decrease extra pyramidal side effects ($F=12.8$, $df=1,56$, $p<0.001$, $ES=-0.85$) and showed a moderate effect on akathisia ($F=2.1$, $df=1,56$, $p=0.16$, $ES=-0.42$). Dizziness was higher with L-THP relative to placebo. Secondary results regarding the peripheral venous blood measurements showed a significant increase in ICAM-1 with L-THP compared to placebo ($F=9.04$, $p=0.004$), but did not show any effect on other peripheral cytokines.

Conclusions: This study failed to show an antipsychotic effect in a 4-week trial. However, there was a trend for a mild effect on the CGI and 24% had a robust response (compared to 9% on placebo). The significant increase in ICAM-1 found with L-THP relative to placebo does not replicate previous reports and our data suggests that L-THP may have pro-inflammatory effects related to its lack of robust effect on treatment. Still, L-THP was well tolerated overall. These results suggest a potential subgroup exists for which L-THP may be effective while not effective for the overall schizophrenia diagnosis and longer studies may be needed to see an improvement.

Funding: This study was supported by the Stanley Medical Research Institute.

Keywords: Schizophrenia, L-thp, Clinical Trial
Disclosure: Nothing to disclose.

M161

An Assessment of the Impact of Prior Antipsychotic Treatment on the Duration of Treatment With RBP-7000 During a Long-Term Safety Study

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Background: RBP-7000 is a once-monthly subcutaneous extended-release risperidone formulation that has been approved by the Food and Drug Administration for the treatment of schizophrenia in adults. The phase III program for RBP-7000 consisted of an 8-week, double-blind, placebo-controlled study followed by a 52-week open-label study of monthly RBP-7000 120 mg. The primary objective of the open-label study was to evaluate the long-term safety and tolerability of RBP-7000. This post hoc analysis was conducted to assess the impact of prior antipsychotic treatment on the duration of treatment with RBP-7000.

Methods: The 52-week open-label study (NCT02203838) enrolled men and women 18–65 years of age with a DSM-IV diagnosis of schizophrenia. This post hoc analysis focused on 408 stable (Positive and Negative Syndrome Scale [PANSS] total score ≤ 70) patients from the open-label study who did not participate in the double-blind, placebo-controlled study (de novo). De novo patients transitioned from their prior antipsychotic treatment to oral risperidone 3 or 4 mg during the run-in phase prior to the first dose of RBP-7000. Patients then received up to 13 monthly subcutaneous injections of RBP-7000 120 mg, which could be down-titrated once to 90 mg per clinical status. Data related to the discontinuation of RBP-7000 (eg, discontinuation rates, number of injections) were calculated for those who previously received risperidone and those who received other antipsychotics (ie, non-risperidone). These analyses will focus on patients who were treated with a single agent prior to receiving RBP-7000. Other outcomes related to discontinuation were analyzed for these groups.

Results: Oral risperidone was the most frequently used previous treatment (129 [32%]), followed by aripiprazole (62 [15%]), quetiapine (58 [14%]), olanzapine (39 [10%]), and haloperidol (19 [5%]). Demographic variables were essentially similar between both the risperidone and non-risperidone groups. One hundred ninety-eight (49%) patients completed the open-label study. The primary reason for discontinuation in the total population was withdrawal of consent (83 [20%]), followed by adverse events (46 [11%]) and lost to follow-up (39 [10%]). Discontinuation rates were numerically lower among patients who previously received risperidone prior to RBP-7000 (62 [48%]) than those who received other antipsychotics (129 [56%]). Similarly, the risperidone group had a higher median/mean number of injections during the treatment phase (13/9) compared to the non-risperidone group (9/8). A numerically larger number of patients who initially received antipsychotics other than risperidone discontinued RBP-7000 due to adverse events (31 [13%]) compared to those initially treated with risperidone (11 [9%]). Discontinuation rates for adverse events related to study treatment were 18 (8%) for the non-risperidone group and 6 (5%) for the risperidone group. After beginning RBP-7000 treatment, mean (SD) prolactin levels decreased to a numerically greater level for those who initially received risperidone (-4.0 [29.9] ng/mL) compared to those who were treated with other antipsychotics (-0.9 [33.5] ng/mL). In contrast, those who previously received risperidone had a slightly

greater increase in mean (SD) body weight (risperidone: 4 [10.7] kg; non-risperidone: 2 [13.6] kg). Both groups remained symptomatically stable throughout the study.

Conclusions: In general, the discontinuation rates and number of injections for RBP-7000 patients in the open-label trial were higher for those who were initially treated with risperidone compared to those who received other antipsychotics. The reasons for the differences in discontinuation rates are not clear, but they may in part be driven by the open-label design and improved tolerability in those who had been previously exposed to risperidone.

Keywords: Antipsychotic, Schizophrenia, Extended-Release Depot, Long-Term Safety, Discontinuation

Disclosure: Indivior, Inc., Employee

M162

New Peripheral Dopaminergic Mechanisms of Antipsychotic Drug-Induced Metabolic Disturbances

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Background: Antipsychotic drugs (APDs) are used to treat highly prevalent psychiatric illnesses including schizophrenia, bipolar disorder and major depressive disorder, making them among the most widely prescribed psychiatric medications today. However, these drugs also cause profound metabolic disturbances including weight gain, glucose intolerance, and insulin resistance and increase the risks of developing type 2 diabetes (T2D) and cardiovascular disease. Significantly, all APDs cause metabolic side effects to differing degrees and current treatments to reduce these metabolic symptoms have only limited efficacy. To date, the mechanisms for APD-induced metabolic disturbances are poorly understood. Nonetheless, the single unifying property of all APDs is their blockade of dopamine (DA) D2-like receptors including D2 (D2R) and D3 (D3R) receptors, suggesting a potential role for these receptors in APD-induced metabolic dysfunction. Importantly, APD-induced changes in glucose homeostasis occur even in the absence of increased food intake, or psychiatric disease. Indeed, as little as a single administration of olanzapine is sufficient to alter glucose homeostasis independent of weight changes in healthy human subjects. This raises the possibility that APDs may act directly on metabolically-relevant peripheral targets to cause metabolic disturbances. Consistent with this, we and others discovered that D2R and D3R are expressed peripherally in both human and rodent insulin-secreting pancreatic beta cells, key regulators of glucose metabolism. While considerably less studied than beta cells, there is also evidence that D2R and D3R are expressed in glucagon-secreting pancreatic alpha cells. We therefore hypothesize that APD-induced metabolic disturbances are driven by the direct actions of APDs on pancreatic alpha cell and beta cell D2-like receptors.

Methods: Human pancreatic islet transcriptome analysis: De-identified human islet alpha cells and beta cells ($n = 5$: 3 females, 2 males) were purified by FACS sorting; alpha cells and beta cells were distinguished via indirect antibody labeling with alpha- and beta cell markers (Brissova et al., 2018).

For DA measurements, mouse alpha cell-derived alpha TC1-6 cell supernatants and cell lysates were collected and run on HPLC (Farino et al., 2019).

Insulin and glucagon homogenous time-resolved resonance energy transfer (HTRF) assays: De-identified cadaveric human islets

and BALB/c mouse islets were collected and cultured overnight prior to use. Islets were glucose-stimulated and supernatants collected for insulin and glucagon measurement via HTRF (Farino et al. 2019; Aslanoglou et al., 2019).

All human and mouse islet studies were IRB- and IACUC-approved. All studies were conducted in triplicate on $n > 3$ experimental days.

Results: We conducted a comprehensive transcriptome analysis to characterize the DA signaling and biosynthetic machinery in human pancreatic alpha and beta cells followed by RNA-sequencing analysis (RNAseq). We found that human alpha and beta cells express the complete DA biosynthetic, catabolic and signaling machinery. Consistent with this, HPLC analyses demonstrated that alpha cells both synthesize and secrete DA. Unlike beta cells, alpha cells also secrete DA precursor L-DOPA. Our transcriptome data additionally showed that both human alpha and beta cells express all five DA receptors, with D2R and D3R being the predominantly expressed DA receptors. These data suggest that APDs may target D2-like receptors in beta cells and alpha cells.

To study APD actions on alpha and beta cell D2R and D3R, we developed a novel rapid optical glucagon detection assay based on HTRF technology, similar to our existing HTRF insulin assay (Farino et al., 2016). This approach eliminates all washing steps, making our assays rapid and high-throughput. We first examined the roles of DA signaling on glucagon release from human pancreatic islets, discovering that low DA concentrations potently decreased glucagon release, in addition to DA's inhibition of beta cell glucose stimulated insulin secretion (GSIS). These data suggest that DA modulates both glucagon and insulin secretion in islets. We next examined whether APDs disrupt coordinated secretion of glucagon and insulin during glucose stimulation of human islets. We showed that both clozapine and olanzapine substantially increased alpha cell glucagon secretion relative to vehicle controls; haloperidol also raised glucagon secretion, albeit to a lesser degree compared to olanzapine and clozapine. All three APDs also significantly increased GSIS from the same islets. Our results suggest that APDs enhance insulin and glucagon release, contributing to systemic metabolic dysfunction.

Conclusions: Overall, we show that pancreatic alpha cells may provide a key source of pancreatic DA which signals locally at alpha and beta cell receptors to modulate insulin and glucagon release. APDs disrupt this peripheral DA signaling at alpha and beta cells to significantly disturb secretion of key hormonal regulators of metabolism. Specifically, APDs disrupt dopaminergic inhibition of GSIS in beta cells, leading to excessive insulin secretion in islets – a driver of insulin resistance in type 2 diabetes. Similarly, APD blockade of alpha cell D2R/D3R also profoundly elevates glucagon secretion – a key driver of hyperglycemia. Ultimately, our work suggests that APDs act directly on both alpha cell and beta cell DA signaling to significantly disturb metabolism.

Keywords: Dopamine, Dopamine (D2, D3) Receptors, Insulin Resistance, Diabetes, Antipsychotic Induced Weight Gain

Disclosure: Nothing to disclose.

M163

ENHANCE: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study of Adjunctive Pimavanserin for Treatment of Schizophrenia in Patients With an Inadequate Response to Antipsychotic Treatment

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Background: Second-generation antipsychotics (APs) are the current gold standard for treatment of schizophrenia, offering advantages over the older first-generation. However, both efficacy and safety remain a concern for many patients. Clinical response to AP treatment for schizophrenia with these dopaminergic agents is often characterized by limited control of psychotic symptoms, leading to poor functioning, polypharmacy, and relapse. There is a strong unmet need for treatment that will improve overall outcomes and can safely be given adjunctively to current APs. Pimavanserin (PIM), a non-dopaminergic inverse agonist/antagonist of 5-HT_{2A} serotonin receptors, and to a lesser extent 5-HT_{2C} receptors, has been approved for treatment of Parkinson's disease psychosis and is now under investigation for treatment of hallucinations and delusions associated with several other psychiatric conditions as well as for treatment of negative symptoms in schizophrenia. Here we report the findings from the Phase 3 ENHANCE study of adjunctive PIM for treatment of schizophrenia in patients with inadequate response to their current AP treatment.

Methods: This was a 6-week, randomized, double-blind, placebo-controlled multicenter study conducted in North America and Europe. Adult DSM-5-diagnosed schizophrenia outpatients demonstrating an inadequate clinical response to their background AP treatment were randomized to receive PIM 20 mg/day or placebo added to their ongoing AP treatment for 6 weeks. Inclusion criteria required moderate-to-severe psychotic symptoms, defined as a Positive and Negative Syndrome Scale (PANSS) total score ≥ 65 and ≤ 110 , and an item score ≥ 4 (moderate or worse) on at least 2 items (Delusions, Hallucinations, and Suspiciousness/Persecution), at both screening and baseline, and a Clinical Global Impressions–Severity (CGI-S) score ≥ 4 . Daily PIM dose was initiated at 20 mg/day but could be increased to 34 mg or decreased to 10 mg as needed for efficacy or tolerability during the first 3 weeks of treatment. The primary efficacy measure was the 6-week change from baseline in PANSS total score. Key secondary outcome was Clinical Global Impressions–Severity (CGI-S) score. Other secondary and exploratory outcomes included Week 6 changes in PANSS factor subscores, Marder Factor scores, and change from baseline in PANSS total score by region.

Results: A total of 396 patients were randomized to PIM ($n = 198$) or placebo ($n = 198$) as adjunct to their current AP. The average daily dose for the PIM group was 25.63 mg/day ($SD = 5.37$). A consistent improvement of symptoms was observed in the PIM group, but improvement on the primary endpoint, the PANSS total score, was not statistically significant ($P = 0.0940$) relative to placebo at Week 6. The key secondary analysis of CGI-S, showed improvement consistent with the primary result (unadjusted $P = 0.0543$). Secondary and exploratory analyses showed an effect on negative symptoms: PANSS Negative Symptoms subscale (unadjusted $P = 0.0474$) and PANSS Marder Negative Factor score (unadjusted $P = 0.0362$). The majority of patients (81.5%) were enrolled in European sites, and a pre-specified analysis by region showed an effect for PIM on both the PANSS total (unadjusted $P = 0.0234$) and the CGI-S (unadjusted $P = 0.0214$) in the European subgroup.

Study completion was achieved by 88% of PIM and 96% of placebo patients. PIM was well tolerated, with a treatment-emergent adverse event (TEAE) rate (40.4%) similar to that for placebo (36.9%), and few patients in either group having at least 1 severe TEAE (PIM 1.0%, placebo 1.5%) or discontinuing due to a TEAE (PIM 2.5%, placebo 0%). No deaths occurred in either group. Use of adjunctive PIM did not result in clinically significant differences from placebo in vital signs, body weight, metabolic syndrome, electrocardiography (including QT interval), or extrapyramidal symptoms.

Conclusions: Although statistical significance was not achieved on the primary endpoint, a consistent trend of antipsychotic effect was observed for adjunctive PIM among patients with

schizophrenia with an inadequate response to current AP treatment, including an effect on negative symptoms. Treatment was well tolerated. Additional results from this study will be presented in the future.

Keywords: treatment-resistant schizophrenia, Serotonin 5-HT_{2A} Receptor, Negative Symptoms, pimavanserin

Disclosure: ACADIA Pharmaceuticals Inc, Employee

M164

Double Blind, Two Dose, Cross-Over Clinical Trial of the Positive Allosteric Modulator at the Alpha7 Nicotinic Cholinergic Receptor AVL-3288 in Schizophrenia Patients

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Background: Despite promise, studies of nicotinic alpha-7 acetylcholine (n-alpha7) receptor agonists, have largely failed to separate from placebo in schizophrenia. We describe AVL-3288 is a n-alpha7 positive allosteric modulator (PAM), which is only active in the presence of the endogenous ligand (acetylcholine), and theoretically less likely to cause receptor desensitization. We describe the first multiple dose AVL-3288 trial and the first in a patient population.

Methods: 24 non-smoking, medicated, outpatient men and women with schizophrenia or schizoaffective disorder and an RBANS > 61 were enrolled into this Phase 1b, single-center, randomized, double-blind, placebo-controlled, 3-treatment-phase (10, 30 mg or placebo), cross-over study.

The principal outcome measures were the RBANS Total Scale Score, with auditory P50 evoked potential suppression the key target engagement biomarker. Secondary outcome measures include task-based fMRI (RISE task), mismatch negativity, the Scale for the Assessment of Negative Symptoms of Schizophrenia [SANS] and the Brief Psychiatric Rating Scale [BPRS].

Results: 24 subjects were randomized without any clinically significant treatment emergent adverse effects. Baseline RBANS (82 +/- 17) and BPRS (41 +/- 13) scores were consistent with moderate impairment. Primary outcomes were negative, with non-significant worsening for both active groups vs. placebo in the P50 and minimal between group changes on the RBANS and RISE task.

Conclusions: The lack of target engagement changes are consistent with a lack of clinical effect, and are consistent both with failing the AVL-3288 compound. We are unaware of active studies using this mechanism, and when viewed with the totality of negative meta-analysis and failed Phase III studies, these results raise questions about the utility of further study of the n-alpha7 receptor as a viable target in schizophrenia. Although our results are negative, this study is a successful example of the NIMH Fast-Fail approach.

Keywords: Alpha-7 Nicotinic Acetylcholine Receptor, P50, Clinical Trial Design, Target Engagement, Schizophrenia Novel Treatment

Disclosure: Nothing to disclose.

M165

Reorganization of the Functional Connectome From Rest to Task in Schizophrenia and Bipolar Disorder

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Background: Neuroimaging and graph analyses suggests an aberrant organization of the whole-brain functional connectome in schizophrenia (SZ) and bipolar disorder (BD). There are two principles of network organization: segregation (i.e., the local organization of the brain in functionally specialized units) and integration (i.e., short pathways between every brain region). A balance of the two is associated with more effective neuronal processing. Segregation can be measured using the clustering coefficient and integration using characteristic path length and global efficiency. Importantly, network segregation and integration change from resting to task states in healthy participants (HC). Studies using resting state functional magnetic resonance imaging (fMRI) in SZ showed a reduced network segregation compared to HC, while integration seemed to be largely preserved. Studies using task fMRI showed no differences between SZ and HC. However, in SZ and BD studies it is very rare to examine changes in segregation and integration at rest versus during a task on the same participants in the same session. Therefore, the questions arise as to whether the organization of the functional connectome adapts to task demands in severe mental illness and whether the extent of change from resting to task states is impaired in patients compared to HC. The current fMRI study addressed these questions in a reasonably large sample of patients with SZ and BD as well as HC participants.

Methods: 44 individuals with SZ, 44 with BD, and 43 HC participants were eligible for analysis. Diagnoses were confirmed with the SCID-I. Participants provided written informed consent prior to participation. Task (400 s) and resting state fMRI (300 s) were acquired on a Siemens Tim Trio 3 T MRI scanner using a T2*-weighted echo planar imaging (EPI) gradient-echo pulse sequence (TR 2500 ms; TE 35 ms; voxel size 3x3x3.3 mm). fMRI data (pre-) processing was performed in FSL in accordance with current standards. Nuisance regression was performed (6 motion regressors, cerebrospinal fluid and white matter signal, one regressor for each TR with a relative FD > 0.5 mm). Task related activity was additionally regressed out. The residual NiftIs were used to extract mean time series for each node (anatomical Destrieux atlas; functional Power atlas). Nodes that were not covered in the fMRI scans were excluded [N(anat) = 26; N(func) = 60]. Pearson correlation coefficients (r) were computed for each pair of nodes. Negative r's were set to 0. Participants with > 50 % negative r's were excluded (N = 1). Connectivity matrices were Fisher-z-transformed. Graph analyses were performed using the R package 'brainGraph'. Clustering coefficient ('Cp'), characteristic path length ('Lp') and global efficiency ('E.global') were calculated across a range of density thresholds (0.1 to 0.5, steps of 0.02). The area under the curve (AUC) across densities was computed for each graph index, participant and condition (task vs. rest). For statistical analysis, mixed-design analysis of variance was performed with the between-subject factor group (SZ vs. BD vs. HC) and the within-subject factor condition.

Results: One graph index for segregation (Cp) and two for integration (Lp, E.global) were assessed separately.

Cp: There was no significant group X condition interaction [F(2,127) = 0.92, p = 0.4, η^2_G < 0.01]. There were significant main effects of group [F(2,127) = 4.71, p = 0.01, η^2_G = 0.05] and condition [F(1,127) = 21.37, p < 0.01, η^2_G = 0.04]. Both SZ [t(85) = -2.94, p < 0.01] and BD [t(84) = -2.1, p = 0.04] showed lower Cp than HC across conditions. There was no significant difference in Cp between BD and SZ [t(85) = 1.17, p = 0.25]. Regardless of group, Cp increased during a task compared to rest (i.e.,

segregation increased) [$t(127) = 4.62, p < 0.01$]. Results were consistent for the functional atlas (at $\alpha = 0.05$).

Lp: There was no significant group X condition interaction [$F(2,127) = 1.87, p = 0.16, \eta^2G < 0.01$] and no significant main effect of group [$F(2,127) = 0.22, p = 0.81, \eta^2G < 0.01$]. There was a significant main effect of condition [$F(1,127) = 10.48, p < 0.01, \eta^2G = 0.02$]. Lp decreased equally (i.e., integration increased) for all groups during a task compared to rest [$t(127) = -3.24, p < 0.01$]. Results were consistent for the functional atlas (at $\alpha = 0.05$).

E.global: There was no significant group X condition interaction [$F(2,127) = 1.29, p = 0.28, \eta^2G < 0.01$]. There were significant main effects of group [$F(2,127) = 5.86, p < 0.01, \eta^2G = 0.06$] and condition [$F(1,127) = 8.44, p < 0.01, \eta^2G = 0.02$]. Both SZ [$t(85) = 3.34, p < 0.01$] and BD [$t(84) = 2.1, p = 0.04$] showed higher E.global than HC across conditions. There was no significant difference in E.global between BD and SZ [$t(85) = 1.35, p = 0.18$]. Regardless of group, E.global decreased during a task compared to rest [$t(127) = -2.91, p < 0.01$]. None of the analyses were significant for the functional atlas (at $\alpha = 0.05$).

Conclusions: This study examined the functional connectome both during rest and task in SZ, BD and HC. Our results show that the functional connectome equally well adapts to task demands in patients and HC. While there were clear differences between patients and HC in network segregation for both resting and task states, group differences for integration varied with regard to the graph index and atlas used. Our findings indicate that a less efficient overall local organization of the brain and not an aberrant adaption of the functional connectome to task demands accounts for impaired neuronal processing in BD and SZ.

Keywords: fMRI Functional Connectivity, Schizophrenia, Bipolar Disorder, Graph-based Analysis, Task vs Rest

Disclosure: Nothing to disclose.

M166

Plasma Anthranilic Acid Correlates With Obesity but Not Insulin Resistance Markers in Schizophrenia

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Background: Dysregulation of tryptophan (Trp) – kynurenine (Kyn) pathway was suggested as a common mechanism underlying increased risk of insulin resistance (IR) and obesity in schizophrenia patients (and their first-degree relatives). Downstream metabolism of Kyn is trifurcated into formation of 3-hydroxykynurenine (3HK), kynurenic acid (KYNA) and anthranilic acid (ANA). Over-production of KYNA was suggested to be causally linked to major psychopathology in schizophrenia [Erhardt et al. 2007]. Up-regulated formation of KYNA in schizophrenia is triggered by the deficiency of kynurenine-3-monooxygenase (KMO), enzyme that catalyses Kyn conversion into 3HK [Schwarcz et al. 2001]. Since Kyn conversion into KYNA is catalysed by substrate-unsaturated enzyme, KMO inhibition elevates production of KYNA by increasing availability of Kyn as a substrate for KYNA formation from Kyn. Concurrently with up-regulation of KYNA production, KMO deficiency increases Kyn conversion into anthranilic acid (ANA) catalysed by unsaturated enzyme as well. Literature and our recent data suggested the involvement of peripherally originated KYNA in the mechanisms of IR and obesity in schizophrenia. However, we are not aware of studies of plasma ANA in a context of IR and obesity in schizophrenia.

Methods: Fasting plasma samples were obtained from 52 [19 drug-naïve first-episode and 33 previously-treated, but not medicated for, at least, 6 weeks] acutely ill DSM-IV schizophrenia patients and fifty-two healthy members of hospital staff and their relatives matched for gender, body mass index (BMI), and waist circumference (WC). There were 34 men and 18 women in each of studied groups. IR and obesity markers were previously assessed [Steiner et al., 2017]. ANA was evaluated by HPLC–mass spectrometry [Oxenkrug et al., 2015]. Statistical analysis: data was presented as mean + s.e.(nM). Mann-Whitney U test and Spearman's rank correlations ($p < 0.05$ considered as significant). Study was approved by the University of Magdeburg Review Board and by Tufts Medical Center IRB, and written informed consent was obtained.

Results: There were no statistically significant differences between ANA plasma levels in schizophrenia patients (14.05 ± 5.54) and healthy subjects (16.28 ± 7.53).

Markers of IR. In healthy subjects ANA correlated with insulin levels (but not with HOMA-IR) ($r = 0.25, p = 0.04$). In schizophrenia patients ANA did not correlate with HOMA-IR and insulin.

Markers of obesity. Plasma concentrations of ANA did not correlate with WC and BMI in healthy subjects. In schizophrenia patients ANA correlated with BMI ($r = 0.34, p = 0.01$) and WC ($r = 0.37, p = 0.01$) and leptin ($r = 0.44, p = 0.006$).

Conclusions: Contrary to positive correlations of ANA with markers of IR (insulin) in healthy subjects, we found no correlations between ANA and HOMA-IR and insulin in schizophrenia patients. In contrast, plasma ANA levels were correlated with markers of obesity (BMI, WC and, especially, leptin) in schizophrenia patients (but not in healthy subjects). Notably, we previously reported elevated serum ANA in leptin receptor deficient Zucker fatty rats [Oxenkrug et al. 2016]. Present findings suggest different involvement of peripherally originated ANA in mechanism(s) of IR and obesity in schizophrenia patients and non-schizophrenia subjects. Notably, ANA, in difference with KYNA, penetrates blood-brain barrier. Assessment of plasma ANA might be utilized as peripheral biomarker of increased risk of development of obesity in schizophrenia patients.

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Keywords: Antipsychotic-Naïve First-Episode Schizophrenia, Anthranilic Acid, Obesity, Insulin Resistance, Leptin

Disclosure: Nothing to disclose.

M167

Cognitive Functioning in Psychotic Disorders Across the Lifespan and Illness-Stages

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Background: Current models of cognition in schizophrenia are mainly based on the idea that the largest cognitive decline occurs prior to- or in the first years of overt clinical psychotic symptoms. While some research suggests a second 'peak' in decline during the late chronic stages, the consensus view is that there is relative

stability in the later phases of the illness. However, studies examining cognition mostly do so in relation to illness- status (i.e. early onset schizophrenia, UHR, FEP, chronic schizophrenia). Surprisingly few, if any, examined the possibility that the cognitive decline is actually an age-related rather than illness-course related process.

With the present study we aimed to test the hypotheses that cognitive decline starts early on but continues to deteriorate and diverge from the healthy population as patients get older, possibly accelerated by symptoms, antipsychotic medication and cannabis use. In addition, we aim to explore familiarity of cognitive decline.

Methods: Data analyzed in this study were collected in 30 centers across 13 countries (England, the Netherlands, Spain, France, Italy, Serbia, Turkey, Austria, Switzerland, Germany, Australia, Denmark and Brazil), and were part of the baseline assessment from the large-scale multinational EUropean Gene-Environment Interactions (EU-GEI) study, which ran from May 1 2010 to April 30 2015, or the Genetic Risk and Outcome of Psychosis (GROUP) study, which ran from April, 2004 to December, 2013. Combined, the studies included 3,341 controls, 2,347 siblings and 3,170 cases between the age 18 and 65, who were either in the prodromal, recent-onset or established stages of illness. Cognitive functioning, our primary study outcome, was measured using an abbreviated version of the WAIS-III, which consisted of the Digit Symbol Coding, Block Design, Information and Arithmetic subtests.

By means of multilevel linear regression models that account for the nested structure of our data (i.e. individuals within centers/countries and within families) we examined: (i) severity, (ii) relation to age versus illness stage, (iii) effect of cannabis use, symptom severity, functional disability and antipsychotic medication), and (iv) familiarity of cognitive impairments.

Results: Our preliminary results revealed cognitive impairments in patients across domains. Siblings showed mild impairments in Arithmetic ($Z = -3.83$, $p < 0.001$) and Information ($Z = -7.12$, $p < 0.001$), with scores in between those of patients and controls, but did not differ from controls on Block Design and Digit Symbol Substitution.

Within the patient group, illness duration and cannabis use did not have a negative effect on cognitive performance. Instead, cannabis users had significantly higher scores on the Information subtest than non-users ($Z = 4.91$, $p < 0.001$). Cognitive performance was negatively associated with GAF functioning scores, use of antipsychotics and age (range $p = 0.016$ – $p < 0.001$). Antipsychotics did not affect the performance on the Information subtest.

Linear multilevel regression analyses revealed that age-associated decline was apparent in patients, siblings and controls and across most cognitive measures, with the exception of the Information subtest (where no age-related decline was detected). However, age-associated group differences were also observed; patients and siblings appeared to show less decline than controls by age on Arithmetic (siblings: $Z = 2.62$, $p = 0.009$; patients: $Z = 2.15$, $p = 0.031$) and Information ($Z = 4.89$, $p < 0.001$; $Z = 2.91$, $p = 0.004$). No interaction effect was detectable for Digit Symbol. For Block Design, siblings but not patients showed relatively less age-related decline than controls ($Z = 3.35$, $p = 0.001$).

Conclusions: Results of this largest schizophrenia study to date underscore that greatest cognitive decline in patients with schizophrenia has already occurred prior to onset of overt clinical psychotic symptoms. Siblings showed impairments in Arithmetic and Information tests, suggesting familiarity in verbal cognitive performance. Age-related decline was apparent in all groups, although differences in cognitive performance between patients and siblings compared to controls diminished at older ages. Importantly, our findings suggest that after illness-onset, cognitive impairment has little to do with the stage of illness or illness-duration.

Keywords: Cognition, Familiarity, Age Effects

Disclosure: Nothing to disclose.

M168

Robust Hierarchically Organized Whole-Brain Patterns of Dysconnectivity in Schizophrenia Spectrum Disorders Observed After Personalized Intrinsic Network Topography

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Background: Spatial patterns of brain functional connectivity can vary substantially at the individual level. Applying cortical surface-based approaches with individualized rather than group templates may accelerate the discovery of biological markers related to psychiatric disorders. We report new results from multi-cohort data in people with schizophrenia spectrum disorders (SSDs) and healthy controls using individualized connectivity profiles in cortical-subcortical and cortical-cortical networks.

Methods: We utilized resting state and anatomical MRI data from $n = 494$ participants ($n = 202$ SSD, $n = 292$ healthy controls (HC), age $M(SD) = 28.8(8.9)$, 294 Males) from four cohorts: 1) Centre for Addiction and Mental Health 2) Zucker Hillside Hospital 3) The Center for Biomedical Research Excellence (Christensen et al 2014), and 4) UCLA Consortium for Neuropsychiatric Phenomics LA5c Study (Poldrack et al 2016). For each participant, functional timeseries were extracted from 80 cortical regions of interest, representing 6 intrinsic networks using 1) a volume-based approach 2) a surface-based group atlas approach, and 3) Personalized Intrinsic Network Topography (PINT), a personalized surface-based approach (Dickie et al., 2018). Timeseries were also extracted from previously defined intrinsic network subregions of the striatum (Choi et al 2011), thalamus (Ji et al 2019), and cerebellum (Buckner et al 2011).

Results: Compared to a volume-based approach, the correlations between all cortical networks and the expected subregions of the striatum, cerebellum, and thalamus were increased using a surface-based approach (Cohen's $D = 0.27$ – 1.00 , all $p < 10^{-6}$) and further increased after PINT (Cohen's $D = 0.18$ – 0.96 , all $p < 10^{-4}$). In SSD vs HC comparisons, controlling for age, sex, scanner and in-scanner motion, we observed robust patterns of dysconnectivity that were strengthened using a surface-based approach and PINT (Number of differing pairwise-correlations: volume: 357, surface: 562, PINT: 630, FDR corrected). These patterns were found from four different cortical networks - frontal-parietal, sensory-motor, visual, and default mode -- to subcortical regions.

Conclusions: Our results indicate that individualized approaches can optimize cortical network dysconnectivity differences in people with SSDs. These robust patterns of dysconnectivity were visibly organized in accordance with the cortical hierarchy, as predicted by computations models (Murray et al 2019). Our results also change our understanding of the specific network-network functional connectivity alterations in people with SSDs, and the extent of those alterations. Future work will examine these new patterns of dysconnectivity with behaviour using dimensional models.

Keywords: Cortical Circuit Function, Schizophrenia, Functional MRI (fMRI), Corticostriatal Networks, Thalamo-Cortical Interactions

Disclosure: Nothing to disclose.

M169

The Medial Septum Enhances Reversal Learning via Opposing Actions on Ventral Tegmental Area and Substantia Nigra Dopamine Neurons

Abstract not included.

M170

One vs Two-Hits: Investigating the Impact of Adolescent Alcohol Exposure on Adulthood Drinking and Local Field Potential Oscillations in a Rodent Model of Schizophrenia

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Background: Individuals with schizophrenia (SCZ) are far more likely to abuse alcohol than the general population, which adversely affects disease morbidity. The precise etiology of SCZ and co-occurring alcohol use disorder is unclear, but it is theorized that early life stressors combined with adolescent drug exposure may lead to exacerbated symptoms, including increased substance use. In clinical samples, prenatal exposure to infection is a significant risk factor for SCZ, and can be modeled in rodents using maternal immune activation (MIA). MIA offspring demonstrate multiple behavioral and neurobiological phenotypes reflective of neuropsychiatric illness, but the ability of MIA to induce increased alcohol drinking has not been investigated. Furthermore, it is unknown whether a “second-hit” in adolescence (such as exposure to alcohol) is necessary to increase alcohol drinking in adulthood, as well as what the neurobiological consequences of one- vs. two-hits are in this model. The current experiment therefore aimed to investigate the impact of MIA, adolescent alcohol exposure (AE), and MIA + AE combined (DUAL) on adulthood drinking behavior and local field potential (LFP) oscillations recorded from the striatum, frontal cortex, and hippocampus (regions heavily involved in SCZ and alcohol reward).

Methods: Pregnant Sprague-Dawley dams were injected intravenously with polyinosinic:polycytidylic acid [poly(I:C); 4mg/kg] or saline (1 mL/kg) on gestational day 15. Poly(I:C) is a synthetic analog of double-stranded RNA, which leads to a heightened immune response in rats. Both male and female pups were allowed to develop normally and weaned on post-natal day (P) 21. Half of the rats were allowed to drink 10% alcohol in their home cage from P28-42 (24 hr access). On P70, one group of rats were again given access to alcohol in their home cage for 90 min/day, 5 days/week (n = 18-20/group) for 3 weeks in order to measure adulthood alcohol consumption. The other group of rats were implanted with electrodes targeting the bilateral nucleus accumbens shell (NacSh), infralimbic (IL) and prelimbic (PL) medial prefrontal cortex, and the CA1 region of the hippocampus. Following recovery, LFPs were recorded from each awake, freely-behaving rat (n = 8-18/group) during two 30-minute sessions. Data from each recording were analyzed using established frequency ranges (delta = 1-4 Hz, theta = 5-10 Hz, alpha = 11-14 Hz, beta = 15-30 Hz, low gamma = 45-65 Hz, and high gamma = 70-90 Hz) from the rodent literature. Standard LFP signal processing to characterize the power spectral densities within, and coherence between brain regions for each rat was calculated using custom code written for Matlab. Using the machine-learning algorithm lasso, we built predictive models to classify rats based on group assignment, (e.g., Control, MIA, AE, or DUAL). We compared the model performance from real data to the performance of models built and tested on data permutations (which estimates chance). If the lasso indicated that information existed in the LFP signal, we implemented exhaustive single

feature regressions using each LFP predictor to determine the relative information content of each neural feature.

Results: A repeated measures ANOVA revealed that MIA did not impact g/kg of alcohol consumed in adolescence ($p = 0.33$, $n2p = .03$). However, DUAL exposure led to increased alcohol consumption in adulthood ($p = 0.015$, $n2p = .08$), with no effect of MIA ($p = 0.14$, $n2p = 0.03$) or AE ($p = 0.19$, $n2p = 0.02$) alone. For the LFP data, models predicting the DUAL group from all other groups outperformed chance (77% vs. 52%, respectively). Single features regression models indicated that high gamma coherence between the NAcSh and IL, and CA1 and IL contained significant information differentiating the DUAL group from all other groups.

Conclusions: The current experiment suggests that MIA or AE alone does not impact alcohol drinking, but that “two-hits” (e.g., MIA + AE) leads to enhanced alcohol consumption in adult offspring. These data align well with what is observed in clinical populations, where early alcohol/substance use increases the risk of developing SCZ. Additionally, high frequency oscillations between regions known to be dysfunctional in SCZ and addiction differentiated the DUAL rats from all other rats, suggesting that “two-hits” may lead to abnormal connectivity between these regions. Our current work aims to determine whether the neural features that differentiate DUAL rats are also related to alcohol intake levels, in order to identify potential neural targets for future therapeutic development aimed at reducing drinking in SCZ.

Keywords: Alcohol, Machine Learning, Local Field Potentials, Maternal Immune Activation, Schizophrenia-like Behavior

Disclosure: Nothing to disclose.

M171

Normalizing Glycogen Synthase Kinase 3 (GSK3) Activity in Fast-Spiking Neurons Rescues Gamma Oscillation Deficits in an NMDAR Hypofunction Model of Schizophrenia

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Background: Auditory steady-state responses (ASSRs), click trains-evoked EEG oscillations at 40-Hz in the temporal cortex, are known to be compromised in patients with schizophrenia, which may be associated with their cognitive dysfunction. ASSRs are also severely impaired in our schizophrenia mouse model in which NMDA receptor subunit GluN1 is deleted in ~50% of cortical and hippocampal GABA neurons in early postnatal development (Ppp1r2cre/GluN1 knockout (KO) mice; Belforte et al, 2010; Nakao and Nakazawa, 2014). Furthermore, we recently found that immunoreactivity (IR) against a phosphorylated form of glycogen synthase kinase 3 (GSK3; at Y216 in GSK3 β), which is an auto-activated form of GSK3 α/β , is augmented in the PV neurons (presumably GluN1-deleted), but not pyramidal neurons of the mutant mPFC. To assess the impact of predictive GSK3 β over-activity on the in vivo action potential (AP) spike synchrony of cortical pyramidal neurons and in vivo tone-evoked LFP gamma oscillation, and cognitive behavior, we took a pharmacological and genetic approach.

Methods: ANIMAL Ppp1r2-cre(+ / -)/fGluN1(f/f) KO mice were generated as previously described (Belforte et al., 2010), and were tested with various GSK3 inhibitors under two in vivo electrophysiology tests (Cross-correlation and ASSR) and two behavioral tests [spontaneous alternation in Y-maze and prepulse inhibition (PPI)]. To achieve GSK3 α - or GSK3 β -specific genetic inhibition in

G-

luN1-deleted GABA neurons, either floxed-GSK3 α or floxed-GSK3 β mouse strain was bred to Ppp1r2cre/GluN1 KO mice to generate the GABA neuron-selective GSK3 heterozygous KO mice. In those triple transgenic mutant mice, the kinase activity could be normalized in the GluN1-deleted PV neurons. IN VIVO MULTIUNIT RECORDING. Animals (both sex, 10-15 weeks old) implanted with a microarray carrying 6 tetrodes on the somatosensory cortex were subjected to a linear track to record the unit activity from somatosensory cortex. To analyze the level of synchronization of local excitatory circuits, after cell-clustering to isolate the pyramidal neurons, pairs of cells recorded in the same tetrode were subjected to cross-correlation analysis. IN VIVO LFP RECORDING. LFP recording was performed from A1 cortex of awake, head-restrained mice in an auditory isolation chamber (background sound level, 35 dB SPL). 500-ms long click trains consisting of 80 dB white-noise pulses presented at 40Hz (40-Hz ASSR stimuli) were applied 50 times with an inter-stimulus interval of 20 sec. BEHAVIORAL TEST: A different cohort of animals were subjected to Y-maze spontaneous alternation test (for assessment of spatial working memory) and prepulse inhibition (PPI) of acoustic startle reflex as described previously.

Results: Pretreatment with TDZD-8 (nonselective GSK3 inhibitor, 2.5 mg/kg, IP) alleviates in vivo AP spike synchrony deficits (21 pairs, $p=0.0004$, paired t-test) and diminished tone-evoked gamma oscillations in the GluN1 mutant mice (9 electrode channels, $p=0.021$, paired t-test). To determine which isoform of GSK3, GSK3 α or GSK3 β , elicits the impairment via over-activity in the PV neurons, we used the GSK3 β -paralog selective inhibitor, BRD3731 (30 mg/kg, IP) and GSK3 α -paralog selective inhibitor, BRD0705 (30 mg/kg, IP). Administration of BRD3731, but not BRD0705, alleviated the defective gamma oscillation in the GluN1 mutant mice (9 channels before and after BRD3731, $p=0.006$; 6 channels before and after BRD0705, $p=0.34$, paired t-test). To address the role of GSK3 β -specific inhibition in GABA neurons, we bred a floxed-GSK3 β mouse strain to the Ppp1r2cre/GluN1 KO mice to generate the GABA neuron-selective GSK3 β heterozygous knockout (GABA neuron-selective knockdown). Genetic GSK3 β knockdown reverses in vivo AP spike synchrony deficits (31 neuron-pairs from GSK3 β knockdown mice vs 24 pairs from the original KO mutants, $p=0.0013$, t-test), but GSK3 α knockdown did not reverse the deficits (10 pairs from GSK3 α knockdown mice vs 24 pairs from KO mutants, $p=0.99$, t-test). Genetic GSK3 β knockdown mice also alleviated the defective gamma oscillation (7 channels for GSK3 β knockdown vs 13 channels for original mutants, $p=0.02$, t-test). We assessed whether GSK3 inhibition restored the cognitive function in the GluN1 mutant mice. TDZD-8 restored the spontaneous alternation in spatial Y-maze ($n=6$ for TDZD8 vs $n=8$ for saline, $p<0.05$, t-test) and PPI of the GluN1 mutant mice ($n=12$ for TDZD8 vs $n=20$ for saline, $p<0.05$, two-way ANOVA post hoc test). Pretreatment of BRD3731, but not BRD0705, ameliorated the defective spontaneous alternation in the spatial Y-maze ($n=9$ for BRD3731, $n=6$ for BRD0705, $n=8$ for saline, $p=0.03$ for BRD3731 vs saline; $p=0.53$ for BRD0705 vs saline, t-test) and PPI ($n=8$ for BRD3731, $n=6$ for BRD0705, $n=20$ for saline, $p=0.001$ for BRD3731 vs saline, $p>0.05$ for BRD0705 vs saline, two-way ANOVA post hoc test) of the GluN1 mutant mice. Genetic GSK3 β knockdown also restored the spontaneous alternation in the spatial Y-maze ($n=9$ for GSK3 β knockdown vs $n=8$ for original mutants, $p=0.04$, t-test) and PPI of the GluN1 mutant mice ($n=10$ for GSK3 β knockdown vs $n=20$ for original mutants, $p<0.05$, two-way ANOVA post hoc test).

Conclusions: Our results suggest that inhibition of GSK3 β , but not GSK3 α , in cortical GABA neurons ameliorates the cognitive dysfunction in schizophrenia, presumably by the restoration of neuronal gamma synchronous oscillatory activity.

Keywords: GSK3, NMDA Receptor, Parvalbumin Neurons, Gamma Oscillation, Transgenic Mice

Disclosure: Nothing to disclose.

M172

An Independent Replication Supporting Corticopallidal Contributions to Functional Impairment in Schizophrenia

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Background: Abnormalities between the prefrontal cortex and basal ganglia have been described by numerous studies of schizophrenia (SZ). We recently reported that individuals with first-episode SZ who developed greater vocational and social impairments showed lower baseline functional connectivity between the globus pallidus (GP) and regions of the salience network (Tarcijonas et al., 2019). The following study aimed to extend these findings in a cohort of individuals with more chronic illness.

Methods: All data were obtained from a publicly available Center for Biomedical Research Excellence (COBRE) dataset (http://fcon_1000.projects.nitrc.org/indi/retro/cobre.html), which included resting-state fMRI and structural scans obtained on a 3T scanner, and an array of clinical and neuropsychological measures. Individuals with SZ ($N=61$, 14F, mean age = 38 years) and matched healthy controls ($N=73$, 23F, mean age = 36 years) were examined in this analysis. Patients were divided into high- or low-functioning groups based on scores across measures of psychopathology and cognition. Seed regions of interest in bilateral GP interna and externa were drawn based on anatomical location. Resting-state connectivity was examined between these seeds and regions of the salience network (including the dorsal anterior cingulate, and left and right insula) that were significant in our previous study (Tarcijonas et al., 2019). Functional connectivity was examined between low- and high-functioning individuals with SZ and controls.

Results: Consistent with our previous findings, low-functioning individuals with SZ demonstrated significantly reduced connectivity between bilateral GP and the salience network, relative to healthy controls ($P<0.05$, Bonferroni corrected). No connectivity differences were observed between higher functioning individuals with SZ and healthy controls.

Conclusions: These results replicate our previous findings in a more chronic cohort of individuals with SZ. Our findings further advance corticopallidal connectivity as a biomarker of functional impairments in SZ and contribute to a foundation for treatment-based studies.

Keywords: Resting State Functional Connectivity, Schizophrenia, Globus Pallidus, Salience Network, Functional Impairments

Disclosure: Nothing to disclose.

M173

Synaptic Elimination in First-Episode Psychosis Subjects

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Background: Schizophrenia may be related to a reduced cortical synapse density according to postmortem studies. Clinically high-risk subjects show a steeper decrease in grey matter thickness, and in vitro modeling using patient-derived cells implicate excessive synaptic pruning during neurodevelopment as a part

of the schizophrenia pathophysiology. However, it is unclear to what extent synapse elimination is present during various stages of the disease, which is of clinical importance as in a real-world setting most subjects received their schizophrenia diagnosis, not until their mid-twenties. This study aims to assess if a synapse elimination process is present during first-episode psychosis (FEP) diagnosis.

Methods: Immunoprecipitation mass spectrometry was used to measure cerebrospinal fluid (CSF) concentrations of the two pre-synaptic proteins synaptosomal-associated protein 25 (SNAP-25) and synaptotagmin-1 (SYT-1), in 44 FEP subjects (mean age 29.9 years) and 21 healthy controls (25.9 years).

Results: No significant differences in CSF SNAP-25 or SYT-1 between FEP patients and healthy controls (SNAP-25tot $P = 0.104$, 95%CI -1.08~11.4, SYT-1 $P = 0.137$, 95%CI -19.18~136.03) were observed. Neither protein showed a correlation with symptom ratings, cognitive performance, or antipsychotic medication.

Conclusions: No evidence for increased synaptic elimination in FEP patients was found. Additional studies in high-risk subjects at the early prodromal phase will be needed to address if excessive synapse destruction occurs before the development of overt psychotic symptoms.

Keywords: First Episode Psychosis, Schizophrenia, Pruning

Disclosure: Nothing to disclose.

M174

Circuit Mechanism Mediates Sub-Chronic Ketamine-Induced Increase in Dopamine Synthesis

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Background: Patients with schizophrenia show increased striatal dopamine synthesis capacity which is linked to symptoms in imaging studies (Howes et al., 2012). N-methyl-D-aspartate receptor (NMDAR) blockers such as ketamine induce psychotic symptoms in healthy humans (Krystal et al., 1994). Schizophrenia is associated with a reduction in parvalbumin (PV) expressing GABAergic interneurons, which are regulated by NMDAR, in the cortex and hippocampus (Benes et al., 1991, Zhang et al., 2002). It has been suggested that impaired PV neuronal function may lead to disinhibition of mesostriatal dopamine neuron activity (Grace 2016). However, the circuit mechanisms underlying increased dopamine synthesis capacity and how it is regulated by changes in PV neurons, is unknown. Therefore, we tested the effect of the sub-chronic ketamine on dopamine synthesis capacity using the same [18F]-FDOPA PET imaging approach which has shown elevated dopamine synthesis capacity in patients, and investigated the underlying circuitry.

Methods: All procedures were conducted under license in accordance with the UK Animals (Scientific Procedures) Act of 1986. Male mice received sub-chronic administration of 30mg/kg ketamine or saline. They underwent a dynamic [18F]-FDOPA PET scan to measure striatal dopamine synthesis capacity. PV interneurons in PLc and ventral subiculum (VSub) of the hippocampus in PV::Cre mice were transduced with Cre-dependent hM3Dq-mCherry designer receptors exclusively activated by designer drugs (DREADDs). Mice received clozapine N-oxide (CNO) or saline before the administration of ketamine and

they underwent a PET scan. Data were analysed by two-tailed independent samples t-tests or two-way ANOVAs. $P < 0.05$ was considered statistically significant.

Results: Sub-chronic ketamine administration significantly increased striatal dopamine synthesis capacity compared to saline controls ($p < 0.05$). In vivo activation of PV interneurons in the PLc and VSub, prior to ketamine administration, significantly reduced the elevation in striatal dopamine synthesis capacity ($P < 0.01$).

Conclusions: We showed that sub-chronic ketamine leads to an increase in striatal dopamine synthesis capacity in the mouse, resembling the dopaminergic alteration seen in patients with schizophrenia. Our data suggest that ketamine's effects on dopamine synthesis capacity are mediated by the inhibition of PV interneurons in the cortex and VSub of the hippocampus.

Keywords: Ketamine, Schizophrenia, Depression, PET Imaging, Chemogenetics

Disclosure: Nothing to disclose.

M175

A Genetics Perspective on the Role of the Neuro(Immune) System in Schizophrenia

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Background: The role of the immune system in schizophrenia has been the subject of widespread debate with numerous past reports proffering conflicting evidence, without definitive resolution, from diverse investigatory approaches including neuroimaging, pharmacology, and molecular genetics. Recent reports however, of credible schizophrenia-associated genetic variants with potential effect on immune function have further reinvigorated debate of the role of the immune system in schizophrenia, currently warranting renewed inquiry from a systematic genomics perspective.

Methods: The current analysis expands upon a previous report of 700 critical immune genes culled from 20 canonical immune pathways. Using RNA Sequencing of post-mortem dorsolateral prefrontal cortex (DLPFC) and hippocampus, schizophrenia case-control differential expression of the immune gene sets are compared to the transcriptome, by a Wilcoxon rank sum test of t-statistic distribution.

Results: In DLPFC PolyA + RNA-Seq ($n = 155$ SCZ, $n = 196$ CONT) the culled immune set ($n = 501$ genes, $p = 2.11 \times 10^{-8}$), adaptive immunity ($n = 55$ genes, $p = 0.002$) and innate immunity ($n = 122$ genes, $p = 5.1 \times 10^{-5}$) were decreased in expression compared to the overall transcriptome ($n = 24,122$ genes). Likewise, the finding of decreased expression was replicated in DLPFC RiboZero RNA-Seq ($n = 153$ SCZ, $n = 226$ CONT) for the immune set ($n = 442$ genes, $p = 1.2 \times 10^{-5}$). In addition, select canonical immune pathways were relatively decreased, including microglia ($n = 27$ genes, $p = 4.7 \times 10^{-3}$) and complement ($n = 21$ genes, $p = 0.048$). Further, the pattern of relatively decreased immune expression was observed in Hippocampus RiboZero RNA-Seq ($n = 133$ SCZ, $n = 200$ CONT) for the immune set ($n = 442$ genes, $p = 1.46 \times 10^{-5}$) and select immune pathways. In both DLPFC and Hippocampus, the eigenvector of immune genes was not significantly associated with schizophrenia polygenic risk (as defined by most recent PGC report). The current results are contrasted with reported pathway analyses of the PGC schizophrenia working group.

Conclusions: A set of critical immune genes appears to be decreased in differential expression compared to the overall transcriptome in post-mortem DLPFC and Hippocampus, seemingly inconsistent with a currently prevalent view of increased expression or 'activation' of the immune system in schizophrenia. Other ongoing analyses are extending the current analyses to other brain regions, and querying the expression of immune genes within co-expression networks.

Keywords: Schizophrenia, Genomics, Neuroimmune

Disclosure: Nothing to disclose.

M176

Cell Type-Specific Genetic Regulation of Expression in the Granule Cell Layer of the Human Dentate Gyrus

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Background: Extensive effort has been spent over the past ten years to more fully characterize the human brain transcriptome within and across brain regions and cell types, and to better understand changes in RNA expression associated with brain development and aging, developmental or psychiatric brain disorders, and local genetic variation. Here we survey gene expression in two preparations from postmortem human brain: 1) bulk tissue from hundreds of subjects and 2) hundreds of thousands of cells from a smaller sample by deeply profiling gene expression from a specific cell population.

Methods: We performed laser capture microdissection (LCM) to extract the DG-GCL in postmortem human hippocampal tissue from 263 human subjects, including 75 donors with schizophrenia, 66 with bipolar disorder, 29 with major depression, and 93 neurotypical controls, all with genome-wide genotype data. We combined these data with 333 age-matched RNA-seq samples from the homogenate hippocampal formation. We performed differential expression analyses for age, genotype, and the different psychiatric diagnoses using linear regression analysis.

Results: We identified 1337 genes with expression that only associated with age across the lifespan in the DG-GCL ($FDR < 0.05$) and these genes were enriched for diverse neuronal processes. We further identified ~9 million SNP-feature eQTL pairs in the DG-GCL ($FDR < 0.01$), of which 15% were not even marginally significant ($p > 0.05$) in bulk hippocampus. Using these eQTL maps, we identified novel schizophrenia-associated genes and their features that were completely missed in bulk brain tissue, including associations to GRM3 and CACNA1C. We lastly found a small number of genes differentially expressed in the DG-GCL in patients with schizophrenia, bipolar disorder or major depression compared to neurotypical individuals that were largely missed in bulk tissue.

Conclusions: Overall, we demonstrate that the LCM-based enrichment strategy detects signals unique to the granule cell layer that were completely missed in homogenate tissue and generates TWAS evidence of novel schizophrenia risk associated loci that also were dependent on gene expression data in DG-GCL in contrast to bulk hippocampal tissue. This strategy of deeply sequencing target cell populations provides a powerful balance between unbiased single cell and homogenate tissue sequencing that can provide cell type-specific associations to common molecular and clinical traits.

Keywords: RNA-Sequencing, Hippocampus, Dentate Gyrus, Genomics, Schizophrenia Genetics

Disclosure: Nothing to disclose.

M177

Circadian Patterns of Hallucinatory Experiences in Patients With Schizophrenia: Potentials for Chrono-Pharmacology

Abstract not included.

M178

The Novel, Non-D2 Psychotropic Agent SEP-363856 Modulates Presynaptic Dopamine Function in Mice

Abstract not included.

M179

Linking Echoic Memory to Primary Auditory Cortex in the Macaque Monkey to Better Understand Echoic Memory Deficits and Primary Auditory Cortex Pathology in Schizophrenia

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Background: Auditory information is briefly stored in a passive sensory buffer (echoic memory) for comparison with subsequent sounds. It has been suggested that impaired encoding into echoic memory lies at the heart of auditory deficits in individuals with schizophrenia. However, because the neural substrate of echoic memory is unclear, it limits our ability to ameliorate auditory deficits in schizophrenia. Given the sensory nature of echoic memory, it is commonly assumed to reside in early sensory regions such as primary auditory cortex (A1). This is consistent with the prominent pathological changes that point to an involvement of Heschl's Gyrus in auditory deficits in schizophrenia. A mechanistic understanding of the neural substrate of echoic memory necessarily depends on granular measures of neural activity in an appropriate animal model. Macaque monkeys have been shown to exhibit a passive, pre-categorical and short-lived component of auditory short-term memory that most likely corresponds to human echoic memory. So far, however, single-cell recordings in the macaque have not been able to establish a firm link between echoic memory and A1. To provide a functional link between echoic memory and A1, we tested if echoic memory in the macaque is affected by the same stimulus properties that are known to affect neural responses in A1. In particular, we tested if echoic memory is affected by tone intensity which increases response amplitudes and widens receptive fields in A1, or if it is insensitive to tone duration which has only minor effects on the mostly phasic responses in A1.

Methods: To better understand the potential involvement of A1 in echoic memory, two macaque monkeys were trained to release a lever if and when a series of identical pure tone pips was interrupted by a deviant pip of different tonal frequency. Across different trials, the pips were presented at one of 5 intensities (40, 50, 60, 70 or 80dB SPL) and one of six durations (25, 50, 75, 100, 150, or 200 ms). Inter-stimulus intervals were kept between 500 and 1000 ms, a range in which task performance was shown to depend mostly on

information in echoic memory. EEG responses were recorded from arrays of up to 32 chronically implanted cranial EEG electrodes. Echoic memory function was quantified as the slope of the psychometric function relating the frequency-difference between standard and target tone to lever release probability.

Results: Except for the softest tones, echoic memory was invariant across a wide range of stimulus intensities. In contrast, echoic memory improved significantly and substantially for longer tone durations. The improvement was approximately linear across the presented durations. Follow-up experiments suggested that the effect was driven by more accurate encoding of information into echoic memory rather than prolonged maintenance. The effect of tone intensity and duration on neural responses was quantified via auditory evoked EEG potentials. As expected, EEG potentials increased with intensity. In contrast, only one EEG component (the N1 homolog) of one animal was modulated by tone duration. All other EEG components, including the ones believed to originate in A1, were insensitive to tone duration. These findings reveal an interesting double-dissociation between echoic memory function and neural responses in A1.

Conclusions: Our findings provide two novel insights into echoic memory function in the macaque: (1) Echoic memory is largely indifferent to tone intensity despite its well-known effect on neural response amplitude and receptive field width in A1. It remains to be tested how this intensity-invariance is implemented at the neural level. (2) Encoding of information into echoic memory improves with tone duration suggesting a temporal integration window above 200 ms. It remains to be tested if and how temporal integration is related to the relatively small fraction of neurons in A1 that exhibit sustained responses to their preferred stimulus, and how information contained in sustained firing is integrated over time. We will discuss short-term pre-synaptic depression as one candidate mechanism of echoic memory that can account for temporal integration of stimulus information.

Keywords: Auditory Short-Term Memory, Macaque Monkey, EEG Biomarkers, Psychophysics, Temporal Integration

Disclosure: Nothing to disclose.

M180

Grey Matter and Cell Type-Specific Transcriptomic Profiling of Mitochondrial Functional Pathways: Differences Between Schizophrenia and Bipolar Disorder

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Background: Schizophrenia (SZ) and bipolar disorder (BD) share some genetic and environmental risk factors, as well as certain clinical features. These shared factors and features in SZ and BP may be linked via similar functional, morphological and brain alterations, many of which have been associated with evidence of mitochondrial dysfunction.

The primary role of mitochondria is the synthesis of ATP via oxidative phosphorylation (OXPHOS). Mitochondria also participate in other biological processes integral to neuronal functioning, including mediating oxidative stress, Ca²⁺ buffering and apoptosis. Although mitochondrial perturbations have been reported in both SZ and BP, the severity of these alterations and/or the affected mitochondrial functions might differ between diagnoses, especially at the level of individual cell types. For example, a cell type-specific transcriptomic profiling study directly comparing SZ and BP subjects found significant OXPHOS-related alterations in PFC pyramidal neurons (PNs) collected from layer 3

(L3PNs) and layer 5 (L5PNs) in SZ subjects but not in BP subjects. Moreover, because mitochondria are responsible for multiple distinct but interdependent biological processes, analysis of the higher-order gene expression relationships within and between biological pathways may prove informative.

Thus, we explored whether transcriptomic analyses of mitochondrial-related gene expression and co-expression relationships would provide insight into the nature of mitochondrial alterations in these disorders. We analyzed a large gene set that indexes a multitude of mitochondrial functions to interrogate disease-related alterations within total grey matter and L3PNs and L5PNs from the PFC of subjects with SZ or BP using a dual strategy: 1) identification of differentially-expressed genes (DEGs) and assessment of their functional pathway enrichment, and 2) application of weighted gene co-expression network analysis (WGCNA) for an unbiased examination of how differences in gene expression affect the presence and preservation of higher-order co-expression relationships.

Methods: PFC grey matter data were analyzed from the RNA sequencing (RNASeq) studies completed as part of the Common-Mind Consortium from the SZ (N = 57), BP (N = 35), and unaffected comparison (CON; N = 82) subjects obtained from the University of Pittsburgh. We also re-analyzed data from two previously published microarray studies of PFC L3PNs and L5PNs. The first study included 36 pairs of CON and SZ subjects, and the second study included a separate cohort of 19 triads of CON, SZ and BP subjects. Genes within the pathway defined by Gene Ontology (GO) as 'mitochondria' (GOMito) were included for analysis. DEG analysis was performed in grey matter using a basic linear regression model with covariate correction and was performed in PNs using a random intercept model with variable covariate selection. Functionally-related pathway enrichment analysis was performed via INGENUIT Pathway Analysis (IPA). The co-expression network for healthy subjects was constructed using WGCNA, and a module preservation algorithm was implemented to compare network structures across diagnoses.

Results: In PFC grey matter, 871 GOMito genes were detected by RNAseq; 41% of these GOMito genes were differentially-expressed in SZ whereas only 8% were differentially-expressed in BP. In SZ, 83% of DEGs were lower and 17% were higher, whereas in BP, 99% of DEGs were lower. DEGs in SZ subjects were enriched for the annotated pathways OXPHOS, mitochondrial dysfunction and sirtuin signaling. In contrast, no pathways were identified as significantly enriched for the DEGs of BP subjects. However, comparison of differential-expression test statistics showed a significant correlation between SZ and BP subjects ($r = 0.5$, $p < 0.00001$). WGCNA identified five co-expression modules in CON subjects which were all preserved in both SZ and BP subjects.

In PNs, 662 GOMito genes were detected by microarray; 28% of GOMito genes were differentially-expressed in L3PNs and 25% were differentially-expressed in L5PNs in SZ subjects. In both cell populations, 97% of DEGs were lower, and were enriched for OXPHOS, mitochondrial dysfunction and sirtuin signaling pathways. In L3PNs and L5PNs from BP subjects, no GOMito genes were differentially-expressed. However, comparison of differential-expression test statistics showed a significant correlation between SZ and BP subjects (L3PN: $r = 0.3$, $p < 0.0001$; L5PN: $r = 0.2$, $p < 0.0001$). Similar to grey matter findings, the co-expression modules identified by WGCNA in PNs from CON subjects were preserved in SZ and BP subjects.

Conclusions: Transcriptomic differential-expression and co-expression network analyses of a large and specific gene set that indexes multiple mitochondrial-related functions indicate that pathways related to energy production are significantly affected in PFC grey matter and L3PNs and L5PNs in SZ subjects. Despite the significantly altered gene expression, the higher-order co-expression networks were preserved in SZ, demonstrating a coordinated reduction in genes related to energy metabolism. In contrast, few

to no GOMito genes showed altered expression in BP subjects, but the significant relationship between SZ and BP subject test statistics suggests similar mitochondrial-related alterations may occur in both disorders but are more severe in SZ.

Keywords: Postmortem Brain Tissue, Mitochondria, Dorsolateral Prefrontal Cortex, Pyramidal Cell, Transcriptome

Disclosure: Nothing to disclose.

M181

Basal Forebrain Volumes Predict Circuit Specific Functional Sensitivity to Muscarinic M1 Receptor Modulation on Cognitive Function in Healthy Controls and Patients with Psychotic Disorders

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Background: The cholinergic neurons of the basal forebrain (BF) provide the major source of cholinergic innervation to the neocortex and hippocampus and play a critical role modulating cognitive processes such as attention, learning and memory, in part through activation of post-synaptic M1 receptors. Post-mortem and in vivo imaging studies have shown decreases in M1/M4 muscarinic receptors in patients with schizophrenia. These decreases may in part be associated with the cognitive deficits observed in patients with schizophrenia and supported by the preliminary pro-cognitive effects of the M1/M4 agonist Xanomeline. However, the relationship between cholinergic neuronal integrity and the sensitivity to M1 receptor modulation is unknown. The BF volume may be an important biomarker of cholinergic neuronal integrity and could potentially modify functional response to cholinergic drugs including M1 and/or M4 agonists. The objective of this study was to examine the profile of cognitive impairment associated with M1 receptor antagonism by biperiden in healthy volunteers and medication free patients diagnose with a psychotic disorder and determine whether BF volumes predict cognitive sensitivity to biperiden. A secondary objective was to determine whether BF cholinergic cell groups such as the CH4 (i.e. nucleus basalis) volume predicts biperiden effect on cortically mediated cognitive domains, and whether CH1-3 (septal/diagonal band of Broca) volumes predict biperiden effects on hippocampal mediated episodic memory.

Methods: A total of 30 control subjects and 29 medication free patients diagnosed with a psychotic disorder were included in the study. BFN volumes were quantified from T1 weighted 3T MRI scans. The stereotactic basal forebrain atlas was used to derive masks to quantify the nucleus basalis (CH4) and the septal nucleus (CH1), vertical (CH2), and horizontal limb (CH3). Cognition was assessed twice for all subjects using the Cambridge neuropsychological test automated battery. A randomized, placebo controlled, counter-balanced design was used in which all participants received placebo or 4 mg of M1 antagonist biperiden with a minimal washout period of 7 days. This clinical trial was registered in the Dutch clinical trial registry under ID: NTR5094 (<http://www.trialregister.nl>).

Results: Biperiden significantly impaired planning in controls ($t = -2.431$, $p = 0.041$, $d = 0.47$) and verbal learning and memory ($t = 3.17$, $p = 0.004$, $d = 0.75$) and visual spatial associative learning and memory ($t = 2.20$, $p = 0.040$, $d = 0.40$) in the cognitively impaired psychosis group. Regression analysis showed that CH4 BF volumes significantly predicted biperiden induced impairments in planning, with smaller volumes being associated with greater impairment ($F = 10.38$, $p = 0.033$ $R^2 = 0.27$). Regression analysis also

showed CH1-3 BF volumes significantly predicted biperiden induced impairments in verbal learning and memory ($F = 5.91$, $p = 0.023$, $R^2 = 0.18$), and visual spatial associative learning and memory in the psychosis patients ($F = 4.68$, $p = 0.040$, $R^2 = 0.14$). Smaller CH1-3 BF volume predicted greater impairment in immediate and delayed verbal episodic memory, and smaller CH1-3 BF volumes predicted increased errors in cued recall under biperiden in the paired associative learning task.

Conclusions: Planning and episodic memory (both verbal and visual) were more sensitive to M1 receptor modulation by biperiden than other cognitive domains. The cholinergic CH4 and CH1,2,3 nuclei volumes were associated with circuitry specific cognitive responsivity to M1 receptor antagonism. These findings suggest that M1 receptor modulation of executive functioning and episodic memory (both verbal and visual) may depend on the integrity of basal forebrain cholinergic neurons. BF nuclei volume may be a potential biomarker predictive of superior cognitive efficacy of drugs targeting the cholinergic system, including cholinesterase inhibitors, M1 and M4 receptor agonists and positive allosteric modulators.

Keywords: Cholinergic, Muscarinic, Imaging, Cognition, Basal Forebrain

Disclosure: Nothing to disclose.

M182

Distinct Laminar and Regional Patterns of Markers of GABA Neuron Subtypes in Monkey Prefrontal and Visual Cortices

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Background: Evidence of GABA neuron dysfunction is a common finding in postmortem studies of certain psychiatric illnesses. Although they utilize similar gene products for neurotransmission, subtypes of GABA neurons are distinguished based on the expression of different molecular markers. Transcript levels of many of these gene products are known to differ between prefrontal (PFC) and primary visual (V1) cortices, but whether the laminar patterns of expression are conserved or different between these regions is not known. Moreover, it is not known whether these expression patterns are due to differences in the density of GABA neurons expressing these markers or in the level of expression per neuron. In this study, we examined the expression of multiple GABA-related transcripts in cortical layers 2 and 4, which are enriched for different classes of GABA neurons, of the macaque monkey PFC and V1.

Methods: Layers 2 and 4 were captured by laser microdissection in sections taken from fresh-frozen blocks of PFC and V1 from 15 rhesus macaques (11 male/4 female, 41–101 months of age). All animals were experimentally naïve except for serving as vehicle-exposed animals in separate studies investigating the impact of delta9-tetrahydrocannabinol on cognitive task performance. PCR was used to quantify levels of transcripts that are 1) involved in GABA neurotransmission: GABA synthesizing enzymes (GAD67 and GAD65), vesicular GABA transporter (vGAT), and GABA reuptake transporter (GAT1); 2) present in different GABA neuron subtypes: calretinin (CR), vasoactive intestinal peptide (VIP), calbindin (CB), somatostatin (SST), cholecystokinin (CCK), cannabinoid 1 receptor (CB1R), and parvalbumin (PV); and 3) encode key GABAA receptor subunits: GABRA1 and GABRA2. In a subset of these animals ($n = 6$, 3 male/3 female, ~60 months of age), RNAscope was used to label GAD67, PV, and SST mRNAs in the same tissue sections. The density of GAD67, PV, and SST

neurons and the levels of each transcript per neuron were quantified in layers 2 and 4 of PFC and V1. All studies were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Results: In the PCR study, three regional/laminar patterns of gene expression emerged: First, some transcripts (GAD67, GAD65, vGAT, GAT1) showed no consistent regional or laminar differences. Second, many transcripts were more highly expressed in layer 2 and in PFC (CR, VIP, CB, SST, CCK, CB1R, GABRA2). Third, parvalbumin and GABRA1 were more highly expressed in layer 4 and V1. The within-animal rank order of expression was analyzed for three representative transcripts of these patterns, GAD67, SST, and PV. SST and PV showed highly consistent rank-order patterns for each animal studied, whereas GAD67 exhibited no clear within-animal rank order patterns. To address whether these patterns were due to differences in the relative densities of these GABA neuron subtypes or in the expression of each transcript per neuron, we used triple label RNAscope for GAD67, SST, and PV in a subset of 6 animals. Our study design permits robust quantification of both neuron numbers for SST and PV neurons and the mRNA expression per neuron.

Conclusions: Transcript levels of markers of GABA neurotransmission are highly conserved across layers and regions. In contrast, markers of unique GABA neuron subtypes show regional differences in expression levels and similar laminar patterns within a region. These findings suggest that the circuit organization of GABA neuron subtypes is conserved across regions but that the relative weighting of each type of GABA neuron within a circuit differs by region, differences with potential consequences for circuit function. For example, the high expression of PV in V1 may provide the fast-synaptic inhibition onto pyramidal neurons needed to tune neural ensembles to specific visual stimuli, whereas the high expression of SST in PFC may reflect the need for greater inhibition onto dendrites, secondary to the more complex dendritic arbor of pyramidal neurons in this region. Together, these findings suggest that cortical circuits may be differentially vulnerable to disease effects based on the constitutive GABA neuron subtypes in that region. This differential vulnerability may contribute to the diversity and severity of clinical symptoms observed in psychiatric illnesses.

Keywords: Cortical GABA, Nonhuman Primates, Parvalbumin neurons, Somatostatin

Disclosure: Nothing to disclose.

M183

The Impact of Brain Network Topology on Individual Differences in Cognition and Symptomatology

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Background: Resting state fMRI has allowed in vivo identification of the brain's organization into functionally connected networks. This framework has supplemented historical schema of how cognition, behavior, and symptoms are reflected in brain organization. The spatial organization of these networks demonstrates significant inter-individual variation. We sought to determine if this variation is reflected in cognition and psychiatric symptomatology.

Methods: 105 participants (60 schizophrenia; 45 control) underwent a rsfMRI scan, behavioral, and cognitive assessments. A connectomic multivariate pattern analysis examined participant-

level individual voxel connectivity that corresponds to cognitive / behavioral domains.

Results: Inter-individual differences in network spatial organization demonstrated significant ($p < .001$) effects on multiple measures of cognition and symptom severity. The impact of network topology on these phenotypes was linked to highly circumscribed ($\sim 11\text{mm}^3$) sub-regions of heteromodal association cortices. The topographic organization of networks, within these critical regions, demonstrated large ($r \sim .5$), region-specific effects on behavioral and cognitive measures. In all cases examined, the strongest phenotype-connectivity relationships in the brain were always explained by network topology.

Conclusions: The almost universally accepted practice of group averaging rsfMRI data obfuscates important relationships between network topology and cognitive and behavioral phenotypes. Many previously reported correlations between phenotype and connectivity may be mis-interpretations of individual variation in network topology. Recently proposed individual parcellation solutions still obscure interactions between network topology and anatomy critical to phenotypic variation. We argue that individual network topology is a marker of cytoarchitectonic variation. This variation at critical cortical regions cortices is linked to expression of normative cognition and pathological symptomatology.

Keywords: Psychotic Disorders, Resting State Networks, Negative Symptoms, Social Cognition

Disclosure: Nothing to disclose.

M184

Early-Life Stressful Events and Suicide Attempt in Schizophrenia: Machine Learning Models

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Background: Early-life stressful events are known precursors of suicide attempt in individuals with schizophrenia. Our hypothesis is that both traumatic and non-traumatic stressful events will be important predictors of suicide attempt.

Methods: Lifetime suicide attempt and stressful life events were assessed using the Columbia-Suicide Severity Rating Scale (C-SSRS) and Life Events Inventory (LEI-2) in subjects with schizophrenia. The specific life events were used as predictors of lifetime suicide attempt in logistic regression, random forest, and classification tree machine learning models.

Results: The analysis included 189 individuals with schizophrenia spectrum disorders recruited from a psychiatric teaching hospital in Toronto, including 72 with at least one suicide attempt lifetime (38%).

The three machine learning models showed low sensitivity and high specificity, with the overall the accuracy ranging between 62% and 69%, providing overall a significant prediction. The machine learning models placed the highest importance on sexual molestation and mental illness during early life as predictors of suicide attempt.

Conclusions: Our analyses reiterate the importance of traumatic events in predicting suicide attempt, and make the case for considering "non-traumatic" events when building a better predictive model for suicide behavior. Larger studies with well-defined testing and training datasets are warranted.

Keywords: Suicide Attempt, Schizophrenia, Machine Learning

Disclosure: Nothing to disclose.

M185

Is Body Mass Index and Psychosocial Functioning Status Associated in Long-Term Schizophrenia?**Ramiro Reckziegel, Isadora Bosini Remus, Letícia Sanguinetti Czepielewski, Clarissa Gama****Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, Porto Alegre, Brazil*

Background: It is well established that patients with schizophrenia have a higher mean body mass index (BMI) than the general population, inducing increased cardiovascular risk and mortality. Among other severe mental illness such as bipolar disorder increased BMI is correlated with overall worse clinical outcomes and poor psychosocial functioning status. Nonetheless, there is also some evidence that at least in the acute phase of treatment in schizophrenia, beneficial outcome associates with increasing weight or BMI. The results in long-term schizophrenia are controversial. We tested the hypothesis that high BMI associates with poor functioning status in a sample of long-term outpatients with schizophrenia.

Methods: We analyse the weight, height and psychosocial functioning (Functioning Assessment Short Test, FAST) of two hundred ninety four individuals for a clinical interview, consisting in 193 patients with schizophrenia (SCZ) and 102 individuals with no personal or family history of severe mental illness as a control group (CTR). A linear regression model tested the hypothesis controlling for age, sex and years of education. In SCZ group the model also included chlorpromazine equivalents of medication in use as a covariate.

Results: Among CTR, higher BMI predicted worse FAST total scores (Model: $F(4) = 2.63$, $\text{Adj}R^2 = .32$ $p = .039$; BMI Main effect $t = 2.02$ $\beta = .22$ $p = .046$). The FAST subscores specifically predicted by BMI among CTR were workability ($t = 2.84$ $\beta = .31$ $p = .005$) and leisure time ($t = 2.12$ $\beta = .23$ $p = .037$). In SCZ, although the model was positive ($F(5) = 5.08$, $\text{Adj}R^2 = .096$ $p < .001$), BMI showed no effect in predicting total FAST score (Main effect $t = .62$ $\beta = .043$ $p = .534$). Specific subscores were not predicted by BMI in SCZ, except for a trend in leisure time ($t = 1.86$ $\beta = .13$ $p = .064$).

Conclusions: Although higher BMI predict worse functioning status among CTR, no association was seen in SCZ. We hypothesise that patients with higher BMI are more adherent and responsive to the antipsychotic treatment prescribed. In that case, better control over psychiatric symptoms may compensate for possible impairment in functionality due to increased body weight.

Keywords: Obesity, Functional Capacity, Schizophrenia Subtypes

Disclosure: Nothing to disclose.

M186

Duration of Untreated Psychosis Correlates With Resting State Functional Connectivity and Cortical Morphology in Antipsychotic-Naïve First Episode Psychosis Patients**Adrienne Lahti*, J. Omar Maximo, Nina Kraguljac, Eric Nelson, William Armstrong***University of Alabama at Birmingham, Birmingham, Alabama, United States*

Background: Meta-analyses have consistently identified an association between the duration of untreated psychosis (DUP),

the duration between the onset of positive symptoms and treatment, and clinical outcomes. Active psychosis might adversely affect the brain, and several mechanisms have been proposed, such as NMDA receptor hypofunction or increased dopaminergic activity. The DUP also implicates a process by which antipsychotic drug (APD) treatment attenuates the pathophysiological process underlying the DUP. The goal of this longitudinal multimodal imaging study was to examine the relationship between the DUP and the resting state functional connectivity and cortical morphology of three major neuronal networks [default mode (DMN), salience (SN), and central executive (CEN)] in antipsychotic-naïve first episode psychosis patients (AN-FEP), as well as to evaluate their contribution to eventual treatment response following APD treatment.

Methods: Scans were obtained prior to treatment and after 16 weeks of APD treatment in 55 AN-FEP. Resting state (TR = 1550ms; TE = 37.80ms; flip angle = 71°, FOV = 104mm²; voxel size = 2mm³; 225 volumes, and 72 axial slices) and T1-weighted structural (MPRAGE: TR = 2400ms; TE = 2.22ms; inversion time = 1000ms; flip angle = 8°; voxel size = 0.8mm³) scans were acquired. The CONN toolbox and Freesurfer were used to process and analyze resting state functional connectivity data and structural images, respectively. Resting state signal from a priori brain regions of interest from posterior cingulate cortex (DMN), right insula (SN), and bilateral posterior parietal cortex (CEN) were extracted and correlated with the rest of the brain. Second-level analyses were performed for each correlation map using separate general linear models with DUP with age, sex, and frame-wise displacement (FD) as covariates. All analyses were corrected using voxel ($p < 0.01$, uncorrected) and cluster level correction ($p < 0.05$, FDR corrected). For morphometric analyses (cortical thickness, and surface), labels of the DMN, SN, and CEN defined from the CorticalParcellation_Yeo2011 were projected onto each subject. Measures were extracted from right and left hemispheres labels and entered into partial correlations with DUP (log transformed to account for non-normal distribution of data) while controlling for age and estimated Total Intracranial Volume (eTIV). Treatment response (TR) was defined as the percent change in the positive subscale of the Brief Psychiatric Rating Scale (BPRS) from baseline to week 16. Finally, a mediation analysis was performed to examine whether brain morphology and connectivity mediated the relationship between DUP and TR.

Results: Longer DUP was associated with significant lower connectivity in all three networks, although there was a single cluster of increased connectivity in the CEN network. Longer DUP was associated with a significant reduced surface area in the SN and CEN, and a significant increase in cortical thickness in the SN and DMN. Worse treatment response was associated with longer DUP ($p = 0.019$) and reduced DMN functional connectivity ($p = 0.05$). A mediation analysis showed that the direct path from DUP to treatment response was no longer significant ($p = 0.072$) when the DMN functional connectivity was included in the model. This analysis showed a significant mediation effect using the Sobel test ($z = -1.94$, $p = 0.03$).

Conclusions: Longer DUP was associated with altered functional connectivity and cortical morphology in all 3 networks, suggesting that one or several pathophysiological processes underlie the DUP. Importantly, our data empirically support that DMN connectivity mediates the relationship between DUP and treatment response, implicating brain network connectivity as a neurobiological underpinning of the relationship between longer DUP and poorer clinical outcomes. These findings highlight the importance of reducing DUP and initiation of treatment as soon as possible to mitigate the detrimental effects of psychosis on long-term clinical outcomes.

Keywords: Antipsychotic-Naïve First-Episode Schizophrenia, Duration of Untreated Psychosis, Resting State Functional Connectivity, Cortical Morphology, Antipsychotic Treatment

Disclosure: Nothing to disclose.

M187

Cannabidiol Effects on Delta-9-Tetrahydrocannabinoid-Induced Psychotic Symptoms are Related to Serum Levels of Both Compounds in Healthy Volunteers

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Background: There is increasing interest in the effects of cannabidiol (CBD) as an antipsychotic. In this context, it has been suggested that CBD counter-balances the effects of delta-9-tetrahydrocannabinol (THC), the major psychotomimetic compound of Cannabis sativa. We, therefore, studied the interaction of CBD and THC in healthy volunteers in an experimental setting with well-defined doses of both compounds in a four-arm clinical trial.

Methods: We used an experimental approach using a randomized, double-blind, placebo (PLA)-controlled phase I clinical trial design administering oral THC (20mg) and/or CBD (800mg) to 60 healthy male volunteers. The healthy volunteers were randomly allocated to four treatment groups (PLA-PLA, THC-PLA, CBD-PLA, CBD-THC). Psychopathological changes were assessed using the Positive and Negative Syndrome Scale (PANSS), and blood and CSF samples were taken to detect serum levels of both investigated compounds. A linear mixed model was developed and used to predict the psychopathological effects of both compounds based on their serum levels.

Results: In line with previous studies, we found significantly elevated PANSS scores in THC-PLA compared to PLA-PLA (PANSS total score $+14.9 \pm 2.21$; $p = 0.000$) and no effect of CBD-PLA on psychopathology at all. However, contrary to our hypothesis and previously reported data (e.g. Leweke et al., 2000), CBD-THC showed significantly elevated PANSS scores vs PLA-PLA as well (PANSS total score $+15.5 \pm 5.22$; $p = 0.012$). Interestingly, THC serum levels were numerically higher in CBD-THC (19.08 ± 5.386 pmol/ml) than in the THC-PLA (13.85 ± 4.095 pmol/ml). The linear mixed model used to predict the effects of both compounds on psychotic symptoms yielded a partial extinction of the THC effect by CBD when CBD serum levels were altered by the factor 2 and 3 and a complete elimination with four times higher serum levels of CBD.

Conclusions: Our data confirm the psychotomimetic effects of THC in humans and indicate that the influence of CBD hereon is substantially dependent on the used dose-relation of both compounds. In particular, CBD may pronounce the effects of THC under certain circumstances likely related to a pharmacokinetic interaction of both compounds after higher dosage oral administration that calls for further investigation.

Keywords: Psychosis, Delta-9-Tetrahydrocannabinol, Cannabidiol, Serum Levels, Pharmacokinetic and Pharmacodynamic

Disclosure: Curantis UG (Ltd.), Stock / Equity, Acerus Pharmaceuticals, Grant

M188

Predicting Psychosis Risk Using a Specific Measure of Cognitive Control: A 12-Month Longitudinal Study

Abstract not included.

M189

A 7T MRS Study of Early and Late Illness in Schizophrenia: Focus on Neurotransmitters and Bioenergetics

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Background: Proton magnetic resonance spectroscopy (MRS) studies in schizophrenia have shown altered GABAergic, glutamatergic, and more recently, bioenergetics. However, few studies have examined multiple brain regions and explored the role of illness duration in schizophrenia. In this study, we investigated if GABA, glutamate (Glu), glutamine (Gln), lactate, and Gln/Glu from five brain regions were different between adults with schizophrenia with short (less than 5 years ill) and long (greater than 5 years ill) illness duration, healthy controls, and first-degree relatives using 7T MRS.

Methods: Forty adults with schizophrenia, 38 healthy controls, and 11 first degree relatives participated in this study. Spectroscopic data were acquired from the anterior cingulate (AC), left centrum semiovale (CSO), left dorsolateral prefrontal cortex (DLPFC), left hippocampus (HP), and bilateral thalamus using a STEAM sequence with the following parameters: TR/TM/TE= 3000/X/14, 2048 complex points, 5000 Hz spectral width, NEX=64 for all regions. Spectra were fit using LCModel. Psychiatric symptom severity was assessed with the BPRS and the BNSS. Due to non-normality, non-parametric tests (Mann-Whitney or chi-square) were utilized to assess group differences in metabolite levels. The relationship between metabolites and psychiatric symptom severity were analyzed with Pearson product moment correlations.

Results: Glutamate was lower ($p < 0.05$) in the AC in the schizophrenia group compared to controls and relatives and related to negative symptom severity ($r = -0.324$, $p < 0.05$). Gln was higher in adults with schizophrenia in all brain regions and statistically significantly in the CSO and DLPFC (p 's < 0.05). Adults with a longer illness duration had lower glutamate in the AC, CSO, and DLPFC (p 's < 0.05), lower GABA in the HP ($p < 0.05$), and higher lactate in the AC and CSO (p 's < 0.05) compared to adults with shorter illness duration. Higher AC lactate was related to greater negative symptom severity ($r = 0.37$, $p = 0.029$).

Conclusions: This is the first 7T study to show regional metabolite differences between patients with short and long illness duration, first-degree relatives, and controls. Our results indicate that alterations in glutamate, GABA, and lactate may worsen with illness duration or age. Negative symptom severity was strongly related to lower AC glutamate and higher AC lactate, providing evidence of potential targets for intervention. Although we cannot rule out antipsychotic medication effects, controlling for antipsychotic load did not influence the results.

Keywords: 1H-MRS, Glutamate, GABA, Lactate

Disclosure: Otsuka America Pharmaceutical, Inc for PsychU, Consultant

M190

Heterogeneity and Efficacy of Antipsychotic Treatment for Schizophrenia With or Without Treatment Resistance: A Meta-Analysis

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Background: There is a question as to whether clozapine's superior efficacy is more specific to treatment-resistant schizophrenia (TRS), or whether benefits of clozapine apply to a similar degree across schizophrenia in general. The authors address this by examining both magnitude and variability of treatment response in patients treated with clozapine and other antipsychotics for both studies of strictly-defined TRS (TRS studies) and studies of non-resistant schizophrenia (non-TRS studies).

Methods: Double-blind randomised controlled trials comparing clozapine with other antipsychotics in patients with schizophrenia were identified using five databases. Standard deviations and means of change in total, positive, and negative symptoms were extracted. The variability ratio (VR) and coefficient of variation ratio (CVR) were used to quantify relative variability in symptom change between patients receiving clozapine and other antipsychotics. Hedges' *g* was used to quantify mean differences.

Results: Thirty-nine studies consisting of 10 TRS studies (*n* = 822) and 29 non-TRS studies (*n* = 2,566) were meta-analysed. Relative variability in change of total symptoms did not differ significantly between clozapine and other antipsychotics in TRS studies (VR=1.84; 95%CI, 0.85-4.02). These findings were similar with CVR, and for positive and negative symptoms. Clozapine was superior to other antipsychotics in improving total symptoms in both TRS (*g*=0.34; 95%CI, 0.13-0.56) and non-TRS (*g*=0.20; 95%CI, 0.08-0.32) studies. Furthermore, clozapine was superior in improving positive symptoms in both TRS and non-TRS studies, but not for negative symptoms. Pooled effect sizes showed no significant difference between TRS and non-TRS studies.

Conclusions: Clozapine is more effective than other antipsychotics for schizophrenia irrespective of treatment-resistance, and there are no differences in variability of response. Sensitivity analysis indicates that this effect is mainly driven by comparisons with chlorpromazine and haloperidol. These findings argue for further research into the use of clozapine in patients without established treatment-resistance, especially in early stages of illness. Trial Registration: PROSPERO CRD42018086507.

Keywords: Schizophrenia, Antipsychotics, Treatment Resistant Schizophrenia, Clozapine, Meta-Analysis, Individual Variability

Disclosure: Japan Society for the Promotion of Science, Grant, Sumitomo Dainippon Pharma, Honoraria, Bracket, Consultant, MedAvante-ProPhase, Consultant

M191

Brain Microstructure Relates to Functional Brain Activity in Clinical and Healthy Populations

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Background: Cognitive impairment is a core feature of schizophrenia and predicts functional outcome including particularly deficits in working memory (WM) but also in other domains such as emotional processing. Various studies link WM deficits to alterations in dorsolateral prefrontal cortex (DLPFC) brain activation and recent evidence suggests a relationship between WM and DLPFC microstructure in schizophrenia. Similar links between emotional cognition and amygdala activation have been reported.

Despite this converging evidence, the relationship between brain gray matter microstructure and functional activation during cognitive tasks is widely unstudied. In order to better understand the biological mechanisms underlying differences in brain activity in clinical populations, we engaged in a novel multi-modal analysis in schizophrenia and healthy controls using state-of-the-art MRI technology.

Methods: In the present study, we used functional (fMRI) and diffusion-weighted MRI (dMRI) data from two separate studies including both sexes. Sample 1 included baseline MRI data (acquired prior to treatment) from 42 patients with schizophrenia, enrolled in a rTMS treatment trial. Exploratory sample 2 comprised healthy volunteers (HV; *n* = 761) from the human connectome project (HCP), publicly available at <http://www.humanconnectomeproject.org>, to confirm results in a healthy population sample. BOLD (Blood-Oxygen-Level Dependent) imaging of an N-back WM task (sample 1 and 2) or emotional faces recognition task (sample 2 only) was used to estimate task-based brain activation in DLPFC or amygdala, respectively. General linear models were run using SPM and the contrast between high vs low WM load (N-back) and emotional faces vs object recognition (emotional faces task) calculated. Grey matter microstructure was examined using multi-shell dMRI and the neuritic orientation dispersion and density imaging (NODDI) model, which provides indices of neuritic orientation dispersion (ODI) and neuritic density (NDI). Values for NDI, ODI and BOLD contrast for bilateral DLPFC (two parcels with the highest activation were included and called Bp9-46v and B46) and amygdala were extracted using the Glasser and the subcortical connectome workbench parcellation, respectively. Associations were explored using Pearson's linear correlation analysis and corrected for the effect of age.

Results: Preliminary analysis revealed significant associations between BOLD activation and microstructure in the right DLPFC. Both schizophrenia patients (parcel B46 only: *r* = -0.311; *p* = 0.050; parcel Bp9-46v: *p* > 0.05) and HV (parcel Bp9-46v: *r* = -0.105; *p* = 0.0037; parcel B46: *r* = -0.074; *p* = 0.042) with stronger BOLD signal had lower neuritic orientation dispersion. There were no significant associations between BOLD and NDI in right DLPFC and none for the DLPFC parcels on the left hemisphere (*p* > 0.05).

Interestingly, similar associations were apparent in the right and left amygdala in HV. Those with stronger BOLD signal had lower neuritic orientation dispersion (right amygdala: *r* = -0.130; *p* = 0.00035; left amygdala: *r* = -0.072; *p* = 0.048) and neuritic density (right amygdala: *r* = -0.126; *p* = 0.00053; left amygdala: *r* = -0.072; *p* = 0.047).

Conclusions: These findings provide the first direct evidence for an association between brain microstructure and BOLD activation to a WM task in patients with schizophrenia and HV. This suggests that changes in the underlying microstructure may account for some of the observed deficits in functional activity in clinical samples. Future studies should examine brain microstructure as a possible biomarker of response to cognition-enhancing treatments.

Keywords: Brain Microstructure, NODDI, BOLD Imaging, Multi-modal Imaging, Schizophrenia

Disclosure: Nothing to disclose.

M192

The Relationship Between Autism Spectrum Disorder and Prodromal Psychosis in the Adolescent Brain Cognitive Development Cohort

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Background: Multiple studies report an increased rate of schizophrenia diagnosis among individuals with autism spectrum disorder (ASD). However, little is known about how to identify youth within the heterogeneous category of ASD who are at risk of developing psychosis. It is also unclear to what extent the neurocognitive correlates of psychosis, such as impairments in executive function, processing speed, and working memory, differ in the ASD population relative to the non-ASD population. We explored these questions, with a focus on the relationship between existing ASD diagnosis and emerging prodromal psychosis in the Adolescent Brain Cognitive Development (ABCD) sample. We hypothesized that: ASD would be a predictor of prodromal psychotic symptoms in our sample (1), and that neuropsychological profiles would differ among ASD youth without prodromal symptoms, ASD youth with prodromal symptoms, and youth with prodromal symptoms but not ASD (2).

Methods: We examined a sample ($n = 11,875$) of youth (47.84% female; age $M = 9.91$ years, $SD = 0.62$ years) from the ABCD study (data release 2.0.1). We identified youth whose parents reported an ASD diagnosis during study screening, and examined prodromal symptoms using Prodromal Questionnaire – Brief Child Version (PQ-BCV) scores. We defined “potentially significant” symptoms using a PQ-BCV total score cutoff of 6, based on previous literature. We also examined neuropsychological profiles using three NIH Toolbox measures selected in advance to capture core neuropsychological deficits associated with ASD and/or psychosis: Dimensional Change Card Sort (a measure of executive function), Pattern Comparison (a measure of processing speed), and List Sorting (a measure of working memory). To assess ASD diagnosis as a predictor of prodromal symptoms, we estimated a hierarchical linear regression model with family unit and study site as nested random effects and age, sex, ethnicity, and household per capita income as covariates. We excluded 1,262 participants from this model due to missing data. We used one-way analysis of variance to compare mean age-corrected scores along these measures across ASD youth without prodromal symptoms, ASD youth with prodromal symptoms, and prodromal youth without ASD. We made post-hoc pairwise comparisons using Tukey’s method.

Results: In our regression model, ASD was a meaningful predictor of prodromal symptoms, with ASD youth having, on average, PQ-BCV scores 1.38 points higher than non-ASD youth ($\beta = 1.38$, 95% confidence interval = 0.89 to 1.88, $t = 5.46$, $p = 4.94 \times 10^{-8}$). In comparison, the effect sizes of covariates were much smaller (male sex: $\beta = 0.29$, 95% confidence interval = 0.16 to 0.43, $t = 4.41$, $p = 1.03 \times 10^{-5}$; age in months: $\beta = -0.026$, 95% confidence interval = -0.03 to -0.02 , $t = -5.77$, $p = 8.11 \times 10^{-9}$). Regarding the identification of subgroups, we found that 201 of the sample’s 11,875 participants (1.69%) had a parent-reported ASD diagnosis, consistent with national estimates of the ASD rate in the general population. Of these, 60 (29.85%) also had a PQ-BCV summary score of at least 6. 1,938 participants (16.32%) had a PQ-BCV score of at least 6 but no ASD diagnosis. In comparing NIH Toolbox measure scores across groups, we did not find meaningful differences in card sort or processing speed scores. We did, however, find a difference in working memory that approached statistical significance, $F(2,2092) = 2.93$, $p = 0.054$. Post-hoc comparisons showed that working memory scores (for which the normative mean is 100) differed between youth with prodromal symptoms who had and did not have ASD (prodromal with ASD: $M = 92.91$, $SD = 15.22$; prodromal without ASD: $M = 97.53$, $SD = 14.74$). Neither group differed significantly in working memory from youth with ASD but without prodromal symptoms ($M = 98.11$, $SD = 16.75$).

Conclusions: Our finding that ASD diagnosis is associated with elevated levels of prodromal psychosis in youth is consistent with literature suggesting that rates of schizophrenia are greater in individuals with ASD than in the general population. Our finding of decreased working memory in prodromal youth with ASD compared to those without are not conclusive, as we were unable to identify significant differences in working memory between either group and ASD without prodromal symptoms. However, they suggest a possible future direction for research exploring the connection between ASD and schizophrenia. As impairment in working memory is not considered typical of ASD, this dimension may warrant further exploration as a potential early marker of vulnerability to prodromal symptomatology. Longitudinal follow-up of the ABCD sample may also allow us to further understand the relationship between ASD or ASD traits and subsequent diagnoses of chronic psychosis.

Keywords: Autism Spectrum Disorder, Psychosis, Schizophrenia, Neurocognition, Adolescent Brain Cognitive Development Study

Disclosure: Nothing to disclose.

M193

Modulation of Dopaminergic and Glutamatergic Function by GPR52 Agonist Supports Therapeutic Utility as Novel Treatment for Psychosis

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Background: GPR52 is a Gs coupled orphan receptor which is highly expressed in the striatum, exclusively on medium spiny neurons expressing dopamine D2 receptors, and on cortical pyramidal neurons expressing dopamine D1 receptors. Based on its localization and functional coupling, GPR52 may play a role in the modulation of fronto-striatal and limbic dopamine in neuropsychiatric disorders. GPR52 agonists are thought to be particularly relevant to the treatment of psychotic disorders, including schizophrenia, where they are hypothesized to improve cognition and negative symptoms indirectly by potentiating D1 signalling, but alleviate positive symptoms through inhibition of D2-mediated signalling in the striatum.

Biodistribution studies of GPR52 mRNA in mice suggest that the receptor may also be co-localised with cholinergic and glutamatergic systems. GPR52’s potential role in the modulation of cholinergic and glutamatergic signalling has not been fully explored to date but is also highly relevant to the treatment of neuropsychiatric disorders. Currently, no effective therapeutic strategies exist to treat cognitive and negative symptoms in neuropsychiatric disorders, and existing atypical antipsychotics are associated with adverse effects. Given the important implications of early published findings, the current study sought firstly to replicate initial studies performed with the GPR52 agonist, 4-(3-(3-fluoro-5-(trifluoromethyl)-benzyl)-5-methyl-1H-1,2,4-triazol-1-yl)-2-methylbenzamide (FTBMT), where positive effects were noted in mouse psychostimulant-induced hyperlocomotion assays. The potential for tachyphylaxis was also investigated using a subchronic dosing regimen. In addition, emerging data generated using GPR52 tool compounds will be presented to supporting an improved understanding of the mechanism of action and PK/PD relationships.

Methods: FTBMT was tested in d-amphetamine- and MK-801-induced hyperlocomotor studies. Male Sprague-Dawley rats received FTBMT at 1, 3 and 10 mg/kg, PO, 1 hour prior to injection of d-amphetamine (0.5 mg/kg, SC) or MK-80 (0.1 mg/kg,

SC). Locomotor activity was recorded for 2 hours following injection of the psychostimulant. Tachyphylaxis was investigated in a separate experiment by comparing d-amphetamine-stimulated locomotor activity responses following acute versus 10 days' treatment with FTBMT (10 mg/kg, PO). Vehicle control groups were included.

Results: FTBMT dose-dependently decreased the hyperlocomotor response to both d-amphetamine ($p < 0.05$ vs vehicle control at 10 mg/kg) and MK-801 ($p < 0.05$ at 3 and 10 mg/kg). No tachyphylaxis was observed following 10 days of treatment with FTBMT. New data generated with GPR52 tool compounds will be reported.

Conclusions: The results confirm previous published effects of FTBMT on psychostimulant-induced hyperlocomotor responses, further supporting GPR52 as a target in the modulation of dopaminergic and glutamatergic pathways. Importantly, no tachyphylaxis was observed following subchronic treatment with the GPR52 agonist. These data provide further support for GPR52 as a promising target for the development of a novel class of antipsychotic with potential to also improve negative and cognitive symptoms.

Keywords: GPR52, GPCR, Cognition, Psychosis, D1/D2

Disclosure: Sosei Heptares, Employee, AstraZeneca, Employee, (Spouse) Idorsia Pharmaceuticals, Employee (Other Immediate Family Member)

M194

Thalamofrontal Circuits in Timing Behavior

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Background: Effectively timing decisions and actions is critical for daily functioning and is heavily impaired in a variety of neuropsychiatric and neurodegenerative diseases. Timing recruits a diverse set of brain regions and how these areas interact to generate timed behavior is not well understood. The rodent medial frontal cortex and mediodorsal thalamus play a prominent role in cognitive functioning and are thought to mediate these processes via reciprocal interactions. Therefore, we asked whether these areas are necessary for timing individually and, if so, whether they interact to generate well timed behaviors.

Methods: Specifically, we trained rats on an operant task in which they were presented with one of two cues (tone or light). Each cue instructed the rat to make a response after a distinct time interval elapsed, in order to earn reward (e.g., tone-8s / light-16s). In Experiment 1, we implanted cannulas bilaterally and infused muscimol into both the mediodorsal thalamus and medial frontal cortex. In Experiment 2, we attempt to demonstrate that these deficits emerge due to impaired communication between the thalamus and frontal cortex specifically. To assess this, we trained rats on the same task, and infused the inhibitory opsin ArchT3.0 into the mediodorsal thalamus.

Results: Results from Experiment 1 indicate that reversibly inactivating either area with Muscimol heavily impaired timing. Importantly, inactivating either area produced a highly similar deficit relative to saline infusions and although rats maintained equivalent response rates, these responses lacked temporal organization around the cue's target interval. Results from Experiment 2 currently suggest that inhibiting thalamic projections from the mediodorsal thalamus to the frontal cortex produces a similar timing deficit to that seen when either area is inactivated individually.

Conclusions: Collectively, these findings establish the necessity of the mediodorsal thalamus and medial frontal cortex in timing and suggest that these regions may interact to mediate timing behavior.

Keywords: Mediodorsal Thalamus, Timing, Medial Frontal Cortex

Disclosure: Nothing to disclose.

M195

Enhanced NGR/p75/KAL9 Signaling Influences Dendritic Morphogenesis in a Schizophrenia-Relevant Manner

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Background: Kalirin (KAL) is a Rho GEF that is highly involved in regulation of cytoskeletal morphology within dendrites. Nogo receptor (NGR) signaling acts to restrict dendritic growth when it complexes with p75, a process which involves activation of RhoA. The KAL9 isoform is a dual GEF, capable of activating both Rac1 or RhoA. We evaluated a naturally occurring missense mutation in KALRN (KALRN-PT), located near the RhoA GEF in KAL9, and found that it acts as a gain of function mutation for RhoA activation. We hypothesized that KAL9 sits downstream of NGR/p75, and that the enhanced RhoA activity in KALRN-PT leads to increased NGR/p75 signaling, thus impairing dendritic morphogenesis in pyramidal cells (PCs) across development.

Methods: RhoA activation assays were performed using transient expression of a RhoA sensor in in vitro dissociated cortical cultures overexpressing either KALRN-WT or KALRN-PT. Following transfection with either NGR1, KAL9 siRNA, or the combination, dendritic morphology was quantified in PCs in hippocampal slice culture. CRISPR/Cas9 gene editing was used to insert the human KALRN-PT mutation at the endogenous locus of the C57/Bl6J strain. Golgi staining was performed on cortical sections from 4 and 12-week old mice encompassing primary auditory cortex (A1), and full dendritic reconstructions of A1 Layer 3 PCs were performed in NeuroLucida software. Spine density analyses and cortical volume measurements were performed on Golgi stained material using StereoInvestigator software.

Results: KALRN-PT confers enhanced RhoA activation compared to KALRN-WT. siRNA knockdown of KAL9 rescues the dendritic deficits seen with NGR1 overexpression. L3 PCs from A1 in homozygous KALRN-PT mice demonstrate reduced dendritic length and complexity at 12-weeks, but not at 4-weeks. There is no observed change in spine density along secondary apical dendrites between 12-week old KALRN-WT and KALRN-PT mice. Similarly, there is no significant change in cortical volume between genotypes at 12-weeks, although KALRN-PT mice showed a trend towards a 5-6% volume reduction ($p = 0.24$).

Conclusions: The increased RhoA activity arising from the PT mutation results in increased NGR/p75/KAL9 signaling and subsequently leads to reduced dendritic length and complexity in L3 PCs in A1 in early adulthood. Interestingly, this change is not present during the pre-adolescent period and presumably emerges during adolescence, consistent with the timing of onset of clinical symptoms of schizophrenia in humans. This change in dendritic structure is not accompanied by any statistically significant change in spine density or cortical volume.

Keywords: Dendrite, Schizophrenia, Auditory Cortex, Kalirin

Disclosure: Nothing to disclose.

M196

Effects of Antipsychotic Treatment on Insulin Sensitivity are Independent of Adiposity Change

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Background: The primary aim of this study was to evaluate the acute effects of olanzapine or ziprasidone administration on whole-body as well as tissue-specific insulin sensitivity (SI) in antipsychotic-naïve healthy young men, independent of drug-induced changes in adiposity. We hypothesized that olanzapine, but not ziprasidone, would result in acute decreases in SI compared to placebo.

Methods: Participants:

Sedentary healthy males ages 18-45 were randomized in a cross-over design to intramuscular (IM) olanzapine or ziprasidone, each given on a different day than IM saline/placebo, counterbalancing order of administration. Inclusion criteria were sedentary lifestyle as well as clinical and/or laboratory indicators of cardiometabolic risk as follows: body mass index (BMI) ≥ 25 and < 35 ; fasting plasma insulin $\geq$ approximately 15 μ U/ml; triglyceride $\geq$ approximately 130 mg/dl. Exclusion criteria were any DSM-IV Axis I diagnosis (history of substance use disorders were not excluded if they were in full remission), presence of any medical disorder that would confound the assessment of relevant biologic measures or diagnosis (eg diabetes or frank hyperlipidemia), or a condition that would preclude blood sampling (eg clinically significant anemia or coagulopathy).

Study Assessments:

The two separate days of IM treatment (drug, placebo) were each conducted during assessment of SI using hyperinsulinemic-euglycemic clamps with stable isotopomer tracing. Body composition was assessed with Dual Energy X-ray Absorptiometry (DEXA) at both timepoints. SI at adipose tissue was measured by evaluating the rate of appearance (Ra) of labeled glycerol; SI at liver was measured by evaluating rate of appearance (Ra) of labeled glucose; SI at muscle was measured by evaluating the rate of disappearance (Rd) of labeled glucose.

Analytic Approach:

Main effects of time, time x order of exposure (drug vs. placebo first) and drug condition (ziprasidone vs. olanzapine) on SI were assessed with Analysis of Variance (ANOVA). Dependent variables included whole body disposal, measured as D20 infusion rate (mg/kg/min), percent change in glycerol rate of appearance (glycerol Ra), percent change in glucose rate of appearance (glucose Ra), and percent change in glucose rate of disappearance (glucose Rd). Factors used in the analyses are defined as following: drug is a 2 level fixed factor representing the two active drugs studied, olanzapine and ziprasidone; order is a 2 level fixed factor representing the order in which the placebo and active drug were given. In addition, stable body fat across timepoints was assessed using ANOVA with DEXA total fat at the dependent, with time and drug as independents.

Results: Thirty-seven healthy males (mean age: 33.5 ± 8.5 years) participated in the study. In the olanzapine group, 14 participants received active drug first followed by placebo; 5 participants received placebo first followed by active drug. In the ziprasidone group, 12 participants received active drug first followed by placebo; 6 participants received placebo followed by active drug. The mean length of time between clamps was 56.1 days (SD: 38.3) with minimum of 16 days and maximum of 159 days between procedures. Body fat did not change between

sessions, with no main effect of time ($F[1,29] = 0.03$, $p = 0.87$) or interaction between time and drug on DEXA total fat ($F[1,29] = 0.03$, $p = 0.87$). A significant time x order effect was observed for whole body SI (D20 infusion rate in mg/kg/min, $F[1,33] = 15.216$, $p < 0.001$) and for glucose Rd ($F[1,33] = 12.197$, $p = 0.001$). No statistically significant order effect of antipsychotic exposure was observed for glucose Ra or glycerol Ra. No significant 3 way interactions of time x order x drug were detected on any measure of SI.

Conclusions: The magnitude of the observed treatment effect on whole body insulin sensitivity in the present study; approximately 1 mg/kg/min, can be compared to well-established effects of adiposity on clamp-measured insulin sensitivity. In a prior study by our group using a similar hyperinsulinemic-euglycemic clamp protocol, an increase of 1 unit BMI was associated with a 0.428 decrease in whole body insulin sensitivity measured by glucose infusion rate (mg/kg/min). The effect observed in the present study would be equivalent to a 2-unit increase in BMI. These results suggest that there is an adiposity-independent, acute-onset effect of both tested antipsychotic drugs on glucose regulation at the level of skeletal muscle. It remains unclear how long this effect may last, and how it interacts, if at all, with observed effects of adiposity on insulin sensitivity.

Keywords: Insulin Resistance, Atypical Antipsychotics, Antipsychotic Induced Weight Gain

Disclosure: Sunovion, Consultant, Alkermes, Advisory Board, Alkermes, Grant, Otsuka America, Inc., Grant

M197

Auditory Mismatch Negativity, Neurocognition, Brain Structure, and Functioning in First Episode Psychosis Patients

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Background: Patients with psychosis spectrum disorders exhibit deficits in electrophysiology, cognition, brain structure (grey matter volume) as well as hallmark impairments in social and occupation function. The early phase of a psychotic illness is the "critical period" because functional deterioration occurs at the fastest rate, but this period is also a "window of opportunity" in terms of reducing the development of disability and augmenting recovery. There is large variability in the functional outcomes of psychosis patients in the early phase, and the underlying neurobiological mechanisms of such outcomes are not well understood. Thus, there is a critical need to gain a better understanding of the brain changes underlying functional outcomes in first episode psychosis (FEP) patients.

In FEP, the profile of neurocognitive impairment tends to be stable over time and its severity is associated with worse outcomes and a more chronic course. An electroencephalographic (EEG) measure of mismatch negativity (MMN) is also related to functional outcomes and social cognition. FEP patients with the most impaired MMN amplitudes at baseline have the most severe disability at follow-up. In addition, MMN deficits are more pronounced in patients diagnosed with schizophrenia (SZ), highlighting the heterogeneity underlying different diagnoses. Several key brain regions, including the anterior cingulate cortex (ACC) and temporal lobe show significant grey matter volume reduction in FEP. These regions are also linked to MMN generation.

In this study, we investigated: a) whether brain volume in two regions of interest, the rostral anterior cingulate cortex (rACC) and

the Heshl gyrus (HG), were associated with neurocognitive, MMN, and/or functional outcomes in patients, b) whether diagnosis of SZ or bipolar (BP) disorder modulate the effect of brain volumes on functioning and cognition, and c) whether the volumes of specific brain regions could be used to predict cognitive and functional scores.

Methods: Data were collected from 68 participants (33 controls, 35 FEP patients) of both sexes. Structural magnetic resonance imaging (MRI) were acquired on a 3T Siemens scanner. T1-weighted images were converted to NIFTI files, and analyzed with FreeSurfer 6.0 using the Desikan-Killiany Atlas for grey matter parcellation. rACC and HG volumes were normalized to the estimated Total Intracranial Volume. Domains of real-life functioning and cognition were assessed using the Multnomah Community Ability Scale (MCAS) and the MATRICS Consensus Cognitive Battery (MCCB) respectively. MMN was assessed using a duration MMN paradigm and analyzed at Fz site. Exploratory correlation analysis was performed in R 3.5.2 using the Hmisc and corplot packages. Partial correlation analysis and regression analysis were performed in Stata v15.

Results: Analysis within the patient population ($n = 35$) revealed significant correlations between the left rACC and MCAS scores, and between the left HG and MCCB composite t-score ($r = 0.49$, $p = 0.035$). Partial correlation analysis, controlling for age, sex and education, revealed significant correlations between the left rACC and MCAS scores ($r = 0.461$, $p = 0.009$). No significant correlations were found between both brain regions and MMN at the Fz site. Linear regression revealed a significant interaction effect of diagnosis with the left rACC volume on MCAS scores, specifically in SZ patients ($n = 65$, Beta = 104.1563, $p = 0.002$). Interestingly, while there was a significant effect of the left HG volume on MCCB composite scores ($n = 61$, Beta = 301.0444, $p = 0.008$), no such modulatory effect was observed between diagnosis and the left HG volume. Finally, a diagnosis and rACC volume interaction model was able to explain 75% of the variance observed in MCAS scores ($F_{8,56} = 20.54$, $R^2 = 0.7458$). A second model was also built predicting MCCB scores based on the volume of the left HG ($F_{6,54} = 4.97$, $R^2 = 0.3556$).

Conclusions: We have shown that specific brain regions volumes, in particular the left rACC and left HG, are significantly correlated with, and possibly even predictive of, behavioral outcomes such as functioning and cognition. The modulatory effect of diagnosis on the ability of rACC volume to predict MCAS scores suggests changes in neural architecture could underlie the symptoms differentiating between the diagnosis of SZ and BP.

Keywords: First Episode Psychosis, Structural MRI, Functional Outcomes, Neurocognition

Disclosure: Nothing to disclose.

M198

Aberrant Cortical Ensembles Underlie Schizophrenia-Like Phenotypes in *setd1a* Deficiency

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Background: A breakdown of synchrony within neuronal ensembles leading to destabilization of network “attractors” could be a defining aspect of schizophrenia (SZ), representing a common downstream convergence point for the diverse etiological pathways associated with the disease. Here we investigate how such an attractor pathology could mediate sensory cortical processing phenotypes resulting from loss of function mutations

in the *setd1a* gene, a recently identified rare risk genotype with very high penetrance for SZ.

Methods: We employed fast two-photon calcium imaging of neuronal populations (GCaMP6s, 10Hz, 100-250 cells, layer 2/3 of primary visual cortex V1) in awake head-fixed mice (*setd1a* +/- vs wildtype littermate controls) during rest and visual stimulation with moving full-field square-wave gratings (0.04 cpd; 2.0 cps; 100% contrast, 12 directions). Multielectrode recordings were analyzed in the time-frequency domain to assess stimulus induced oscillations and cross-layer phase synchrony.

Results: Activity levels and orientation/direction selectivity at the level of individual neurons were unaffected in *setd1a* +/- . On average, correlations between cell pairs in V1 were not changed but showed altered distributions compared to WT. Further, population-wide “ensemble activations” were markedly less reliable over time during rest and visual stimulation in *setd1a* +/- , resulting in unstable encoding of basic visual information. This alteration of ensembles coincided with reductions in alpha and high-gamma band phase synchrony within and between cortical layers, suggesting a potential mechanism for known EEG biomarkers of SZ.

Conclusions: These results provide new evidence for an attractor theory of SZ and highlight the utility of the *setd1a* +/- mice for modeling sensory processing phenotypes.

Keywords: Neural Circuits, Two-Photon Microscopy, Sensory Processing

Disclosure: Nothing to disclose.

M199

Spatial Gene Expression at Single Cell Resolution in Mouse and Postmortem Human Brain

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Background: Analyzing spatial and temporal gene expression at single cell resolution in the brain can provide insight into cell characteristics, biological functions, and disease pathogenesis. Advanced approaches like multiplex single molecule fluorescence in situ hybridization (smFISH) and spatial transcriptomics can quantify RNA transcripts in single cells while providing spatial information within tissue architecture. These rapidly evolving techniques open possibilities for combining spatial gene expression maps with single cell or single nucleus RNA-sequencing (sn-RNAseq) data to add anatomical dimensions to existing datasets and further refine cell type-specific molecular signatures in the human brain.

Methods: To advance quantitative approaches for spatial gene expression data, we developed and validated “dotdotdot” as an open-source data analysis pipeline for smFISH images generated using RNAScope, a widely-used multiplex smFISH technology. Images from mouse and postmortem human tissue sections were acquired in z-stacks using laser scanning confocal microscopy. Following automated nuclear segmentation, dot detection, and autofluorescence masking, we used k-means clustering for classifying nuclei expression levels (low, medium and high) and CART (Classification and Regression Trees) for classifying cell types (GABAergic neurons, glutamatergic neurons, astrocytes, or oligodendrocytes). smFISH data were further compared to existing transcriptome-wide expression datasets in single cells and intact tissue slices as validation.

Results: We validated the robustness of “dotdotdot” in mouse cortex by demonstrating that activity-induced genes *Arc* and *Bdnf* are differentially regulated in single cells following induction of widespread neural activity by administration of electroconvulsive seizures (ECS). We also validated the effectiveness of “dotdotdot” in postmortem human brain, a heterogeneous tissue with high levels of lipofuscin autofluorescence, by demonstrating high sensitivity and specificity of cell type prediction via inter-rater reliability and independent neuronal signal, as well as recapitulating the expected proportions of canonical cell type markers in different cortical layers. Using images acquired in postmortem human dorsolateral prefrontal cortex (DLPFC), we further demonstrate the feasibility of integrating spatial gene expression with bulk and snRNA-seq datasets to add anatomical context to transcriptomics data acquired in dissociated tissue.

Conclusions: We developed “dotdotdot” as an automated workflow for processing and analyzing spatial gene expression in single cells. We also provide downstream analytic strategies to quantify smFISH data in human and mouse tissue for future integration with spatial transcriptomic and snRNA-seq data sets.

Keywords: Transcriptomics, Postmortem Human Brain, Single-cell RNA Sequencing

Disclosure: Nothing to disclose.

M200

Autosomal Recessive Transmission of Psychosis in a Consanguineous Family

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Background: Finding candidate risk genes for psychiatric disorders is based on several approaches; each with advantages and limitations. Linkage analysis in clusters of pedigrees sharing a DSM diagnoses or phenotype have largely not replicated. Transcriptome and genome wide association studies using many tens-of-thousands of cases have successfully led to hundreds of risk loci; however, the significant loci have low effect size. Perhaps no risk genes with large effect size are causal in psychiatric genetics, i.e., all are highly polygenic. Yet, it is puzzling why all other branches of medicine have found many Mendelian genes associated with disease. In fact, early discovery of such genes played pivotal roles in the initial understanding of the pathophysiology of many common disorders (e.g., dyslipidemia, thalassemia, and other inborn errors of metabolism). Another interpretation is the heterogeneity of psychiatric disorders precludes finding rare Mendelian genes when many distinct diseases under a DSM rubric are lumped together at a syndromal level. Recently, linkage analyses of single consanguineous pedigrees afflicted by a variety of disorders are finding rare variants with large effect sizes associated with disorders providing candidate genes for further study (Rilstone et al., NEJM 2013; Alkuraya et al., 2010). However, because these genes are so rare, causal evidence requires convergent approaches such as molecular dissection within cellular pathways and interactome, 3D modeling, animal models, and directed therapeutics.

Methods: Human Subjects: A Pakistani consanguineous pedigree consisted of two healthy parents who were first cousins and seven offspring. One child died early. Two girls had psychosis with auditory hallucinations and delusions consistent with DSM schizophrenia with onset at ages ~15 and 23 years. The older girl had the more severe affliction. These girls had no history of substance abuse, major affective illness, or intellectual disability. The other children were psychiatrically well. All provided informed

consent. The MINI was used as a screener. Blood was collected and processed for DNA sequencing.

DNA analysis: Whole exome capture used the Agilent SureSelect v5 system. The DNA was sequenced on the Novaseq 6000 with 150 bp paired-end reads. Coverage was 100 fold. Homozygosity mapping used Agilevcmapper. Allele specific PCR was used to determine polymorphism in the local population.

Risk gene identification: The criteria used to identify target variants follows: 1) it should be exonic; 2) its linkage should be consistent with autosomal recessive inheritance; 3) the gene should be rare (MAF < 0.01) given negative selection pressure; 4) it should be completely conserved; 5) it should not show polymorphism in the local population; 6) the variant should be pathogenic based on conservation and current predictive tools for structural impact scores; 7) it should not be homozygous in databases of normal subjects.

Results: There were three large homozygous regions located on chromosomes 4, 12, and 15 in the affected sisters. Only the homozygous region on chromosome 4 contained an exonic variant which affected an amino acid conserved in evolution in all vertebrate species examined and was predicted to be pathogenic by all six software packages used. Only the homozygous region in chromosome 4 (111-128.6 Mb, hg19) showed the correct linkage. The variant linked to the psychosis phenotype localized to Chr:4-120181668 (hg19), in exon 10 of USP53 (NM_019050.2); c.682T > C; p.(Cys228Arg) at 4q26.

Conclusions: This allele's frequency in South Asians is 5.2(10)⁻⁵ (gnomAD) without individuals homozygous for the variant. The variant was not polymorphic in the local population. Given the rarity, it is unlikely this variant will be found in another family.

The functions of USP53 (ubiquitin specific peptidase 53) are not entirely clear. It is a nonprotease homolog of the ubiquitin peptidase family expected to be cytoplasmic. It is expressed at low to medium levels in mouse and human brain. The human immunohistochemistry remains to be clarified. A knockout in mice is associated with deafness and abnormalities of the tight junction (Kazmierczak et al., 2015). Two interacting partners (CRKL, DAB2) are positioned in the reelin pathway thought important in the neurodevelopment of schizophrenia. Another partner is BLMH, a homocysteine-thiolactone hydrolase. Homocysteine has been implicated in schizophrenia and has motivated treatment trials with folic acid and vitamin B12 (Roffman et al., 2013). Abnormalities of the ubiquitin proteasome have also been identified in schizophrenia (Kim et al., 2018).

Limitations of this study include the following: 1) pathogenic copy number variants or non-coding variants were not addressed; 2) limited generalizability in explaining most psychotic illness or the population risk for psychosis; and 3) lack of direct evidence for causality. Given the rarity of the variant, such evidence will require convergent findings in elucidating the biology of USP53 in the brain using molecular and cell biology techniques and their clinical translation.

Keywords: DNA Sequencing, Multiplex Families, Schizophrenia, Bipolar Disorder, Genetics, Ubiquitination, Psychosis, Folic Acid, Whole Exome Sequencing

Disclosure: Nothing to disclose.

M201

β-Arrestins Mediate Rapid 5-HT_{2A} Receptor Endocytosis to Limit Serotonin and Hallucinogen Signaling

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Background: Serotonin (5-HT) type 2A receptors (5-HT_{2A}) modulate mood and perception and are the principal molecular targets for psychedelic hallucinogens and atypical antipsychotic medications. The 5-HT_{2A} canonically activates heterotrimeric Gq G-proteins which, in turn, activate phospholipase C and formation of inositol phosphates to stimulate the intracellular release of calcium. The 5-HT_{2A} may also interact with β -arrestin proteins; however, the importance of β -arrestins in the action of hallucinogens or for 5-HT_{2A} signaling are largely undefined. Here we use CRISPR/Cas9 genome editing to stably knockout (KO) β -arrestins in cells to study β -arrestin contributions to 5-HT_{2A} agonist signaling and receptor trafficking.

Methods: Wildtype (WT) HEK293 parental cells or cells lines in which β -arrestin 1 and 2 were stably knocked out using CRISPR/Cas9 genome editing were generated, validated and used for these studies. WT and KO cells were transfected with human 5-HT_{2A} and receptor signaling and trafficking in response to agonists was assessed using a combination of receptor imaging by confocal microscopy, live cell calcium fluorescence imaging, cell surface ELISA assay and western blotting for ERK phosphorylation. Additional Gq-coupled GPCRs and their signaling responses to agonists were also tested in both WT and KO cells as controls.

Results: We first examined if agonist activation of 5-HT_{2A} induced plasma membrane recruitment of β -arrestin using confocal imaging. Agonist activation of HA-5-HT_{2A} with 10 μ M 5-HT, or the selective agonist and psychedelic hallucinogen 2,5-Dimethoxy-4-iodoamphetamine (DOI), induced robust and rapid (within 30 secs) translocation of β -arrestin2-GFP from cytoplasm to the plasma membrane, where it strongly colocalized with HA-5-HT_{2A}. Live cell confocal imaging of HA-5-HT_{2A} determined rapid endocytosis of receptors within 3 mins of agonist stimulation. To determine if β -arrestins control this rapid receptor endocytosis to impact signaling, we used HEK293 cells lacking both isoforms of β -arrestins. Using a receptor cell surface ELISA, we confirmed 5 min agonist treatment with 5-HT or DOI resulted in rapid (~50%) loss of receptors from the cell surface in parent cells; however, endocytosis was significantly reduced in β -arrestin 1/2 KO cells. Measuring kinetic live cell calcium release using the FLIPR assay and dose responses of selective 5-HT_{2A} agonists, we determined prolonged duration of calcium release in β -arrestin 1/2 KO cells. The maximal 5-HT_{2A} calcium signaling was significantly elevated by 45% (5-HT) and 46 % (DOI) in KO cells vs. WT parent cells; however, agonist potency was unchanged. Re-expression of β -arrestin 1 or 2 in KO cells reduced the elevated 5-HT_{2A} calcium responses to that of parent cells and β -arrestin KO did not affect other Gq-coupled GPCRs (5-HT_{2C}, P2YR), indicating 5-HT_{2A} specific effects from genetic knockout of β -arrestins. In addition, knockout of β -arrestin1/2 increased and prolonged the duration of 5-HT_{2A}-mediated ERK phosphorylation in response to DOI.

Conclusions: This study indicates β -arrestins rapidly interact with 5-HT_{2A} and profoundly limit both intensity and duration of Gq-mediated signal transduction in response to 5-HT and a hallucinogen. Moreover, these results indicate rapid agonist-induced 5-HT_{2A} endocytosis is dependent on β -arrestins which serves to limit calcium signaling. Taken together, these findings reveal that β -arrestins control the rapid phases of 5-HT_{2A} signaling by mediating receptor endocytosis and this may impact the responsiveness to psychedelic hallucinogens.

Keywords: Beta Arrestin, Hallucinogens, Serotonin 5-HT_{2A} Receptor, CRISPR/Cas9, Calcium Imaging

Disclosure: Nothing to disclose.

M202

Multiplexed Single-Nucleus RNA Sequencing Reveals Cellular Subpopulation Specific Pathologies in Psychotic Disorders

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Background: Multiple molecular studies of homogenized post-mortem human brain tissue have identified disrupted GABA signaling in schizophrenia and bipolar disorder, and down-regulation of the GAD1 gene is among the most widely replicated findings in molecular studies of these disorders. Emerging technologies for single-cell transcriptomics now allow for high-throughput assessment of how the GABAergic deficit is partitioned across distinct GABAergic interneuronal subpopulations, advancing our understanding of circuitry-based information processing and its dysfunction in psychotic illness.

Methods: Full-thickness cortical samples were dissected from postmortem human prefrontal cortex (BA 10) tissue from 24 control subjects and 24 individuals with schizophrenia and 24 individuals with bipolar disorder balanced for age and gender (50% male, 50% female). Tissue samples were assessed by multiplexed single-nucleus RNA sequencing using the Multiplexing Using Lipid-Tagged Indices strategy on the 10x Genomics platform producing ~800,000 single-cell transcriptomes across all 72 subjects.

Results: Within this large dataset we identify 18 transcriptomically distinct cellular archetypes corresponding to the known major cell types within the human prefrontal cortex and their prominent subpopulations. Comparison across diagnostic groups using the ACTIONet algorithm reveals cell type and disease-specific gene-expression patterns and enriched pathways, pointing to specific neuronal processes associated with Sz and BD in excitatory and inhibitory neurons.

Conclusions: Advances in single-cell genomics technologies promise to revolutionize our knowledge of the “parts list” of the cellular machinery of the human brain, as well as how molecular pathologies are distributed among those functional units in psychiatric illness. This work demonstrates the power of assessing single-cell transcriptomes across the many neuronal and non-neuronal cell populations present within defined neuronal circuits to elucidate the molecular pathology of psychotic disorders at a resolution not previously possible, offering insights into how this pathology operates within the complex cytoarchitecture of the human brain.

Keywords: Postmortem Human Brain, Single-cell RNA Sequencing, Schizophrenia, Bipolar Disorder

Disclosure: Nothing to disclose.

M203

Spontaneous and Evoked Auditory Gamma Abnormalities in First-Episode Schizophrenia: A Longitudinal Study

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Background: Schizophrenia is generally associated with deficits in the power and phase synchronization of stimulus-evoked gamma-band (30-100 Hz) oscillations in the electro- and magnetoencephalogram (EEG/MEG). The gamma oscillation deficit that has been the most widely replicated is of the 40 Hz auditory steady-state response (ASSR), which has been found to be reduced compared to healthy individuals in the chronic and first episode states. These deficits indicate that gamma-generating neural

circuitry in the auditory system is dysfunctional in schizophrenia. Recent studies have also suggested that spontaneous broadband gamma (30-100 Hz) activity in the auditory cortex is increased in chronic schizophrenia. Both the 40 Hz ASSR deficit and increased spontaneous gamma “noise” in schizophrenia have been attributed to abnormal inhibitory interneuron function, possibly as a consequence of NMDA receptor hypofunction. However, it is unknown whether increased spontaneous gamma is present in the first episode state, and if so, whether this abnormality and the 40 Hz ASSR deficit progress over time. Therefore, in this study we investigated whether 1) spontaneous gamma activity in the auditory cortex is increased in first-episode schizophrenia, and 2) the spontaneous gamma and 40 Hz ASSR abnormalities change over a 1 year interval after the first hospitalization for psychosis.

Methods: Participants were 23 individuals with first-episode schizophrenia (FESZ) and 39 healthy comparison individuals (HC), assessed at two time points (Time 1 and Time 2) one year apart on average. The FESZ and HC groups were matched on gender proportion and age. Participants were presented with 500 ms click trains at 20, 30, and 40 Hz stimulation frequencies while the EEG was recorded with a dense electrode array. EEG artifacts were removed with independent component analysis. Dipole source analysis was used to localize the ASSR to bilateral auditory cortices and to construct a spatial filter for auditory cortex activity, from which spontaneous gamma and the ASSR were derived. Spontaneous gamma activity was computed with the Fast Fourier Transform as the induced gamma power in the baseline (-500 to 0 ms) and ASSR (30-530 ms) periods. ASSR phase locking factor (PLF) and evoked power were computed with the Morlet wavelet transform.

Results: As in our previous study on chronic schizophrenia, induced gamma power was increased overall in FESZ compared to HC ($p < 0.01$), and in the 40 Hz stimulation condition, this increase was particular to the left auditory cortex ($p < 0.05$). Induced gamma power did not change between the assessment time points. Also replicating previous results, 40 Hz ASSR PLF ($p < 0.0001$) and evoked power ($p < 0.01$) were decreased in FESZ compared to HC. In contrast to induced gamma, the 40 Hz ASSR PLF deficit worsened over the 1-year assessment interval ($p < 0.05$).

Conclusions: Spontaneous gamma power was found to be increased in first-episode schizophrenia, showing a similar pattern across experimental conditions as in chronic schizophrenia. Thus, this gamma abnormality appears to be present early in the course of schizophrenia, although the size of the effect did not change over the 1-year assessment interval. In contrast, the 40 Hz ASSR PLF deficit in first-episode schizophrenia did become worse over 1 year. The differential progression of increased spontaneous gamma activity and the 40 Hz ASSR PLF deficit suggest that the underlying neural substrates of these effects differ to some degree. These findings add to our understanding of the auditory cortex abnormalities that occur in schizophrenia and point to additional directions to explore in elucidating the neural circuit disturbances that underlie this disorder.

Keywords: Schizophrenia, Gamma Oscillation, First-Episode, Auditory Steady-State Response

Disclosure: Biogen Inc., Grant

M204

DNA Methylation in the Auditory Cortex Differs at Many Genomic Sites Between Schizophrenia and Control Subjects and May Contribute to Reduced Dendritic Spine Density in Subjects With Schizophrenia

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Background: DNA methylation (DNAm), the addition of a methyl group to a cytosine nucleotide, regulates gene transcription and may play an important role in schizophrenia pathogenesis. In a previous study, we found evidence to suggest that DNAm alterations may contribute to altered dendritic spine density in subjects with schizophrenia.

Methods: We used the Illumina MethylationEPIC Array to characterize DNAm at ~850,000 sites across the genome in the superior temporal gyrus of postmortem brains from 44 control subjects and 44 subjects with schizophrenia. Dendritic spine density had previously been characterized in 40 subjects in each group.

Results: We identified > 250 sites in the genome at which DNAm differed between non-psychiatric control and schizophrenia subjects at FDR of $q < 0.1$. Many of the sites at which differential DNAm was detected were annotated to genes previously associated with schizophrenia and/or dendritic spine structure and function. Further, we found that DNAm levels correlated with DSD at more genomic sites than expected by chance in control subjects but not in subjects with schizophrenia.

Conclusions: Our results suggest a potential role for DNAm alterations in schizophrenia pathogenesis, generally, and reduced dendritic spine density in subjects with schizophrenia, specifically. Current analyses focus on determining the cell types in which DNAm differs between schizophrenia and control subjects. Future studies will focus using a CRISPR/Cas9-based tool to recapitulate schizophrenia-associated DNAm alterations in cell culture and assessing their effect on cellular processes.

Keywords: DNA Methylation, Postmortem Brain Tissue, Dendritic Spine, CRISPR/dCas9, Epigenetics

Disclosure: Nothing to disclose.

M205

Circuit and Cellular Mechanisms of Prefrontocortical Kappa Opioid Receptor-Mediated Disruptions in Working Memory

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Background: Cognitive dysfunction in schizophrenic patients emerges earlier than other symptoms, and persists throughout the duration of illness. While most anti-psychotic drugs are effective at treating the positive symptoms of schizophrenia, the negative and cognitive symptoms are poorly managed by clinically available therapies. Interestingly, pharmacological activation of kappa opioid receptors (KORs) in humans produces visual and auditory hallucinations similar to positive symptoms in schizophrenia, and chronic activation of dynorphin systems (endogenous KOR ligands) in mice can lead to affective changes similar to the negative symptoms of schizophrenia (anhedonia and depression). These observations suggest that endogenous dynorphin activity may be dysregulated in some persons with schizophrenia, but whether selective KOR antagonists could ameliorate cognitive symptoms in schizophrenia has not been adequately explored in pre-clinical studies.

Methods: To study the role of KORs in the PFC in working memory, we trained male C57BL/6J mice in an operant delayed

alternation task to make a response on one retractable lever, wait a specified delay for reinsertion of the levers, and then respond on the alternate lever. Mice were trained until reaching stable performance with a 10s delay for reinsertion and then were injected in the PFC with either artificial cerebrospinal fluid (ACSF) or the long-lasting (~3 weeks) KOR antagonist, norBNI (1.25 µg in 0.5 µL vehicle). Following recovery from surgery, mice were treated with saline, KOR agonist (U50,488; 5 or 10 mg/kg i.p.; nalfurafine 50 µg/kg), or 2-day repeated forced swim stress immediately before a delayed alternation session. Using immunohistochemistry, we examined the expression of KOR and levels of KOR phosphorylation in PFC cell bodies following pharmacological KOR activation or behavioral stressors.

Results: A majority of KOR-expressing neurons in the prefrontal cortex (PFC) co-expressed Ca²⁺/calmodulin-dependent kinase II (CamKII) and systemic administration of a KOR agonist increased KOR phosphorylation in the PFC. KOR-expressing neurons projected to both striatal and thalamic target regions. Saline administration did not change performance in delayed alternation or increase KOR phosphorylation. Mice with PFC ACSF injections had significantly decreased correct responses following systemic KOR activation with U50,488 compared to a baseline day, and these disruptions were blocked with local PFC norBNI microinjection. Repeated forced swim stress, which is known to cause dynorphin release in the dorsal raphe nucleus and ventral tegmental area, did not produce behavioral disruptions in the delayed alternation task or phosphorylation of KOR in the PFC, suggesting that swim stress may not be sufficient to cause dynorphin release in the PFC. Nalfurafine, a G-biased KOR agonist, had no significant effect on performance in the delayed alternation task.

Conclusions: These studies demonstrate that KOR activation in the PFC produces deficits in working memory maintenance that are likely to be arrestin-dependent. Current experiments aim to further characterize the molecular requirements for KOR-mediated cognitive dysfunction and the neural circuits involved in these behaviors.

Keywords: Kappa Opioid Receptor, Dynorphin, Working Memory, Schizophrenia, Prefrontal Cortex

Disclosure: Nothing to disclose.

M206

Activation of Dopamine 1 Receptors Partially Rescues Dynamic Structural Changes Present in Human Monocyte-Derived-Neuronal-Like Cells (MDNCs)

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Background: Despite intense research, the pathophysiology of schizophrenia remains obscure. Among the main obstacles is the lack of adequate models able to replicate critical neurodevelopmental processes in vitro using cells that carry the genetic susceptibility to develop schizophrenia. One of these essential processes is the ability of neurons to constantly modify their structure in response to neurotransmitters and other molecules in order to establish functional connections. A neurotransmitter that has consistently been associated with schizophrenia is dopamine but its exact role as a potential etiological factor remains to be determined. Dopamine's ability to alter the neuronal structure has been scantily studied, hence, here we assess whether dopamine can modify dynamic structural changes presents in MDNCs.

Methods: We have developed a protocol to transdifferentiate blood circulating monocytes into neuronal-like cells in only 20 days and without reprogramming the cell's genome. Instead of inserting viruses into the cell's genome, we utilize different growth factors, antioxidants and conditioned media to promote neuronal differentiation. In order to measure dynamic structural changes in MDNCs we compared exactly the same cells via microphotographs, before and after one hour of incubation, either under control conditions or treated with the dopamine 1 receptor (DR1) agonist DHX with or without pretreatment with SCH a DR1 antagonist. Each structural change encountered is given a value, the sum of all the structural changes is reported as a Structural Dynamic Index (SDI). MDNCs were followed for 48hrs. Pictures before and after 1hr were taken right after differentiation. Another set of before and after pictures were taken at 24 and 48hrs. Experiments were conducted with blood samples from 5 men and one woman. This study was approved by the Penn State Hershey Medical Center IRB (00006911).

Results: MDNCs express several neuronal markers and present spontaneous action potentials as well as postsynaptic inhibitory and excitatory currents. Moreover, transdifferentiation of monocytes delivers reproducible results in sequential samples from the same donors. We have also shown that MDNCs structurally resemble human developing neurons after five days in culture as well as human neuroblastoma cells differentiated with retinoic acid. In addition, we have established that MDNCs as well as human neuroblastoma cells present similar structural dynamic changes as those described in neurons during brain development. Our most recent results indicate that MDNCs continue to extend their longest primary neurite 24hrs after differentiation while 48hrs after, no further growth is observed. Incubations with DHX or DHX + SCH for 1, 24 and 48hrs did not affect the longest primary neurite growth. MDNCs evidenced significant changes in their structure shortly after differentiation. However, its SDI drastically drops 24 and 48hrs after differentiation ($P < 0.005$). Treatment with DHX partially rescues this drop in SDI by day 22 ($P < 0.05$) and this rescue is blocked by pretreating MDNCs with SCH. DHX and SCH did not affect SDI in any of the other incubation times tested.

Conclusions: MDNCs obtained mostly from men, actively modify their structure shortly after differentiation but these dynamic changes diminish after 24 and 48hrs of incubation under control conditions. Activation of DR1 partially reestablishes these dynamic structural changes after 48hrs. DR1 does not affect growth of MDNCs longest primary neurite. These results open the possibility to study structural changes in response to DR1 activation in MDNCs from patients with schizophrenia.

Keywords: Adult Stem Cells, Development, Dendrites, Axons, Schizophrenia

Disclosure: Nothing to disclose.

M207

Transdiagnostic Psychopathology Domains and Neurobiology Associated With Familial High Risk for Psychoses

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Background: Dimensional delineation of psychopathology can help better understand transdiagnostic neurobiology and help predict risk for future dimensional psychopathology. The

Diagnostic and Statistical Manual developed dimensional Cross-Cutting Measures (CCM) modeled as review-of-systems in internal medicine. It is proposed to address transdiagnostic psychopathology within the clinical context. Some domains in the DSM CCM overlap onto the Research Domain Criteria (RDoC) of the National Institute of Mental Health. Relationship of these transdiagnostic clinical measures to neurobiology is unexplored. We derived longitudinal DSM CCM domains from a battery of clinical assessments administered to a cohort of familial high-risk (FHR; $n = 75$; male/female 35/40, age 16.26 ± 3.53 years) and healthy comparison ($n = 53$; male/female 24/31, age 16.79 ± 3.84 years) subjects at baseline, year 1 and year 2.

Methods: Structured Clinical Interview for DSM-IV, Structured Interview for Prodromal Symptom, Youth Self-Report, Chapman's scale, and the K-Schedule for Affective Disorders and Schizophrenia were used to extract 12 DSM CCMs, namely depression, anger, mania, anxiety, somatic symptoms, suicidal ideation, psychosis, sleep problems, memory, repetitive thoughts and behaviors, dissociation, personality functioning, and substance use. Clinical, imaging and neurocognitive data were collected at baseline, year 1 and year 2. Groups were compared on twelve DSM CCM domains using MANOVA and repeated measures MANOVA. Structural imaging data was examined using voxel-based morphometry within SPM12. For longitudinal imaging data, computerized anatomical toolbox 12 (CAT12) was used to map changes in morphometric measures.

Results: A MANOVA model comprising of baseline CCM domain scores was significant (Wilk's $\lambda = 0.57$, $F(1, 126) = 8.13$, $p = 0.001$). Bonferroni-corrected between-subjects' effects showed that FHR scored significantly higher on 10 of the 12 CCM domains (somatic, sleep, inattention, depression, anger, irritability, anxiety, psychosis, repetitive thoughts and behaviors, and suicide) but scored lower on the mania domain compared to HC.

Repeated measures MANOVA showed a significant difference between FHR and HC scores in sleep, inattention, mania, and anxiety over time ($F(1,94) = 10.785$, $p = 0.001$) over 2 years. Over 2 years, scores on mania domain decreased in FHR (HC scores were between a mean of 0.8-1.6 and FHR mania mean scores were between 0.0 — 0.4). Substance Use domain scores were higher in FHR compared to HC at baseline and year 1. At Year 2, HC scored higher in the substance use domain. Overall, there was no difference between the groups in the substance use domain.

Imaging data primarily showed longitudinal decrease in volume of the prefrontal (middle frontal and orbitofrontal) and limbic (amygdala and insula) regions. Multiple comparisons were corrected using 3DClustsim.

Conclusions: FHR show elevated scores on multiple CCM domains suggesting greater morbidity among these youths compared to their peers suggesting that FHR youth are at higher risk for broad psychosis spectrum psychopathology (BPSP) not just for psychosis as evidenced by no significant difference between groups. Thus, FHR youths are developing broad spectrum psychiatric symptoms in other areas that is worthy of clinical attention suggesting that familial risk for psychosis may exert pleiotropic effect on manifest psychopathology. FHR scored low on mania with no difference in depression suggesting decreased drive and motivation. The DSM CCM domains correspond to the RDoC framework in several areas of the matrix. Association of longitudinal changes in inattention and anxiety scores with prefrontal and limbic regions suggests potential role of these regions in the evolution of broad psychosis spectrum psychopathology. This analysis provides evidence that the DSM CCM is a valuable tool in reviewing broad symptomatology in FHR patients, as opposed to focusing solely on categorical diagnoses. Our study supports that DSM CCM domains can be used to integrate RDoC domains within the clinical context and also help in elucidating neurobiology related to broad psychosis spectrum psychopathology.

Keywords: Antipsychotic-Naïve First-Episode Schizophrenia, Familial Risk of Schizophrenia, Research Domain Criteria (RDoC), Transition to Broad Psychosis Spectrum Psychopathology

Disclosure: Nothing to disclose.

M208

Effectiveness of Pimavanserin, a Selective 5-HT_{2A} Inverse Agonism, Alone and in Combination With Atypical Antipsychotic Drugs as Treatment for Positive and Negative Symptoms

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Background: Atypical antipsychotic drugs (AAPD), e.g. clozapine, olanzapine, risperidone, lurasidone, are effective to treat positive and negative symptoms in some patients with schizophrenia (SCZ). We have shown that a key feature of most AAPDs is their more potent 5-HT_{2A} inverse agonism, than D₂ receptor antagonism. Selective 5-HT_{2A} inverse agonists, e.g. M1009077 and SR43469B, are themselves effective to treat positive and negative symptoms in some acutely psychotic SCZ patients. We have also reported that augmentation of low doses of risperidone with pimavanserin (Pim), a selective 5-HT_{2A} inverse agonist approved for treatment of psychosis in patients with Parkinson's disease, leads to more rapid onset of antipsychotic effect with fewer side effects in acutely psychotic SCZ patients. Whether augmentation of standard doses of AAPDs with PIM would lead to beneficial effect on positive and negative symptoms in patients who are resistant to AAPDs is currently under investigation.

Methods: Here we report on the efficacy of PIM, alone, and in combination, with AAPDs, on the phencyclidine (PCP) and amphetamine models of positive symptoms, increased locomotor activity (LMA), and the scPCP model of negative symptoms in C57Bl6 mice, the induction of a deficit in SI. We administered singled doses of either PCP or amph to stimulate LMA. We treated with PCP for 7 days followed by withdrawal to cause a deficit in SI. We attempted to induce treatment resistance to AAPDs by combining acute restraint stress (ARS) or chronic unpredictable stress (CUS).

Results: in accord with prior studies AAPDs were more effective to block PCP-induced (PI) LMA than amphetamine-induced (AI) LMA. The combination of acute restraint stress (ARS) or chronic unpredictable stress (CUS) with scPCP produced resistance to the action of AAPDs to block PI-LMA. PIM alone was ineffective. Several AAPDs were effective to improve SI in scPCP-treated mice. The combination of CUS or ARS and scPCP produced resistance to the action of AAPDs to rescue SI. PIM alone was inactive. Pim, in combination with full doses of some AAPD, restored SI in scPCP + CUS mice, suggesting that these combinations may be effective for negative symptoms. These combinations were less effective in blocking PCP-I LMA.

Conclusions: Mouse studies using PCP and amphetamine can be of value to test treatments for treatment refractory patients with SCZ. Depending on the stage of illness and prior response to APD treatment, pimavanserin may be effective as augmentation of AAPDs for positive and negative symptoms. Results reported here would predict greater efficacy in treatment resistant negative symptoms. The mechanism by which additional 5-HT_{2A} receptor blockade is effective in models of treatment resistance requires further study.

Keywords: Antipsychotic Drug, Pimavanserin, Negative Symptoms

Disclosure: Eli Lilly, Grant, Acadia, Stock / Equity, Allergan, Grant, Sumitomo Dainippon, Grant, Sunovion, Grant

M209

Prediction of Psychiatric Readmission Risk in Psychosis Patients With Natural Language Processing of Electronic Health Records**Elena Alvarez Mellado, Eben Holderness, Nicholas Miller, Kirsten Bolton, Philip Cawkwell, James Pustejovsky, Mei-Hua Hall****McLean Hospital, Belmont, Massachusetts, United States*

Background: Psychotic disorders are one of the leading causes of disability worldwide. A substantial proportion of psychiatric inpatients are re-admitted after discharge. Readmissions are disruptive for patients and families and are a key driver of rising healthcare costs. Reducing readmission risk is therefore a major unmet need of psychiatric care. Electronic health records (EHRs) contain detailed descriptions about a patient's illness presentation, prior course, and treatment plans – all vital information for identifying readmission risk. Developing clinically implementable machine learning tools to enable accurate prediction of risk factors associated with readmission offers opportunities to inform the selection of treatment interventions and implement appropriate preventive measures.

We have previously identified seven clinical important risk factor domains and clinical sentiments as regard to these seven readmission risk factor domains in electronic health records (EHR) associated with readmission (Holderness et al 2019). In this study we develop and test a classification model that predicts the risk of early readmission (readmitted within 30 days from the discharge date) for psychotic patients using prior identified risk factors and additional extracted features from EHR. The classifier establishes whether the most recent admission of a patient will be followed by an early readmission or not.

Methods: Data pipeline for training and evaluating our readmission risk prediction model is shown in Figure 1. The model was trained and tested on a compilation of EHR from 183 psychosis patients who had at least one episode of early readmission in clinical notes. In total, this corpus contained 552 admissions, 280 out of those (50.7%) were early readmissions.

For every admission, 30 features were extracted to feed the classifier. They can be grouped into three categories: general sociodemographic information (e.g., gender, age, marital status), past medical history of the patient (e.g., number of previous admissions, history of previous suicide attempts, average length of stay up until that admission), and information of the most recent admission (e.g., length of stay, number and length of the notes, GAF [Global Assessment Function] score, level of insight, treatment compliance). The classifier includes clinical sentiment analysis scores for seven risk factor domains associated with readmission risk (Appearance, Mood, Interpersonal, Occupation, Thought Content, Thought Process, and Substance). These sentiment scores were automatically obtained through a topic extraction and sentiment analysis pipeline that evaluate the state of the patient concerning each of these risk factor domains for every note in the admission.

Figure 1. Data pipeline for training and evaluating our readmission risk prediction model.

Results: We trained six different classification models: a Stochastic Gradient Descent, a Logistic Regression, a C-Support Vector, a Decision Tree, a Random Forest, and a Multilayer Perceptron. All of them were implemented and fine-tuned using the scikit-learn machine learning toolkit. The Random Forest classifier resulted in the best accuracy (F1= 0.72 and accuracy= 0.70), followed by Decision Tree (F1= 0.62 and accuracy= 0.64). The most useful features to be included in the models were: the

existence of prior history of suicidal attempts, the GAF scores, the insight values, and occupation.

Conclusions: This is, to our knowledge, the first study applying NLP for predicting early readmission risk in psychosis patients using a list of extracted clinical features and sentiment scores of risk domains. Although preliminary, results show the promising possibilities that NLP-based approaches can offer in early readmission prediction. The two best models, Random Forest and Decision Tree models produce clinically explainable features which are useful in clinical practice. We are working on refining the models to improve prediction accuracy.

Keywords: Treatment Outcome Prediction, Readmission Risk, Natural Language Processing (NLP), Psychosis, Electronic Health Record (EHR)

Disclosure: Nothing to disclose.

M210

Anticholinergic Medication Burden Effect on Cognition in Patients With Schizophrenia in the Consortium on the Genetics of Schizophrenia (COGS-2) Study**Yash Joshi*, Juan Molina, Jason Xie, William Hochberger, John Nungaray, Lauren Cardoso, Laura McDonald, COGS Investigators, Raquel Gur, Ruben Gur, David Braff, Gregory Light***University of California, San Diego School of Medicine, San Diego, California, United States*

Background: Longitudinal studies have established that chronic exposure to medications with strong anticholinergic properties increases dementia risk in older adults. Psychotropic medications commonly used in the treatment of schizophrenia (SZ), including antipsychotics, mood stabilizers, antidepressants, and sedative/hypnotics, frequently have strong anticholinergic properties. Since cognitive impairment is a core feature of SZ, excessive anticholinergic burden imparted by medications may further compromise cognitive functioning. This study examined the relationship between anticholinergic medication burden and cognition in a large cohort of SZ patients.

Methods: Anticholinergic medication burden was calculated using the Anticholinergic Cognitive Burden Scale (ACB) in SZ patients (n = 1,113) and healthy subjects (HS, n = 989) enrolled in the cross-sectional Consortium on the Genetics of Schizophrenia (COGS-2) study. Subgroup analyses of SZ patients with high (ACB > 4) vs ACB low (ACB=0) scores were performed. Efficiency scores on the Penn Computerized Neurocognitive Battery Cognitive were used to measure neurocognitive performance.

Results: As predicted, SZ patients had higher ACB scores than HS (2.4 + 2.4; HS = 0.7 + 1.8, p < 0.01), largely driven by antipsychotics medications. When compared with the high ACB subgroup (n = 172), SZ patients in the low ACB subgroup (n = 295) had significantly better efficiency scores across all cognitive domains, (F = 9.1, p < 0.05; average overall cognition z-score, high ACB subgroup = -2.2, low ACB subgroup = -1.7).

Conclusions: Results indicate that nearly one of five SZ patients (~17%) have medication regimens with high anticholinergic burden. Subgroups with higher anticholinergic medication burden have significantly greater cognitive deficits across all domains. Prospective longitudinal studies are needed to clarify cause-effect relationships between anticholinergic burden and cognitive functioning in SZ.

Keywords: Schizophrenia, Cognitive Functioning, Anticholinergic Medication Burden

Disclosure: Nothing to disclose.

M211

Effect of Alcohol Coadministration on the Pharmacodynamics, Pharmacokinetics, and Safety of Lemborexant

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Background: Lemborexant (LEM) is a dual orexin receptor antagonist under investigation for the treatment of insomnia disorder and irregular sleep-wake rhythm disorder. Some sleep-promoting agents, such as suvorexant, zopiclone, and zolpidem have been shown to have additive negative effects on some pharmacodynamic assessments, including cognitive and psychomotor performance, when administered with alcohol. This study was conducted to examine potential interactions of LEM and alcohol on postural stability (falls risk indicator) and cognitive performance, and to assess the safety and tolerability of a single dose of LEM administered with or without alcohol. The effect of alcohol coadministration on LEM pharmacokinetics was also examined.

Methods: This was a single-center, randomized, double-blind, placebo-controlled, single-dose, 4-period crossover drug-alcohol interaction study in healthy volunteers (NCT03483636; E2006-A001-009). Subjects were randomized to 1 of 4 treatment sequences: placebo, LEM 10 mg (LEM10) alone, alcohol (0.6 g/kg, females; 0.7 g/kg, males) alone, or LEM10 with alcohol. An alcohol placebo control (i.e., 1 mL of alcohol floated on top of each aliquot of cranberry beverage provided for drug administration) was included in the placebo and LEM-only conditions. Subjects remained in the clinic for 72h postdose. Each treatment was administered ~2h following a light breakfast. Treatments were separated by a ≥ 14 d washout period.

Postural stability was assessed using an ataxiometer, which measures body sway in units of $1/3^\circ$ angle of arc (units; higher values indicate more body sway, i.e. less postural stability). Cognitive performance was evaluated using a computerized performance assessment battery (CPAB) comprising 9 tasks assessing 4 domains of attention and memory. The primary domain of interest was power of attention (reaction time). Body sway and CPAB assessments were performed predose (baseline) and up to 72h postdose. Change from baseline (CFB) in body sway and each CPAB domain was analyzed using a mixed-effect model for a crossover study adjusted for relevant factors. Pharmacokinetic parameters were calculated by noncompartmental analysis.

Results: Of 32 randomized subjects, 18 (56.3%) completed all 4 treatments (completer analysis set [CAS]). Median (range) age of randomized subjects was 38.5y (26 to 54y); the majority were male (75%) and white (65.6%). The CAS was used for body sway and CPAB analyses. Safety was assessed in subjects who received ≥ 1 dose of study drug.

At 2h post-dose, body sway CFB was significantly higher (worse) for LEM10 with alcohol compared with LEM10 alone (contrast mean difference [95% CI]: 36.2 [17.6-54.7] units; $P < 0.001$). However, body sway CFB values were not significantly different between these groups when assessed at 0.5h and 6-72h postdose. No significant differences in body sway CFB were observed for LEM10 with alcohol vs alcohol alone, or for LEM10 vs placebo at any time point (except at 9h due to 1 subject in the placebo group with an unusually high body sway value at this time point). Alcohol alone worsened postural stability at 2h vs placebo.

The CFB in power of attention was significantly higher (worse) for LEM10 with alcohol vs LEM10 alone at 0.5h (contrast mean

difference [95% CI]: 239.2 [23.3-455.2] msec; $P < 0.05$) and 6.0h (contrast mean difference [95% CI]: 234.7 [25.8-443.6] msec; $P < 0.05$). The CFB in power of attention was also significantly increased for LEM10 with alcohol vs alcohol alone and for LEM10 vs placebo at 0.5h and 2h, but at no other time points.

Compared with LEM10 alone, LEM10 with alcohol worsened performance in the CPAB domains of continuity of attention at 2h; quality of memory at 0.5h and 2h; and speed of memory retrieval at 2h, but no later time points.

Median t_{max} of LEM was 1.5h for LEM10 with alcohol and 1.7h for LEM10 alone. The coadministration of LEM10 with alcohol resulted in 35% and 70% increases in C_{max} and AUC(0-72h) of LEM, respectively, compared with LEM10 alone.

Incidence of treatment-emergent adverse events (TEAEs) was lower with placebo (33.3% [8/24]) compared with LEM10 only (96.2% [25/26]), alcohol only (83.3% [20/24]), or LEM10 with alcohol (95.2% [20/21]). The most common TEAE was somnolence (placebo, 12.5% [3/24]; LEM10 alone, 88.5% [23/26]; alcohol alone, 37.5% [9/24]; LEM10 with alcohol, 85.7% [18/21]). The majority of TEAEs were mild or moderate in severity and considered related to treatment (1 severe TEAE occurred following treatment with alcohol).

Conclusions: LEM10 alone did not affect postural stability (body sway), while alcohol alone significantly worsened postural stability at 2h. LEM10 with alcohol did not show evidence of additivity on postural stability versus alcohol alone. LEM10 with alcohol had additive negative effects on cognitive measures which corresponded with the approximate t_{max} of LEM (~2h). Cognitive performance returned to baseline by 9h post-dose. No synergistic effects of LEM and alcohol coadministration were observed for any outcome. Exposure to LEM was increased when coadministered with alcohol. Overall, this study suggests that LEM should not be taken with alcohol.

Keywords: Lemborexant, Drug-Drug Interaction, Alcohol

Disclosure: Eisai Inc., Employee

M212

Abuse Potential Considerations for Lemborexant, a Dual Orexin Receptor Antagonist

Abstract not included.

M213

Sleep in Autism Spectrum Disorder Without Intellectual Disability

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Background: Autism spectrum disorder (ASD) is characterized by difficulties in communication and social interaction in addition to restricted and repetitive behaviors. Sleep problems are a common concern. This study aims to further elucidate sleep problems in ASD, specifically in individuals without intellectual disability (ASD w/o ID).

Methods: Individuals of both sexes with ASD w/o ID and their relatives were recruited as part of the Autism Spectrum Program of Excellence (ASPE) at the University of Pennsylvania. Among an extensive battery of neurobehavioral assessments, the Social

Responsiveness Scale, Second Edition (SRS-2) was performed on probands and relatives to quantify the level of social impairment. The SRS-2 was compared to various sleep traits using Pearson correlations. The SRS-2 for Adults (SRS-2-A) was completed by an informant known to the participant (153 participants), the SRS-2 Self-Report (SRS-2-SR) was completed by the individual (173 participants), and the SRS-2 for Children (SRS-2-C) was completed by parents (16 participants). Actimetry data was collected via a wrist-worn tri-axial accelerometer for 21 days (data from 76 ASD probands and 110 relatives were considered). The relatives were classified as unaffected (61 participants) or affected (49 participants) if they met Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria for a psychiatric disorder, such as schizophrenia, depression, or other mood disorders. We analyzed sleep data using GGIR, an algorithm based on the distribution of change in z-angle, PennZzz, and ChronoSapiens. We quantified various sleep traits, such as the total sleep time, number of sleep episodes, and sleep efficiency (total sleep time normalized by total time in bed). We also quantified the stability of their activity/sleep pattern across multiple days by computing the interdaily stability.

Results: Although many sleep parameters did not differ between the three groups (probands, affected, and unaffected relatives), we detected a significant variation in sleep traits between and across families. Probands demonstrated significantly lower ($p = <0.001$) interdaily stability than their relatives (both affected and unaffected). Further, the probands had moderately decreased sleep efficiency ($p = 0.05$) and later sleep onset ($p = 0.01$) compared to the unaffected relatives though the other sleep parameters did not differ between the groups.

A key question in our analysis is the relationship between social impairment and sleep traits. We found more indicators of sleep fragmentation in individuals with increased social impairment. For instance, in the SRS-2 Self-Report, increased social impairment was associated with decreased sleep efficiency ($p = 0.04$), higher level of activity during rest phase (5-hour period of lowest activity (L5); $p = 0.02$), and lower interdaily stability ($p = <0.00002$). In the SRS-2 completed for adults by an informant, higher social impairment was also associated with lower interdaily stability ($p = 0.02$) as well as higher intraday variability ($p = 0.03$). These findings were further supported by an association of increased social impairment with earlier onset of activity in all three groups (10-hour period of highest activity (M10); SRS-2-A $p = 0.00003$; SRS-2-SR $p = 0.0001$; SRS-2-C $p = 0.05$). The earlier activity onset signifies individuals being more active during the night thus starting their daily activity earlier. In the SRS-2 Self-Report, increased social impairment was associated with a delayed sleep schedule given a later wake time ($p = 0.04$) and later rest time (5-hour period of lowest activity (L5); $p = 0.04$). In the child SRS-2, higher social impairment was associated with an earlier wake time ($p = 0.01$).

Conclusions: This study focuses on the analysis of sleep traits in ASD subjects including the effect of social impairment on sleep. Thus far, we have found differences in sleep in individuals with ASD w/o ID compared to those without ASD. We have additionally shown that social impairment could play a role in sleep dysfunction. The keystone of this research will be integrating the analysis of sleep traits with a wide range of other behavioral data and whole genome sequencing obtained from ASPE subjects. Moreover, the sleep traits evaluated in this study can be compared to corresponding sleep traits in model organisms with mutations in ASD-related genes to broaden our understanding of sleep and ASD.

Keywords: Autism Spectrum Disorder, Sleep Disturbance, Social Behavior

Disclosure: Nothing to disclose.

M214

Targeting the Gastrointestinal Environment as a Novel Treatment for Opioid Withdrawal

Abstract not included.

M215

A Phase 1 Clinical Trial to Evaluate Pharmacodynamic Interactions After Oral Coadministration of Alcohol and the Highly Selective Glucocorticoid Receptor Antagonist, PT150

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Background: The hypothalamic-pituitary-adrenal (HPA) axis is the body's main stress-response system, and cortisol is the major glucocorticoid hormone produced by the adrenal cortex in humans. A negative feedback system involving glucocorticoid receptors (GRs) regulates HPA axis activity and cortisol secretion. Heavy alcohol use and withdrawal are associated with dysregulation of the HPA axis and disruption of glucocorticoid signaling. In a human laboratory study, the non-selective partial GR receptor antagonist, mifepristone, reduced both excessive drinking and alcohol craving in recently abstinent alcohol-dependent volunteers. In preclinical studies, both mifepristone and selective GR antagonists reduced alcohol self-administration, providing evidence for a specific role of GRs in compulsive-like alcohol intake.

PT150, formerly known as ORG 34517, is a novel, highly selective GR competitive antagonist. In preclinical studies, PT150 reduced the severity of alcohol withdrawal and related HPA axis activation. In contrast to mifepristone, which has a 5.4-fold greater affinity for GR than progesterone receptors (PRs), PT150 has a nearly 500-fold greater affinity for GRs than PRs. Because selective antagonism of GRs may increase tolerance and reduce side effects, we conducted a human laboratory study to determine the 1) safety of concurrent alcohol administration after 5-days of PT150 treatment, and 2) potential efficacy of PT150 for reducing both the acute subjective effects of alcohol and alcohol craving.

Methods: We recently completed a single-center, within-subject, drug-drug interaction study in alcohol-experienced, non-treatment-seeking adults over a 6-day inpatient period. Before treatment with study drug on Day 1, subjects completed two alcohol challenge sessions in which the beverage condition was randomized, separated by four hours: during one session subjects consumed an alcohol beverage (0.8g/kg; 16% ethanol by volume), and during the other session subjects consumed a placebo beverage (1% ethanol by volume). After completion of the alcohol challenge sessions on Day 1, the first dose of PT150 (900mg qd) was administered orally to all subjects. Subjects received study drug for four more days (Days 2-5). Concurrent alcohol administration occurred at steady state of study drug on Day 5. Hence, on Day 5, subjects completed two additional alcohol challenge sessions, conducted the same as on Day 1. Pharmacodynamic endpoints, which included the Biphasic Alcohol Effects Scale (BAES), the Alcohol Urge Questionnaire (AUQ), the Positive Affect and Negative Affect Scale (PANAS), and the Pentobarbital-Chlorpromazine-Alcohol Group (PCAG), Amphetamine (AMP), Morphine-Benzodrine Group (MBG), Lysergic Acid Diethylamide (LSD), and Benzedrine (BG) scales from the Addiction Research Center Inventory (ARCI) were measured before, and at regular intervals for 2-hours after, all 4 alcohol challenge sessions. We measured vital signs, including heart rate and blood pressure, and

the concentration of alcohol (ethanol) in the participant's breath, via breathalyzer, at the same intervals. Additionally, we obtained levels of cortisol, potassium, thyroid-stimulating hormone (TSH), and lipids before administration of PT150 on Day 1, and after administration of PT150 on Day 3 (cortisol and potassium) and Day 6 (cortisol, potassium, TSH, and lipids). Institutional Review Boards at both the Baylor College of Medicine and the Michael E. DeBakey VA Medical Center (MEDVAMC), as well as the Department of Defense, Human Research Protection Office (HRPO) approved all procedures.

Results: We screened 32 subjects, enrolled 11, of which 10 completed all study assessments. The mean age ($\pm$ standard deviation) of the subjects who completed the study was 46.9 years ($\pm$ 11.6; range: 28-63); 100% were male; 70% were African American, 20% were Hispanic, and 10% were Caucasian. Our preliminary data reveal the safety and tolerability of both repeated administrations of PT150 and concurrent administration of PT150 and alcohol in an inpatient setting. We are currently validating pharmacodynamic, cardiovascular, and safety data and will present the results in detail at the conference.

Conclusions: Alcohol use disorder (AUD) is a major public health concern and a considerable risk factor for morbidity and disability yet only a small proportion of individuals with an alcohol use disorder benefit from the currently available FDA-approved medication treatments for AUD, signaling an urgent need for more effective and better-tolerated treatments. Preliminary findings from the current study suggest few side effects and good tolerance following concurrent administration of alcohol and the highly selective GR antagonist PT150. We expect results from the current study to provide important initial clinical data concerning the safety and potential pharmacodynamic interactions when alcohol is consumed concurrently with PT150.

Keywords: Glucocorticoid Antagonists, Alcohol, Pharmacodynamics, Efficacy and Safety

Disclosure: Nothing to disclose.

M216

Associations of Self-Reported Stress in Structural and Functional Connectivity in Chronic Marijuana Users

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Background: Early life and current perceived stress, which are known risk factors for marijuana use, are likely to have differential brain manifestations given their differing behavioral manifestations. To date, however, whether there are distinct neural mechanisms of early life and current perceived stress that impact marijuana use has not yet been examined. This study tested the hypothesis that current and early stress have differential associations with brain white matter integrity and functional connectivity (FC) in adult marijuana users.

Methods: Diffusion tensor and resting-state functional MR images were collected in 98 chronic marijuana users and 95 non-using controls. Measures of white matter integrity included fractional anisotropy (FA), mean diffusivity (MD), axial and radial diffusivity (AD, RD) coefficients of 48 fibers defined in Johns Hopkins University atlas. FC was assessed as the temporal correlation within and between the default mode, central executive and salience networks.

Results: We found a group interaction with current perceived stress and FC such that current perceived stress was negatively correlated with FC within the salience network in the users, but positively correlated in the non-users. Moreover, FC within the

salience network was negatively correlated with delta-9-tetrahydrocannabinol levels in the users. There was no significant group-specific effect of early stress in white matter integrity and FC.

Conclusions: Our findings indicate that current perceived stress, but not early stress, is related to brain network functional, but not structural, connectivity in marijuana users.

Keywords: Cannabis Use, MRI, Connectivity

Disclosure: Nothing to disclose.

M217

Distinct Roles of Dopamine D3 and mGlu5 Receptors in Addiction-Relevant Behaviors in the Rat

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Background: The dopaminergic and glutamatergic dysfunctions that have been observed in substance-dependent individuals may underlie their inflexible decision-making processes. Although these biobehavioral alterations are presumed to be, in part, a consequence of chronic drug use, we hypothesized that alterations may also exist prior to any drug use and lead to a heightened risk for developing addiction-like behaviors.

Methods: To test this hypothesis, we measured dopamine D2/3 receptors and metabotropic glutamate 5 receptors (mGluR5), and decision-making with a probabilistic reversal-learning (PRL) task in the same adult male Long Evans rats before and after they self-administered cocaine (or saline, control) for 21 days ($N = 60$). D2/3 and mGluR5 were quantified with PET neuroimaging and 11C-PHNO and 18F-FPEB, respectively. D3 receptor (DR3) availability was quantified by assessing 11C-PHNO activity in the midbrain, where binding is exclusively due to D3 receptors.

Results: High midbrain DR3 availability prior to cocaine self-administration was associated with deficits in the ability to use positive outcomes to guide choices in the PRL task ($p = 0.04$) and with greater drug-taking behaviors ($p = 0.03$). This effect was limited to DR3 since individual differences in mGluR5 availability before self-administration did not predict future drug-taking behaviors. These findings suggest that high levels of midbrain DR3 lead to greater drug-taking behaviors that could ultimately increase the likelihood of developing an addiction. In contrast, cocaine self-administration significantly disrupted PRL performance by impairing the ability of rats to use negative outcomes to guide their choices, as quantified with a reinforcement-learning model ($p = 0.003$). We also observed a robust increase in mGluR5 receptor availability within the medial prefrontal cortex ($p < 0.001$) which predicted the magnitude of cue-induced reinstatement ($p = 0.03$). DR3 availability, however, was not affected following cocaine self-administration.

Conclusions: Together, these findings implicate distinct decision-making processes and dissociable roles of DR3 and mGluR5 in addiction pathology. Notably, our results suggest that DR3 may be an important target for preventing addiction, while mGluR5 may be a critical substrate in relapse-like behaviors. Our ongoing work is investigating potential therapeutic strategies for reducing DR3 availability to prevent the development of addiction-like behaviors and reducing mGluR5 signaling to treat relapse-like behaviors.

Keywords: Computational Models of Decision-Making, Cocaine Self-Administration and Reinstatement, PET Imaging, Dopamine (D2, D3) Receptors, mGluR5 Receptors

Disclosure: Nothing to disclose.

M218

Separation of D2- And D3-Specific Binding Using [11C]-(+)-PHNO Competition Binding Studies and Independent Component Analysis

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Background: Dysregulation of D2 or D3 receptors is implicated in a number of disorders including addiction, schizophrenia, and Parkinson's disease. Despite structural similarity and overlapping brain distributions, these receptor types have distinct functional roles in healthy brain function and disease. For example, studies in stimulant addiction suggest that D2 receptors are down-regulated while D3 receptors are upregulated [1-3]. Development of subtype-specific medications is therefore an ongoing research priority, but high structural homology presents a challenge for the design and validation of D2- or D3-specific drugs.

The positron emission tomography (PET) radiotracer [11C]-(+)-PHNO binds with high affinity to D2 and D3 receptors and so has been used to estimate relative receptor occupancy at these sites [3,4]. However, accurate quantification of binding at each receptor is challenging, particularly for D3 receptors given the small size of D3-rich regions such as substantia nigra. The objective of this work was to extend our previous research into data-driven analysis of [11C]-(+)-PHNO binding data [1] by evaluating the use of independent component analysis (ICA) to separate D2- and D3-related signals in a competition binding study.

Methods: Eight participants underwent three PET scans with [11C]-(+)-PHNO on a high-resolution PET scanner (HRRT, CTI/Siemens). Participants were scanned at baseline and at two time points following administration of the D3-preferring antagonist ABT-728 (150–1000 mg). Parametric images of binding potential (BP(ND)) were computed using the simplified reference tissue model. Images from all subjects were entered into ICA as four-dimensional time series in order to extract two independent components of spatiotemporally coherent variance in [11C]-(+)-PHNO BP(ND) and corresponding scan-specific loading values. The spatial pattern of each binding component was compared to literature-based [5,6] estimates of D2- and D3-related BP(ND) across brain regions.

For comparison with ICA results, BP(ND) was extracted from seven subcortical regions of interest (ROIs) and an occupancy model was fitted across ROIs to estimate D2- and D3-specific receptor occupancy and proportion of binding attributable to D3 receptors ($f(D3)$). Occupancy in each ICA component was computed as percent displacement in loading values during blocking scans. Concentration-occupancy curves were then constructed for model-based and ICA occupancy estimates to determine site-specific drug EC50. Regional $f(D3)$ was also calculated from ICA data as the ratio of D3-related component loading to total BP(ND).

Results: Two components of [11C]-(+)-PHNO binding were extracted with spatial source maps similar to those in our previous study 1: one comprising caudate and putamen (IC1), and the other including D3-expressing regions such as pallidum, ventral striatum, and substantia nigra (IC2). IC1 was highly correlated with expected D2-related BP(ND) ($r = 0.94$, $p = 0.0018$) and IC2 with expected D3-related BP(ND) ($r = 0.97$, $p = 0.00035$). In

blocking scans, ICA-derived occupancy values closely matched respective model-based estimates across scans (intraclass correlation coefficient [ICC] = 0.98 for IC1 and D2; ICC = 0.95 for IC2 and D3). Fits to a single-site binding model were improved for ICA compared to model-based estimates of D3 occupancy (root mean square error = 4.2 and 6.1, respectively), and EC50 values were similar (for IC1 and D2, EC50 = 49 S.E. 78 μ g/mL and 48 S.E. 71 μ g/mL, respectively; for IC2 and D3, EC50 = 6.8 S.E. 2.0 μ g/mL and 5.6 S.E. 2.6 μ g/mL, respectively). Regional $f(D3)$ values from ICA and occupancy models showed good agreement with each other and with prior literature estimates: ICA-derived $f(D3)$ was 0.06 ± 0.04 in dorsal caudate (model-based, 0.07 ± 0.07), 0.2 ± 0.03 in ventral striatum (model-based, 0.2 ± 0.06), and 0.5 ± 0.04 in globus pallidus (model-based, 0.6 ± 0.09).

Conclusions: Spatiotemporal ICA with competition binding data successfully separated D2- and D3-related binding in the mixed [11C]-(+)-PHNO signal. These measures showed good agreement with existing methods for estimating D2 and D3 binding and provided more precise, less variable estimation of D3 receptor occupancy and $f(D3)$. Altogether, this work demonstrates a novel, data-driven method that allows specific quantification of D2 and D3-related binding without a priori assumptions about receptor distribution. These approaches can improve future research into dopamine dysfunction in psychiatric disorders and facilitate the development and evaluation of D3-targeting treatments. More broadly, these analyses demonstrate the utility of spatiotemporal ICA in analyzing PET data from a tracer with more than one binding site, suggesting new possibilities for precise, simultaneous quantification of multiple targets.

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ATH and PDW contributed equally to this work.

Keywords: Dopamine 3 Receptor Imaging, Dopamine (D2, D3) Receptors, PET Imaging, Independent Component Analysis, Receptor Occupancy

Disclosure: Nothing to disclose.

M219

Simultaneous Measurements of Brain Extracellular GABA and Glutamate In Vivo: Technology and Applications for Neuropsychiatric Disorders

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Background: γ -Aminobutyric acid (GABA) and glutamate (Glu) are the two major neurotransmitters in the central nervous system. Their balance is essential for proper functioning of the brain and imbalance in GABA/Glu systems is implicated in multiple disorders including schizophrenia, ADHD, substance use, depression, and epilepsy. The ability to simultaneously measure GABA and Glu in vivo on a second by second time scale has not previously been possible.

Methods: Ceramic-based microelectrode arrays (MEAs) were used to quantify γ -aminobutyric acid (GABA) by employing a dual-enzyme reaction scheme including GABase and glutamate oxidase (GluOx). Glutamate was simultaneously quantified on adjacent

recording sites coated with GluOx alone. Endogenous glutamate was subtracted from the combined GABA and glutamate signal to yield a pure GABA concentration.

Results: Electrode sensitivity to GABA in conventional, stirred in vitro calibrations at pH 7.4 did not match the in vivo sensitivity due to diffusional losses. Non-stirred calibrations in agarose or stirred calibrations at pH 8.6 were used to match the in vivo GABA sensitivity. In vivo data collected in the rat brain demonstrated feasibility of the GABA/glutamate MEA including uptake of locally applied GABA, KCl-evoked GABA release and modulation of endogenous GABA with vigabatrin.

Conclusions: The initial in vitro and in vivo studies support that the new MEA configuration is a viable platform for combined GABA and glutamate measures in the CNS extending the previous reports to in vivo GABA detection. Potential applications for understanding Neuropsychiatric disorders as well as caveats of the current technology will be discussed.

Keywords: Glutamate Homeostasis, GABA, Multi-Electrode Arrays

Disclosure: Nothing to disclose.

M220

An RCT of Smoking Cessation in African Americans: Self-Report and Objective Outcomes via Tobacco-Specific Nitrosamine Levels

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Background: Compared with Caucasian smokers, African Americans (AA) are disproportionately burdened by smoking related health problems, such as lung cancer, heart disease, and stroke, and in some low-income AA communities in Chicago, smoking rates are as high as 40-60%, i.e., 2-4 times the national rate. While most smokers want to quit smoking, self-efficacy for change is often low and many remain in the contemplation stage for years.

Methods: We conducted an RCT in 204 low-income AA smokers of a novel Enhanced Care (EC) condition consisting of brief single-session motivational, culturally-tailored feedback intervention with a one-week starter kit of nicotine replacement versus a Treatment As Usual (TAU) condition with distribution of self-help materials and pamphlets. Smoking behavior, use of nicotine replacement, and urinary levels of the tobacco specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were the main dependent variables examined at baseline and 1-month follow-up. NNAL levels were assayed by LC-MS/MS at NicoTAR® labs at the Roswell Park Cancer Center. Retention was high with 203 participants (99.5%) completing the 1-month follow-up. The average age was 53.8 years, 50% were female, and 76% were not working (i.e., unemployed, retired, or disabled). Smoking level at enrollment was 8.8 (range: 1-37) cigarettes per day with 96% preferring mentholated cigarettes.

Results: The EC (vs. TAU) intervention produced a significant reduction in participants' self-reported cigarettes smoked per day at 1-month (reduced smoking: 62% EC vs. 38% TAU; $X^2(1) = 22.27$ $p < .001$), greater likelihood of making a serious quit attempt (57% vs 34%, respectively; $X^2(1) = 10.02$ $p < .01$), and higher usage of nicotine replacement (patch or lozenge: 77% EC v. 15%; $X^2(1) = 49.47$ $p < .01$). However, levels of NNAL were similar between treatment conditions, with no reduction in EC vs. TAU (group $\times$ time; $\beta(\text{SE}) = 0.19(19.58)$, $p = .99$).

Conclusions: In sum, a brief motivational and culturally-tailored smoking feedback was effective in reducing non treatment-

seeking low-income AA smokers' self-reported smoking behaviors and use of approved pharmacotherapy. However, self-reported smoking reduction was not associated with a reduction in cancer-specific nitrosamine levels. This finding may be due to lack of accuracy in self-report, compensatory smoking behaviors, and/or longer intervals needed to show change in cancer-relevant objective measures. Continued research to reduce the health burden of tobacco use in underserved smokers is warranted.

Keywords: Smoking Cessation, African Americans, Tobacco-Specific Nitrosamine

Disclosure: Nothing to disclose.

M221

Differential Relationship Between Behavioral Approach and Inhibition Motivation Systems (BIS/BAS) and Intrinsic Brain Connectivity in Adult Cannabis Users and Non-Users

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Background: Motivation mediates goal-directed behavior (Koob, Everitt, & Robbins, 2013) and is an important component of the addiction process (Bell, 1995; Robinson & Berridge, 1993). Specifically, an imbalance between increased drug-oriented motivation and decreased inhibitory control is considered to contribute towards the development and maintenance of substance using disorders (Everitt & Robbins, 2005; Koob & Volkow, 2010). Dampened behavioral inhibition and overactive behavioral approach motivational systems (i.e., BIS/BAS) have been identified in cannabis use disorder (CUD; Silins et al., 2013), although the underlying neural mechanisms of these alterations have not yet been examined. In this study, given the findings of increased resting state functional connectivity (rsFC) in the brain's executive control network (ECN) (Krpotich et al., 2013) with increased approach motivation and a lateralization effect of BIS/BAS within the ECN (Schutter, Weijer, Meuwese, Morgan, & Honk, 2008), we will test the hypothesis that altered ECN rsFC compared to non-users underlies drug-oriented motivation and decreased inhibitory control in cannabis users.

Methods: To that end, we tested potential differences in the relationship between ECN rsFC and BIS/BAS in cannabis using ($N = 86$, mean age = 30.54, 42 males) and non-using adults ($N = 59$, mean age = 29.42, 31 males). To compare total BIS and the three BAS subscale scores between users and non-users we ran a MANOVA. We also modeled the relationship between the motivation systems as a BIS/BAS ratio calculated according to Schutter and colleagues [(Schutter et al., 2008): $\text{BIS/BAS} = (\text{BIS-BAS})/(\text{BIS} + \text{BAS})$]. Using this equation, positive ratios reflect an imbalance towards BIS, while negative values reflect an imbalance towards BAS. Independent component analysis (ICA) was performed in FSL's MELODIC (FMRIB's Software Library <http://fsl.fmrib.ox.ac.uk/fsl/>) to determine participants' rsFC. Then, left and right ECN masks (Shirer et al., 2012) were used to identify which group ICA components were spatially correlated to the a priori networks of interest. Further analysis was limited to the components identified as correlates. Next, a general linear model (GLM) was performed to compare the strength of the ECN, as determined by ICA, with BIS/BAS scores. Finally, a second GLM was constructed to detect significant variation between users and controls in the contrast parameter estimates (COPEs) of BIS/BAS scores within the ECN. Where significant correlations between BIS/BAS scores and ECN strength were compared between groups.

Results: There was a main effect of group such that cannabis users had increased BAS-fun-seeking scores and more negative

BIS/BAS ratios compared to non-using controls ($p < .01$ and $p < .05$, respectively). Two group ICA components each were spatially correlated to the left and right ECN. In each group, the rsFC results showed a lateralized relationship such that total BIS scores were negatively correlated with the right ECN rsFC in users, while total BIS scores were negatively correlated with the left ECN rsFC in controls ($p < .05$). Differential relationships between BAS subscale scores and ECN rsFC were also found where COPE values of BAS-reward responsiveness were increased for right ECN rsFC in users (vs. controls; $p < .05$), while COPE values of BAS-fun-seeking in the right ECN rsFC were increased in controls (vs. users; $p = .05$). The BIS/BAS ratio was not related to ECN rsFC in either group.

Conclusions: In cannabis users compared to non-users, correlation of the BIS scores with right ECN rsFC combined with increased BAS-fun-seeking scores and a more negative BIS/BAS ratio may be mechanisms driving decreased inhibitory control in cannabis users. In addition, cannabis users' increased COPE values of BAS-reward responsiveness in the right ECN rsFC compared to non-users suggests right ECN functionality is influenced by their level of reward responsivity. Therefore, cannabis users who are conditioned to expect and crave drug-related stimuli (as evidenced by increased BAS-reward responsiveness) should also have increased right ECN rsFC. Cannabis users' BIS/BAS ratios were more negative compared to controls suggesting an imbalance towards BAS. However, due to the inconsistency with our findings between the participants' BIS/BAS ratios and ECN rsFC compared to others in the literature (Schutter et al., 2008; Rutherford & Lindell, 2011), further examination is needed to investigate other possible neural correlates of the BIS/BAS ratio. This differential connectivity between cannabis users and non-users could indicate that ECN rsFC is a contributing factor to CUD symptomatology, as indicated in its correlation to BIS and BAS scores in this study. As such, these results suggest that a relationship between brain network connectivity and motivation systems influences cannabis use behavior.

Keywords: Cannabis Use Disorder, Motivation, Behavioral Inhibition, Behavioral Approach, Resting State Functional Connectivity

Disclosure: Nothing to disclose.

M222

Altered Hypothalamic Response to Intrinsic Motivation in Cocaine Dependence

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Background: Individuals with cocaine dependence are characterized by motivation deficits and under-responsiveness to natural reinforcers. Preclinical studies showed that repeated administration of cocaine altered dopaminergic signaling and motivational behaviors. As part of the limbic system, the hypothalamus receives extensive dopaminergic projections from the midbrain and is implicated in a wide range of motivated behaviors. Many studies have described hypothalamic response to arousal, food intake, sexual drive, extrinsic reward and affective responses. However, it remains unclear how hypothalamic dysfunction contributes to motivation deficits in addiction.

A critical component of motivation lies with the internal drive to engage in actions without apparent reward. Intrinsic motivation refers to the spontaneous tendency to seek out challenges and to explore. A recent work examined the neural correlates of intrinsic motivation using a stopwatch task (SWT) in which participants were to stop the watch within a specified time interval. By having

participants choose (self-determined condition or SD) or be assigned (forced condition or FC) a SW in alternative trials, authors examined how motivation influences cerebral activations while keeping task difficulty and performance outcomes identical. The hypothalamus showed increased activation during self- as compared to forced-choices. In the current study, we examined the hypothalamic response to intrinsic motivation using SWT.

Methods: We examined regional responses in 15 cocaine dependents (CD) and 15 healthy controls (HC) matched in demographics and body mass index (BMI) in SWT during fMRI. In SWT, participants pressed button to stop the time within a 3 s time point. Two types of trials were presented. In self-choice (SC) trials, participants were prompted to choose one of the two stopwatches (with varying pairs) to "play" with. In forced-choice (FC) trials, one of the stopwatches was pre-selected for the participants. Following response, participants' total score was displayed in the upper right corner, with one point added and score panel flashed if won and no score change if lost. No monetary reward was associated with the total score. Participants performed four 10-m runs, yielding a total of 56 SC and 56 FC trials. We examined differences in BOLD responses to SC vs. FC trials to identify the neural correlates of intrinsic motivation using SPM 12. In region of interest (ROI) analysis, we used MarsBaR (<http://marsbar.sourceforge.net/>) to derive for each individual subject the activity difference (β contrast) for the ROIs.

Results: In behavior, ANOVA of accuracy rate (CD: 36% for SC and 38% for FC; HC: 47% for SC and 43% for FC) showed a significant interaction effect ($p = 0.027$), but not a group main ($p = 0.11$) or condition main ($p = 0.67$) effect. In post-hoc analyses, HC showed higher accuracy rate during SC ($p = 0.038$) but not FC ($p = 0.35$) as compared to CD. In whole brain voxel-wise analysis, CD showed diminished activation in the hypothalamus ($x = 5, y = -6, z = -12, 135 \text{ mm}^3, Z = 3.34$) compared to HC in SC vs. FC at voxel $p < 0.001$ and cluster-level $p < 0.05$ FWE corrected for the hypothalamus mask, controlling for age, sex, BMI, years of drinking and years of smoking. Further, the hypothalamic activation was negatively correlated with tonic craving, as assessed by the Cocaine Craving Questionnaire (CCQ) score ($r = -0.67, p = 0.0064$) and days of cocaine use in the past month ($r = -0.66, p = 0.0071$) in CD. With age, sex, BMI, years of drinking and years of smoking as covariates, hypothalamic activation remained negatively correlated with CCQ score ($r = -0.83, p = 0.0027$).

Conclusions: We demonstrated for the first time diminished hypothalamic activation during intrinsic motivation in CD as compared to HC. This diminished hypothalamic response was associated with daily cocaine craving and days of cocaine use in the past month. These findings support intrinsic motivation deficits as well as hypothalamic under-activation during intrinsic motivation challenges in cocaine addiction.

Keywords: Hypothalamus, Cocaine Addiction, Intrinsic Motivation, Functional MRI (fMRI), Craving

Disclosure: Nothing to disclose.

M223

Acute Methamphetamine Increases Striatal Response to Reward in Healthy Humans

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Background: Anticipation and receipt of rewarding outcomes are aspects of the decision-making process that are often disrupted in patients with substance use disorders. Prior studies with healthy

volunteers indicate that both anticipation and receipt of rewarding outcomes increase activation in areas of the ventral striatum and prefrontal cortex. However, only a few studies have examined the effect of stimulant drugs on striatal functioning during these processes.

Methods: Healthy young adults ($N = 20$) completed two fMRI scanning sessions during which they received methamphetamine (20 mg) or placebo, in counterbalanced order, one hour before their scan. While in the scanner participants completed a modified version of the Monetary Incentive Delay task, where they responded to a target to earn rewards and avoid losses.

Results: ROI analyses of the striatum parcellated into functional subregions (see Tziortzi et al., 2014 Cereb Cortex) indicated that relative to placebo, methamphetamine increased striatum response to positive feedback (i.e., reward earned/loss avoided). This was the case for all frontally-connected subregions in the dorsal and ventral striatum, including: limbic ($t(19) = 2.52$, $p = .021$), executive ($t(19) = 3.48$, $p = .002$), rostral motor ($t(19) = 2.65$, $p = .016$), and caudal motor ($t(19) = 3.07$, $p = .006$). Methamphetamine did not alter striatal activation during anticipation of reward trials, but did increase activation in the limbic subregion during anticipation of potential loss trials ($t(19) = .243$, $p = .025$).

Conclusions: These results help further our understanding of how methamphetamine affects brain function in healthy humans during the anticipation and receipt of monetary rewards. Importantly, our findings implicate subregions of both the dorsal and ventral striatum, particularly in response to rewarding outcomes.

Keywords: Human Neuroimaging, Methamphetamine, Monetary Incentive Delay Task

Disclosure: Nothing to disclose.

M224

fMRI Response and Memory Dysfunction in Emerging Adult Marijuana Users: Evidence of Sex Differences

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Background: Marijuana (MJ) use rates are highest among emerging adults ages 18-22. Given ongoing neurodevelopment during this age range, emerging adults may be especially vulnerable to MJ-related cognitive dysfunction. Memory decrements are one of the most consistently observed cognitive deficits associated with MJ use, yet the neural substrates of MJ-related memory dysfunction are poorly understood. Of critical importance, some research has suggested sex differences in the influence of MJ on memory function. For instance, our previous work found preliminary evidence for sex differences in functional magnetic resonance imaging (fMRI) response during a nonverbal item recognition task among relatively light MJ users, with male MJ users showing reduced hippocampal and inferior frontal gyrus (IFG) response, but female users showing no differences. These findings need to be replicated in larger samples of heavier users. To this end, we ascertained fMRI response during an object recognition task among male and female emerging adult heavy MJ users and controls. We predicted that male MJ users would show reduced hippocampal and IFG response compared to control males during item recognition, whereas female MJ users would show no differences compared to female controls.

Methods: Participants were 17 (7 females) current heavy MJ users and 16 (8 females) controls, ages 18-22. All participants were free from psychiatric diagnoses (with the exception of cannabis use disorder in MJ users). MJ use was similar between male and female MJ users, with males using 24 days per month (range 12-30) and females using 22 days per month (range 8-30). Male and female MJ users were also matched on alcohol use. During fMRI, participants performed the Relational and Item-Specific Encoding (RISE) task (Ragland et al., 2012), which ascertains both item- and relational-encoding and recognition of object pairs. BOLD response contrast was examined for item recognition. Region of interest analyses obtained fMRI response in right and left hippocampus, and right and left IFG. These regions were chosen because they have been consistently implicated in item recognition on this task in healthy volunteers, as well as in our prior work in MJ users on a similar task. ANOVA analyzed the effects of group, sex, and their interaction for each region.

Results: There were no effects of group, sex, or their interaction on behavioral performance on the task. There was a group x sex effect on fMRI response in right hippocampus ($F=5.0$, $p=.033$), right IFG ($F=4.9$, $p=.035$), and left IFG ($F=5.1$, $p=.031$), and there was a trend for a group x sex effect in left hippocampus ($F=3.5$, $p=.072$). In each region, male MJ users showed reduced fMRI response compared to male controls, while females showed no difference between MJ users and controls.

Conclusions: Consistent with our hypothesis, among emerging adults, male heavy MJ users showed reduced hippocampal and prefrontal fMRI response during item recognition compared to male controls, but female MJ users did not show differences. These results parallel our earlier preliminary findings using a different nonverbal item recognition task in more moderate MJ users. It is possible that these sex differences are subserved, in part, by interactions between MJ and varying hormone levels in females. Future research in larger samples should attempt to elucidate the underlying mechanism of these sex differences.

Funded by NIDA (DA038207, Dager).

Keywords: Cannabis, Fmri, Sex, Marijuana, Memory

Disclosure: Nothing to disclose.

M225

Alterations in the Endocannabinoid Signaling Complex After Incubation of Cocaine Craving

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Background: Relapse to cocaine abuse is often induced by exposure to drug-associated cues. Cue-induced cravings are known to escalate over the period of withdrawal, a phenomenon termed the incubation of drug craving. Expression of incubated cocaine craving is ultimately mediated by the synaptic strengthening of excitatory inputs onto medium spiny neurons (MSN) in the nucleus accumbens (NAc) core. Specifically, during withdrawal, post-synaptic accumulation of high conductance Ca^{2+} -permeable AMPA receptors (CP-AMPA) enhances MSN reactivity to glutamatergic inputs and drug-associated cues to drive the expression of incubation. Additionally, we have shown that the regulation of glutamate release may be impaired after prolonged withdrawal, as mGlu5- and CB1R-mediated synaptic depression induced by DHPG is abolished. Our previous studies suggest that this impairment stems from alterations in the post-synaptic

endocannabinoid signaling complex referred to as the 2-AG signalosome. Here, we investigated mechanisms underlying this impairment.

Methods: All procedures were approved by the Rosalind Franklin University of Medicine and Science and the Oregon Health & Science University IACUCs in accordance with the US Public Health Service Guide for Care and Use of Laboratory Animals. Rats self-administered cocaine for 10 days and subsequently underwent forced abstinence in home cages until tests of operant responding or use in biochemical assays. Bilateral NAC core was dissected for co-immunoprecipitation (co-IP) studies, while NAC core with some shell were used for synaptoneurosomes and DGL activity assays. Co-IP studies involved immunoprecipitation of DGL- α followed by SDS-PAGE and detection of mGlu5 and Homer2 associations with immunoblotting. Synaptoneurosomes were prepared by sequentially passing homogenates through 100 μ m and 5 μ m filters and analyzed by SDS-PAGE and immunoblotting. DGL activity was assayed in homogenized NAC tissue with a synthetic DGL substrate (MRJ20) fitted with a fluorescence resonance energy transfer (FRET) pair on the N and C terminals to measure activity in a FRET plate assay.

Results: While the components of the 2-AG signalosome were expressed at normal levels after incubation of cocaine craving, co-IP studies revealed an increase in the association between total and phosphorylated Ca2+ /calmodulin-dependent protein kinase II (CaMKII) and diacylglycerol lipase- α (DGL), an association that, based on earlier studies, would predict the inhibition of DGL activity and therefore, impaired production of 2-AG. Loss of DGL activity after incubation of craving was confirmed through assay of DGL activity, which also revealed a strong negative correlation of DGL activity with the magnitude of incubation.

Conclusions: These studies clarify the nature of impaired mGlu5-mediated synaptic depression during cocaine withdrawal and establish a role for CaMKII in the synaptic alterations associated with incubation of cocaine craving. Future studies will further assess the role of CaMKII in the loss of mGlu5-mediated synaptic depression after incubation of cocaine craving.

Support: DA009621 (MW)

Keywords: Incubation of Cocaine Craving, Endocannabinoid System, CaMKII

Disclosure: Nothing to disclose.

M226

The Acute Effects of Inhaled Salvinorin A on Resting State Functional Connectivity in Humans

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Background: Salvinorin A is a potent κ -opioid receptor agonist and the main psychoactive constituent of *Salvia divinorum*, an atypical dissociative hallucinogen that is used recreationally and remains unscheduled in many countries. Inhaled SA leads to a rapid onset and short duration of subjective effects that include a sense of depersonalization and derealization. Additionally, some evidence suggests a rapid antidepressant effect of SA similar to that of ketamine and psilocybin, drugs with noteworthy effects on default mode network (DMN) connectivity. Here, we conducted the first functional magnetic resonance imaging study with acute administration of inhaled salvinorin A to explore its effects on resting state functional connectivity in humans.

Methods: In a single-blind, placebo-controlled design, 12 healthy male participants inhaled placebo (hot air) or vaporized SA (15 μ g/kg) at the beginning of two separate 20-minute resting state scans. Volunteers listened to ambient music and wore eyeshades during each scan. We used a validated brain atlas to look at drug effects on whole-brain connectivity and specific resting state networks.

Results: Across the whole brain, SA (compared to placebo) decreased the number of significant static functional connections. This reduction in static connectivity was especially robust within the DMN during the first half of the SA scan ($d = .95$), and persisting attenuation of the DMN during the second half of the SA scan correlated with the duration of subjective drug strength ($r = .59$). SA was also found to decrease static connectivity within the frontoparietal network ($p = .037$) and a subcortical network that includes the salience network ($p = .020$). An increase in functional connectivity was found between medial and lateral visual networks during SA scans ($p = .002$), perhaps reflecting changes in visual processing. Analyses on functional connectivity dynamics, specifically variance and entropy, revealed that SA reduced variability but increased entropy across the brain, including within and between most canonical networks. However, these effects on connectivity dynamics were small, and using a leave one subject out classification procedure, we found that static connectivity alone best predicted whether a scan was collected during placebo or SA (75% accuracy), with variability and entropy adding little and sometimes even reducing classification accuracy.

Conclusions: These findings suggest that some neural effects of SA resemble those of other hallucinogens, whereas other neural effects are unique to the altered state produced by SA.

Keywords: Functional MRI (fMRI), Salvinorin A, Kappa Agonist, Psychedelics, Hallucinogens

Disclosure: Nothing to disclose.

M227

Sex Differences in the Generalization of an Interoceptive State Elicited by a Morphine Occasion Setter

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Background: Research on substance use and misuse typically revolves around the drug as a reinforcer, but drugs of abuse also elicit internal stimulus effects that can be learned as guides of behaviour. Whereas the bulk of that work has used operant two-lever drug discrimination tasks, the Pavlovian associations that are so central to triggering motivated behaviours associated with substance abuse remain understudied. Occasion setters disambiguate associations between conditioned stimuli (CSs) and unconditioned stimuli (USs) and can activate or inhibit US seeking in response to a CS presentation, depending on the associative structure in place. Several drugs with varying levels of abuse potential have been established as effective feature negative (FN) and/or feature positive (FP) occasion setters in male rats, including nicotine, cocaine, methamphetamine, chlordiazepoxide, and caffeine. Given the massive, and growing, public health concern surrounding opioids, we've begun this line of research using morphine as a positive feature that indicates a CS—US relation is active and morphine as a negative feature that indicates a CS—US relation is inactive in both male and female rats to assess whether the specificity of the drug stimulus to guide behaviour is similar for both sexes.

Methods: Twenty male and twenty female Sprague-Dawley rats, weighing ~250 and 200g, respectively, upon arrival from Charles River, were randomly assigned to FP or FN training ($n = 10/\text{group}$) with morphine as the occasion setter. All rats were injected intraperitoneally with either morphine (3.2 mg/kg; 1 ml/kg) or saline 15-min before 20-min daily training sessions in standard operant conditioning chambers. During these sessions, there were eight 15-sec white noise (WN) presentations (80 dB). For FP rats, on morphine sessions, each WN offset was followed by 4-sec access to sucrose (0.01 ml; 26% w/v); on saline sessions, the WN was still presented, but sucrose was withheld. For FN rats, sucrose was delivered on saline sessions and withheld on morphine sessions. Saline and morphine sessions were intermixed such that no more than two of a session type occurred in a row. The measure of conditioning was the first dipper entry difference score: the number of anticipatory dipper entries during the 15-sec WN CS minus the number of dipper entries during the 15-sec pre-CS interval. Only the first WN presentation of each session was used as the measure of learning because the first sucrose delivery (or lack of sucrose delivery) of any given session provides insight into how all subsequent CS presentations in that session would be followed. After 32 total training sessions (16 morphine; 16 saline), all rats entered stimulus generalization testing. For this phase, each rat was assessed in one standard morphine and one saline session. If its dipper entry difference score was at least 4 higher on its CS-active session than its CS-inactive session, then it would go on to test the next day. Test sessions were 4 min and contained only one WN presentation and no sucrose delivery. Each rat was challenged with each of a series of morphine doses in a latin-square order (0.0, 0.5, 1.0, 2.1, 3.2, 5.4 mg/kg). Due to the observed response patterns as rats worked their way through their testing cycles, higher doses of 6.5 and 7.5 mg/kg were added to the female testing orders midway through the testing cycle.

Results: Male and female rats trained with morphine as FP and FN occasion setters all learned the Pavlovian drug discrimination. Regardless of sex, morphine FP-assigned rats learned the discrimination more quickly than FN-assigned rats, and FN-assigned rats were less stable in their discrimination than FP rats. There was no difference in drug generalization between male and female rats trained with morphine as a FN occasion setter; ED50 drug values were 1.26 for males and 1.57 for females. However, for rats trained with morphine as a FP occasion setter, the dose response curve for females was far shifted to the right compared to that of males; the ED50 values were 0.54 for males and 1.94 for females.

Conclusions: The slower acquisition for FN rats likely reflects the added cognitive pressure of learning about an inhibitory stimulus regardless of sex. Sex differences did become a factor with the morphine generalization, with females generally shifted to the right compared to males and that effect much stronger for FP training than FN training. Female rats assigned to FP training did not show the standard drift downward from their 3.2 mg/kg training drug that the male rats showed, but rather increased their sucrose-seeking in response to the WN CS at the 5.4 mg/kg test dose, suggesting the drug was perceived as more "training-drug-like" than the training drug itself. This finding cannot be explained by tolerance to the training drug, as intervening training sessions required a standard baseline discrimination to qualify to test. This finding also exemplifies the need to reassess our notions of drug stimuli that guide appropriate associative behaviours from the perspective of sex differences. Such differences may have profound effects on acquired motivational value of an appetitive drug occasion setter.

Keywords: Morphine, Drug Discrimination, Sex Differences

Disclosure: Nothing to disclose.

M228

Adolescent Stress Results in Sex-Specific Reprogramming of the Reward Circuitry Transcriptome in Adulthood

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Background: Adolescence is a time of heightened sensitivity to rewarding stimuli and is associated with vulnerability to psychiatric disorders. Male rodents that experience adolescent social isolation stress (SI) form stronger preferences for drugs of abuse in adulthood. However, little is known about how females respond to SI. Our findings suggest that SI reverses sex differences in adult reward-associated behaviors and permanently reduces baseline sex differences in anxiety-related behaviors. Given these behavioral alterations, we tested the hypothesis that SI alters the transcriptome in a persistent and sex-specific manner in the nucleus accumbens (NAc), ventral tegmental area (VTA), and prefrontal cortex (PFC).

Methods: Male and female mice were isolated or group housed (GH) from P22 - P42, then GH until ~P90. Transcriptome-wide changes in NAc, VTA, and PFC were investigated by RNA-seq after acute or chronic cocaine or saline administration.

Results: SI reduces sexually dimorphic gene expression across all three brain regions. Further analysis revealed that SI results in expression profiles in males that more closely resemble GH females, suggesting that SI "feminizes" the male transcriptome. Importantly, when SI females are exposed to the first dose of cocaine, their transcriptional profiles resembled GH males in the NAc and PFC but not VTA, suggesting that SI "masculinizes" the female transcriptional response to acute cocaine in the former brain regions. This effect is lost after chronic exposure to cocaine in the PFC.

Conclusions: Together, these data suggest that SI has region-specific effects on sex-specific transcriptional responses to cocaine. Currently, we are utilizing gene co-expression network analysis to identify reward-circuitry conserved key drivers of the sex-specific transcriptional responses to cocaine which are reversed by SI. We predict that these key driver genes will have powerful sex-specific therapeutic potential given their conservation across multiple brain regions. Together, these data show that SI disrupts sex-specific adolescent development of transcription throughout the reward circuitry and reprograms an individual's responses to acute or chronic cocaine.

Keywords: Gene Expression, Cocaine, Sex Differences, Stress in Adolescence

Disclosure: Nothing to disclose.

M229

Cellular and Behavioral Consequences of Acute Exposure to Electronically Vaporized Nicotine in Adult Male C57Bl6j Mice

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Background: The use of electronically vaporized nicotine

('vaping') is increasing in prevalence and popularity, particularly among younger populations. However, the effect of vaping on neuronal function and/or central neuroadaptations underlying behaviors associated with nicotine use remains unclear. Our objectives are to determine the effect of electronic nicotine vapor exposure on neuronal populations implicated in the reinforcing properties of nicotine and on behaviors associated with nicotine exposure.

Methods: We exposed male C57Bl6j mice to electronic nicotine vapor [12% nicotine in 30:70 polyethylene glycol I(PG)/vegetable glycerol (VG)] or PG/VG vapor alone for a three-hour session (3 sec vapes, 10 minute intervals between vapes). Immediately following vapor exposure, we performed assessments of thermoregulation and locomotion and in separate cohorts collected brains for electrophysiological recordings of central amygdala (CeA) and ventral tegmental area (VTA) neurons.

Results: Immediately following a single three-hour session of intermittent nicotine vapor inhalation, mice exposed to electronically vaporized nicotine displayed significantly higher serum nicotine and cotinine levels as compared to PG/VG controls. Mice exposed to electronically vaporized nicotine also displayed a significant reduction in core body temperature and significant reductions in distance traveled in an open field as compared to PG/VG controls. Acute exposure to electronically vaporized nicotine did not significantly alter the passive membrane properties or baseline inhibitory transmission in CeA or VTA neurons, however CeA neurons from mice exposed to electronically vaporized nicotine did display significantly higher baseline firing rates as compared to PG/VG controls.

Conclusions: Acute exposure to electronically vaporized nicotine produces short term deficits in thermoregulation and locomotion. In addition, acute nicotine significantly increases the baseline excitability of CeA neurons, which may contribute to the reinforcing effects of nicotine vapor exposure.

Keywords: Nicotine, Vaping, Amygdala

Disclosure: Nothing to disclose.

M230

Inhibition of the Prelimbic to Nucleus Accumbens Core Pathway Decreases Methamphetamine Cued Reinstatement

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Background: Methamphetamine (meth) causes enduring changes within the medial prefrontal cortex (mPFC) to nucleus accumbens (NAc) circuitry. Projections from the mPFC to the NAc have a distinct dorsal-ventral distribution; neurons originating in the prelimbic (PL) mPFC project to the NAc core, whereas, those originating in the infralimbic (IL) mPFC project to the NAc shell. Inhibition of these parallel pathways has opposing effects on cocaine relapse. Inhibition of PL-NAc core reduces cued reinstatement of cocaine seeking following extinction and IL-NAc shell inhibition reinstates previously extinguished cocaine seeking. Meth, however, exhibits a different profile, as pharmacological inhibition of both the PL and IL decrease cued reinstatement of meth-seeking. Given these contrasting findings, the opposing roles of the PL-NAc core and IL-NAc shell found with cocaine remains unclear in regard to meth seeking.

Methods: To begin to address this issue, we used a combinational viral approach that employs a retrograde traveling AAV-Cre (AAVrg-Cre) injected in the terminal area and a Cre-dependent

AAV inhibitory DREADD (DIO-hM4Di) in the cell body locus. Specifically, one group of rats received AAVrg-Cre in the NAc core and DIO-hM4Di in the PL and another had AAVrg-Cre in the NAc shell and DIO-hM4Di in the IL. Male and female rats self-administered meth (2 hr daily) then went through extinction and reinstatement testing. Tests were conducted with ip injections of the following: 1) DMSO (veh control), 2) 3 mg/kg CNO, 3) 10 mg/kg CNO, or 4) 0.1 mg/kg clozapine.

Results: All rats robustly reinstated in the presence of meth cues following vehicle and this was significantly decreased by inhibition of the PL-NAc core circuit (10 mg/kg CNO) only. Reinstatement of meth seeking was not interrupted by 3 mg/kg CNO or 0.1 mg/kg clozapine. Inhibition of the IL-NAc shell circuit had no effect on cued meth seeking. A follow up study of the positive findings showed that a control AAV, DIO-mCherry, had no effects on lever responding.

Conclusions: Combined, these studies show that inhibition of the PL-NAc core circuit can inhibit reinstated meth seeking in a manner similar to cocaine. The next study will determine if activation of the IL-NAc shell circuit can reduce reinstatement of meth seeking.

Keywords: Addiction, Dual Viral Approach, Relapse, Methamphetamine, Relapse Circuits

Disclosure: Nothing to disclose.

M231

Intravenous Self-Administration of Δ -9-Tetrahydrocannabinol by Adolescent Rats Produces Opposing Sex-Specific Effects on Working Memory in Adulthood

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Background: Adolescence is associated with ongoing brain development and often coincides with initiation of drug use. Indeed, cannabis is one of the most commonly used drugs in adolescents, and exposure to Δ -9-tetrahydrocannabinol (THC), the primary psychoactive component of marijuana may produce intracellular and synaptic perturbations that disrupt the process of brain maturation and persist into adulthood. For example, the prefrontal cortex (PFC) is integral to cognitive functions such as the representation of working memory (WM), and this region undergoes robust developmental changes during adolescence. Human, primate, and rodent studies suggest that chronic adolescent exposure to high levels of cannabinoids like THC can impact PFC function to produce WM deficits. However, clinical studies are not able to control for a number of associated variables, and preclinical studies have often only identified deficits at high experimenter-administered doses that likely produce aversive rather than rewarding effects. Thus, we endeavored to develop a paradigm to achieve self-administration of high doses of THC in adolescents, and determine if this pattern of adolescent exposure may dose-dependently affect WM performance in adulthood. We hypothesized that chronic exposure to THC at voluntarily self-administered doses would result in enhanced drug-seeking behaviors and altered performance on a delay-match-to-sample working memory task.

Methods: Male and female Sprague-Dawley rats were implanted with indwelling catheters on PND 25. Rats then began operant self-administration of escalating doses of THC, reaching a final dose of 30 (moderate dose) or 100 μ g/kg/infusion (high dose) over 20 days (PND 32-51, n = 10-12 per group). A subset of animals were assessed for analgesia using a

tail-flick test immediately following a self-administration test to confirm exposure to physiologically relevant doses of THC. Next, lever pressing behavior was extinguished during 9 days of lever extinction (PND 52-60). Rats were then tested for cue-induced reinstatement and incubation of THC-seeking after 10 and 30 days of abstinence. Concurrently during abstinence, rats were trained and tested on an operant-based delay-match-to-sample working memory task. During this task, rats learned to nose poke into one of 5 illuminated sample ports to receive a sucrose pellet reward. After learning to respond into a specific sample port, rats were trained to perform the task when a variable delay period (0-24s) elapsed before the originally sampled port and 2 adjacent ports were illuminated. The rat then had to choose the originally sampled port to receive a reward. Accuracy on this task was analyzed at increasingly difficult delays to compare the effects of THC exposure on working memory performance. Group differences were assessed using two-way repeated measures ANOVA.

Results: Using this escalating dose procedure, we found that both male and female adolescent rats self-administered moderate and high doses of THC. No difference between males and females emerged for lever presses, infusions, or average mg/kg THC taken ($p > 0.05$ for all analyses). Comparisons of tail-flick latency before and after THC self-administration indicate an increase in analgesia regardless of sex. After completing lever extinction, animals in both the 30 $\mu\text{g/kg}$ and 100 $\mu\text{g/kg}$ group demonstrated reinstatement and incubation of THC-seeking during abstinence with females showing a slightly stronger incubation effect on abstinence day 30. Finally, adolescent self-administration of the moderate dose of THC produced no effect on adult working memory, while high-dose self-administration led to improved performance in males and impaired performance in females.

Conclusions: Intravenous THC self-administration in adolescent rats produced measurable, dose-dependent effects on addiction-associated behaviors and working memory performance. Ongoing studies continue to investigate putative sex and brain region specific differences in neuronal activity and protein expression that may mediate these effects.

Keywords: Adolescent, Working Memory, THC, Drug Self-Administration

Disclosure: Nothing to disclose.

M232

Ketamine Enantiomers Differentially Interact With Opioid Receptors to Modulate Opioid-Dependent Behavior and Opioid Abuse Liability

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Background: The mechanism of action of ketamine is generally attributed to noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonism. However, classic NMDAR antagonists do not recapitulate the full behavioral and pharmacological profile of ketamine, which is also known to bind to other targets in the CNS. 'Ketamine' is a racemic mixture of the R- and S- enantiomers. S-ketamine was recently approved by the FDA for treatment of depression. Interestingly, R-ketamine exhibits ~4 times reduced affinity for NMDAR compared to S-ketamine but is more potent in preclinical antidepressant models. Furthermore, the ketamine

metabolite (2R,6R)-hydroxynorketamine (HNK) lacks appreciable binding to NMDAR yet maintains a preclinical antidepressant profile. Several clinical and preclinical studies have reported interactions between ketamine and the opioid system. Opioid antagonists can block the positive effects of low doses of ketamine and higher doses of ketamine produce dissociative effects similar to those caused by kappa-opioid receptor (KOR) agonists. The objective of this study was to characterize the precise pharmacological action of the different ketamine enantiomers and HNK on opioid receptors and to examine whether any such interactions were relevant to ketamine's abuse liability and/or its potential for modulating opioid-dependent behaviors.

Methods: R-ketamine, S-ketamine and HNK and dizolcipine (MK-801) were screened for their ability to bind to a wide variety of CNS targets. Radioligand binding competition assays were performed in rat brain membranes to evaluate the affinities of the compounds for mu-opioid (MOR), KOR and other CNS targets related to drug abuse liability. Opioid receptors transfected in HEK-293 cells and BRET reporter systems were used to evaluate the potency of the different compounds for the activation of MOR and KOR. S- and R-ketamine self-administration experiments were undertaken in opiate-naïve rats or in rats with a prior history of heroin self-administration. Finally, acute systemic effects of S- and R-ketamine were evaluated in rats undergoing heroin self-administration or deprived of heroin after several days of sensitization.

Results: R-ketamine, S-ketamine and HNK did not bind to any CNS targets with high affinity. R-ketamine and S-ketamine, but not HNK, bound to MOR and KOR with low affinity with S-ketamine being the most potent of the two enantiomers. Surprisingly, the archetypical NMDAR allosteric antagonist MK-801, with structural similarities to ketamine, was also able to bind opioid receptors with affinities similar to S-ketamine. Both R- and S-ketamine were partial agonists at MOR while MK-801 was a full agonist. S-ketamine and MK-801 were full agonists at KOR while R-ketamine was only a partial agonist. None of the drugs were able to activate DOR and remarkably HNK was unable to activate either opioid receptor. Neither S-ketamine, R-ketamine nor HNK acutely affected the responses of rats self-administering food or heroin, which was expected given the difference in potencies between drugs. However, when heroin was substituted for S-ketamine, rats maintained and escalated their intake of S-ketamine over consecutive exposure days. In contrast, when substituted for R-ketamine, rats maintained a low but constant responding rate during several sessions. On the other hand, when heroin was substituted for HNK rats quickly extinguished their lever-pressing responses.

Conclusions: Here we elucidated the pharmacology of ketamine's variants and metabolite on the opioid system. The potency of the drugs for MOR and KOR correlated with their abuse liability potential in an animal model of opiate abuse. The above data suggests that S-ketamine has profound abuse potential and should be used cautiously in opioid-dependent populations. On the other hand, R-ketamine could be a promising candidate for treatment of opioid abuse disorders.

Supported by the NIDA, NIMH, and NINDS-IRPs.

Keywords: Opioid Addiction, Ketamine, Drug Abuse, Substance Use Disorders, Depression

Disclosure: Nothing to disclose.

M233

Investigation of the Reinforcing and Discriminative Properties of Plant-Derived, Highly Purified Cannabidiol in Rats and Monkeys

Abstract not included.

M234

Rapid and Sensitive Detection of Endogenous Opioid Peptides

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Background: Endogenous opioid peptides are critical for analgesia, reward processing, and negative affect, however, research on their function has been challenging due to an inability to detect dynamic *in vivo* release. To begin to address this we have developed two complementary methods to allow both rapid and sensitive detection of opioid peptides. We are further increasing the sensitivity and reproducibility of our microdialysis/nano-liquid chromatography-mass spectrometry (nLC-MS) approach to allow for the quantification of opioid peptide release during behaviors such as drug withdrawal. To allow for increased spatiotemporal resolution we are developing an electrochemical approach using microimmuno-electrodes (MIEs). This approach enables detection of opioid peptides changes over a number of seconds both in brain slices and *in vivo*.

Methods: nLC/MS method development: We used a Q-Exactive LC-MS to optimize the utility of charged standards for dynorphin 1-8, Leu-Enkephalin and Met-Enkephalin by comparing the ratio of these standards to light peptides (synthetic version of endogenous peptides). We were able to stabilize methionine from further oxidation during analysis by modifying it to its sulfone form. We developed a solid phase extraction process to allow improved reproducibility and sensitivity without compromising the limits of detection. We are now beginning to pilot *in vivo* studies measuring changes in opioid peptides following fentanyl withdrawal from mice implanted with a min pump for two weeks, following by naloxone-precipitated withdrawal.

MIEs: Opioid peptides contain an electroactive tyrosine residue, which we oxidize using square-wave voltammetry to measure their presence. To do this we custom-make carbon fiber based electrodes, which are coated with antibody selective to the opioid peptide of interest. To confirm specificity, oxidative current is also measured from tyrosine and other opioid peptides.

Results: Our limits of detection are now in the subfmol range for dynorphin 1-8, Leu-Enk and Met-Enk. We hope to make the charged standards we have used commercially available to the scientific community. We have now chemically stabilized methionine to prevent further oxidation during detection. The inclusion of solid phase extraction process allows for improved reproducibility without compromising detection of all three opioid peptides. We show that dynorphin MIEs are sensitive to increasing concentrations of dynorphin and are optimal at detecting low concentrations of dynorphin. We are currently minimizing non-selective signals to ensure we are able to distinguish each of our three opioid peptides.

Conclusions: Here we show the development and optimization of two methods to detect endogenous opioid peptides at a subfmol range with spatiotemporal resolution. We plan to expand this to not only include other opioid peptides but also neuropeptides in general. This will give much needed insight into role and/or changes in endogenous neuropeptides that occur in neuropsychiatric diseases such as addiction.

Keywords: Endogenous Opioids, Dynorphin, Liquid Chromatography/Mass Spectrometry, Electrochemistry

Disclosure: Nothing to disclose.

M235

Increased Neuroglial Coupling in the PFC is Associated With Cocaine-Induced Cognitive Deficits

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Background: The prefrontal cortex (PFC) is crucial for maintaining goal-directed behavioral strategies. Repeated cocaine use induces maladaptive neuroadaptations in the PFC that have been associated with deficient decision-making. Synaptic glutamate signaling at PFC neurons has been a focus of many studies. However, the influence of astrocytic glutamate on cocaine-induced neuroadaptations is not clear. We hypothesized that cocaine experience will trigger increased PFC neuron sensitivity to astrocyte-derived glutamate due, at least in part, to increased activation of neuronal extrasynaptic NMDA receptors. We further hypothesized that reduced neuroglial coupling may ameliorate cocaine-induced cognitive deficits.

Methods: Rats underwent training to self-administer cocaine for 10 days on a long access (6hrs/day) fixed ratio schedule of reinforcement and training on an operant cognitive flexibility task. Twenty-four hours after the last behavioral session, we monitored the extent of neuroglial coupling by recording extrasynaptic NMDA receptor-mediated slow inward currents (SICs) triggered by glutamate release from astrocytes. In another group of rats, we evaluated whether reduction of extrasynaptic NMDA receptor signaling by viral targeting of an anchoring protein, GIPC1, impacted neuroglial coupling and cognitive flexibility.

Results: Cocaine self-administration significantly increased the frequency of neuronal SIC events in cocaine experienced animals ($p < 0.05$). Conversely, GIPC1 knock-down attenuated SIC frequencies in cocaine treated animals to levels observed in cocaine-naïve groups ($p < 0.05$). No significant differences were found in spontaneous excitatory postsynaptic current amplitude or frequency regardless of group. When examining cognitive flexibility, cocaine self-administration was associated with significant impairment in ability to adopt a new behavioral strategy and significantly more errors compared to control groups ($p < 0.01$). These cognitive deficits were not observed in cocaine animals after the GIPC1 knock-down.

Conclusions: Cocaine self-administration increases PFC neuroglial coupling and results in decision-making impairments. Viral knock-down of GIPC1 attenuates eNMDAR signaling, normalizes neuronal sensitivity to astrocytic glutamate, and prevents cocaine-induced cognitive deficits. Etiology of cocaine use disorder may therefore involve aberrant astrocyte-neuron interactions in the PFC.

Keywords: Cocaine, Prefrontal Cortex, Astrocyte, NMDA Glutamate Receptors, Cognitive Impairments

Disclosure: Nothing to disclose.

M236

Impact of Opioid Dependence and Withdrawal on the Relative Reinforcing Effects of Fentanyl, Methamphetamine, and Cocaine in Rats

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Background: The use of multiple substances, particularly stimulants and opioids, is not a new phenomenon, and there is increasing awareness polysubstance abuse is the norm rather than the exception. Indeed, although the co-injection of cocaine and heroin has been common for decades, recent estimates suggest that the popularity of stimulant-opioid mixtures is growing, with over 50% of treatment-seeking opioid users reporting regular stimulant use. The goal of the current study was to determine how opioid dependence and withdrawal affect the relative reinforcing effects of opioids (e.g., fentanyl), as well as stimulant (e.g., methamphetamine and cocaine).

Methods: Adult male Sprague Dawley rats ($n=8$) were trained to self-administer 3.2 $\mu\text{g/kg/inf}$ fentanyl under a progressive ration (PR) schedule of reinforcement. Once stable, dose substitution was used to generate full PR dose-response curves for fentanyl (0.032–10 $\mu\text{g/kg/inf}$), methamphetamine (0.0032–0.32 mg/kg/inf), and cocaine (0.032–1.78 mg/kg/inf). Next, opioid dependence was established by administering escalating doses of morphine (10, 20, 30, and 40 mg/kg ; SC) twice-daily (every 12hrs) for four days, and maintained by once-daily injections of 40 mg/kg morphine. In order to evaluate the impact of opioid dependence and withdrawal the daily self-administration session occurred 12hrs, or 20hrs after the maintenance dose of morphine. During this phase, PR dose-response curves for fentanyl (0.032–32 $\mu\text{g/kg/inf}$) were pre-determined, with the reinforcing effects of methamphetamine (0.032 mg/kg/inf) or cocaine (0.32 mg/kg/inf) evaluated approximately every 10 days.

Results: Under baseline conditions, fentanyl ($\text{ED}_{50} = 6 \mu\text{g/kg/inf}$; $\text{E}_{\text{max}} = 8.9 \text{ inf}$), cocaine ($\text{ED}_{50} = 0.17 \text{ mg/kg/inf}$; $\text{E}_{\text{max}} = 18.1 \text{ inf}$), and methamphetamine ($\text{ED}_{50} = 0.037 \text{ mg/kg/inf}$; $\text{E}_{\text{max}} = 19 \text{ inf}$) all functioned as reinforcers. After establishing opioid dependence with twice daily morphine, once-daily injections of 40 mg/kg morphine resulted in rats exhibited the emergence of withdrawal signs (weight loss, wet dog shakes, ptosis, diarrhea, vocalization, and mechanical hyperalgesia) by 18hrs (withdrawal condition; WD), but not 12hrs (morphine dependence condition; MD) after their last dose of morphine. When evaluated during WD, the fentanyl dose-response curve was shifted upward, whereas the fentanyl dose-response curve was shifted downward and to the right when evaluated during MD. The reinforcing effects of 0.32 mg/kg cocaine and 0.032 mg/kg/inf methamphetamine were unchanged by either condition.

Conclusions: Polysubstance abuse involving opioids and stimulants is widespread, however, relatively little is known about how opioid dependence and withdrawal impact the reinforcing effects of these commonly co-abused drugs. The current studies provide direct evidence that rats in a state of opioid withdrawal work harder to obtain fentanyl compared to rats that are not physically dependent on opioids, and that the reinforcing effects of fentanyl are suppressed in rats currently dependent on opioids. Opioid dependence and withdrawal did not affect the reinforcing effectiveness of methamphetamine or cocaine. Taken together, these findings suggest that motivations to use opioids are highly dependent on the state of the individual, and influenced by negative reinforcing effects of the opioid, whereas stimulants retain their positive reinforcing effects regardless of whether the individual is in a state of opioid dependence or withdrawal. The latter could contribute to the growing popularity of stimulants among opioid users, as well as the increasing prevalence of overdose deaths attributed to the co-use of opioids and stimulants.

Keywords: Opioid Dependence, Opioid Withdrawal, Fentanyl, Methamphetamine, Intravenous Drug Self-Administration

Disclosure: Nothing to disclose.

M237

Exploring the Role of the Ser9Gly (rs6280) Dopamine D3 Receptor Polymorphism in Nicotine Reinforcement and Cue-Elicited Craving

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Background: The dopamine D3 receptor (D3R) has been shown in preclinical studies to control reinstatement of drug seeking and motivation for drugs. A D3R gene variant, Ser9Gly (rs6280) has been linked to nicotine dependence, yet the mechanisms underlying its involvement in nicotine dependence is unclear. This study investigated the relationship between the Ser9Gly variant and measures of both nicotine reinforcement and cue-elicited craving.

Methods: Phenotypes of smoking behaviors were assessed in genetically grouped (Glycine vs. No Glycine groups) current smokers ($n=103$, cigarettes per day ≥ 10). Laboratory measures included a forced choice session, to measure relative reinforcement of nicotine (nicotinized vs. denicotinized cigarette), and a cue-reactivity session, to measure cue-elicited craving (smoking vs. neutral cues).

Results: The forced choice procedure revealed that subjective ratings were significantly higher in response to nicotinized compared to denicotinized cigarettes; however, the Ser9Gly variant did not significantly influence this effect. By comparison, smoking cues elicited greater craving over time compared to neutral cues, and Glycine carriers of the Ser9Gly D3R variant seem to experience a significant blunted cue-elicited craving effect.

Conclusions: Results support D3R involvement in nicotine cue reactivity. However, more research is needed to illuminate the mechanistic properties of this variant in various aspects of nicotine dependence.

Keywords: Dopamine (D2, D3) Receptors, Genetic Association Study, Cue-Exposure, Cue-Induced Craving, Reinforcement

Disclosure: Canopy, Grant, Bioprojet, Grant, ACS, Grant, Alkermes, Grant

M238

Pharmacological Evaluations of a Novel Chemical Series of Serotonin 5-HT_{2C} Receptor (5-HT_{2CR}) Positive Allosteric Modulators

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Background: The 5-HT_{2C} receptor (5-HT_{2CR}), a receptor subtype in the 5-HT_{2R} family (5-HT_{2AR}, 5-HT_{2BR}, 5-HT_{2CR}), is mechanistically involved in obesity, depression, schizophrenia, and substance use disorders. The selective chemotype targeting of the 5-HT_{2CR} is challenging given the similarity of the orthosteric sites across the 5-HT_{2R} family, such that 5-HT_{2AR} or 5-HT_{2BR} agonists are expected to evoke hallucinations or cardiac valvulopathy, respectively. Targeting 5-HT_{2CR} allosteric site(s), which differ from the orthosteric site for endogenous 5-HT, creates new

opportunities to optimize 5-HT₂CR signaling in disorders marked by hypofunctional 5-HT₂CR signaling. We have reported the discovery of several 5-HT₂CR PAMs and have subsequently focused on chemical optimizations imperative to ultimate clinical success. The aim of this study is to address synthetic feasibility and isomer separation via replacement of our 5-HT₂CR PAM pharmacophore piperidine core, while maintaining the functional activity, off-target profile, and in vitro pharmacokinetics of our lead 5-HT₂CR PAMs.

Methods: A new series of molecules was designed and optimized using structure-activity relationship studies integrating molecular descriptors, biological activity, and pharmacokinetic parameters. In vitro characterization of G protein activation was achieved via an intracellular calcium (Ca²⁺) mobilization assay in stably transfected human (h) 5-HT₂CR-expressing cells, while h5-HT₂AR-expressing cells were employed for counter-screening with secondary competition binding studies via the Psychoactive Drug Screening Program (PDSP). Structure-based design refinements were made using the ergotamine (ERG)-5-HT₂CR X-ray crystallographic structure complex (PDB: 6BQG). In silico assessment of PAMs was accomplished using Schrödinger computational chemistry tools and the aforementioned 5-HT₂CR complex.

Results: The feasibility of pharmacophore core replacement was assessed in silico, wherein 5-HT was dynamically docked to the ERG-5-HT₂CR complex, resulting in a 5-HT-bound model and allowing for the energy minimization of previously ERG-bound residues. Molecular docking of our current 5-HT₂CR PAM lead compound (CTW0415) to the model resulted in the discovery of a putative PAM binding site with interactions spanning transmembrane domain 6 (TM6), extracellular loop 2 (ECL2), and a hydrophobic pocket between TM2 and TM3. In accordance with in silico data showing that pyridine core scaffolds retain the required docking pose, several molecules lacking the chiral piperidine were synthesized (e.g., CTW0508) and found to enhance 5-HT-evoked signaling in h5-HT₂CR cells; a signature upward shift (E_{max} ≥ 120%) was observed and no effect was observed in h5-HT₂AR cells. In vitro pharmacokinetic analyses of CTW0508 demonstrate high solubility, low metabolic liability, and an adequate half-life while PDSP profiling supports the lack of interaction at the 5-HT₂CR or the 5-HT₂AR.

Conclusions: We conclude that our core 5-HT₂CR PAM pharmacophore is amenable to replacing the substituted piperidine ring core with an achiral pyridine ring. Importantly, these new 5-HT₂CR PAMs maintain functional activity and excellent pharmacokinetics as achieved by our lead compound CTW0415. The pyridine-based 5-HT₂CR PAM CTW0508 displays minimal interactions with 5-HT₂AR orthosteric sites; and ongoing docking simulations suggest a topographically distinct binding site. These data open the door to explore and establish the PAM mode of action and describe necessary molecular interactions for further PAM optimization.

Keywords: Serotonin 5-HT₂CR Receptor, Positive Allosteric Modulators, Drug Discovery/Development

Disclosure: Nothing to disclose.

M239

Differential Efficacy of Cue Extinction “Therapy” for Reducing Goal-Directed Versus Habitual Cocaine Seeking

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Background: Cocaine use disorder causes significant health and financial burdens to society; however, no effective treatments

currently exist. One of the biggest hurdles for successful treatment is the high likelihood of relapse in abstinent individuals. Relapse is often triggered by exposure to the environmental stimuli or cues that were associated with prior cocaine use. Thus, a potential treatment strategy is to reduce the strength of cocaine-associated memories, thus reducing the likelihood of relapse. One such strategy, exposure therapy, involves a process known as memory extinction, where cues are presented repeatedly in the absence of drug reinforcement. Unfortunately, clinical application of exposure therapy has met with limited success. One possible reason for the disappointing clinical efficacy of exposure therapy is that in later stages of addiction use can become habitual or compulsive and this may lead to the execution of drug taking actions independent of the current value of the cue that predicts the outcome of those actions. In other words, we hypothesized that habit systems may occlude the ability of cue extinction to reduce cue motivated drug seeking behavior.

Methods: In order to test this hypothesis, we trained Sprague-Dawley rats to self-administer intravenous cocaine paired with an audiovisual cue on either a fixed ratio (FR) schedule of reinforcement known to promote goal-directed drug seeking or on a second order (SO) schedule of reinforcement known to promote putatively habitual drug seeking. We first verified that FR and SO training were differentially sensitive to inhibition of dopamine seeking in the dorsal lateral striatum (DLS) to verify the use of goal-directed versus habitual response strategies, respectively. Intracranial guide cannula were implanted in the DLS and the dopamine receptor antagonist alpha-flupenthixol was infused prior to a test of cue-motivated cocaine seeking in both groups. We next tested the efficacy of cue extinction learning to reduce cue-induced reinstatement by dividing animals under each training schedule into groups exposed to 0, 120, or 240 cues using a passive exposure procedure. Cue-induced reinstatement was tested the following day. Finally, we determined if restoring goal-directed behavior by infusing the AMPA antagonist NBQX in the DLS could reveal the effects of cue extinction learning in SO trained rats. Data were analyzed using ANOVAs with DLS infusion, schedule, and extinction conditions as separate between subjects factors. Bonferroni's post-hoc tests followed significant interactions.

Results: We confirmed that rats trained on an FR schedule maintained DLS dopamine-independent drug seeking, indicative of goal-directed behavior, while dopamine antagonism in the DLS did disrupt drug seeking in rats trained on SO schedule, indicative of habit. Next, we found that while cue extinction training was effective in significantly reducing cue-induced reinstatement in FR trained rats, as previously reported, cue extinction was ineffective in SO trained rats. Finally, we found that inhibition of DLS glutamate signaling at the time of the reinstatement test restored goal-directed behavior and revealed the effect of cue extinction, where 0 cue exposed control rats showed high levels of drug seeking after NBQX, while 120 cue exposed rats infused with NBQX showed low levels of drug seeking, consistent with effective extinction learning.

Conclusions: These studies reveal that under specific schedules of reinforcement, cue motivated cocaine seeking can be controlled by goal-directed or habitual response strategies, and that if cocaine seeking becomes dependent on DLS dopamine signaling (i.e., “habitual”) that cue extinction-based treatments to reduce relapse are ineffective. Thus, successful treatment may require a combination of exposure therapy to reduce the value of cocaine cues and the restoration of goal-directed response strategies in order to use this updated value representation to control behavior.

Keywords: Cocaine Self-Administration, Extinction Learning, Habitual Decision-Making, Amygdala, Dorsolateral Striatum

Disclosure: Nothing to disclose.

M240

Levodopa Reduces Drug Consumption Across Multiple Classes of Abused Substances

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Background: Using a rodent experimental system for the investigation of cocaine use, we previously demonstrated that a dopamine feedback signal elicited in the nucleus accumbens on successful completion of drug seeking was diminished in animals that escalated their drug consumption (Willuhn et al, 2014). Restoration of that signal with co-administration of levodopa (L-DOPA) and benserazide (a peripheral decarboxylase inhibitor) returned drug taking to pre-escalation levels. We posit that this loss of dopamine-mediated feedback could generalize to other classes of abused substances, contributing to excessive drug consumption across substance-use disorders. Accordingly, levodopa could have therapeutic utility by reducing high levels of drug consumption. Levodopa administered with a peripheral decarboxylase inhibitor is an FDA-approved pharmacotherapy indicated in the treatment of the symptoms of idiopathic Parkinson's disease, post-encephalitic parkinsonism, and symptomatic parkinsonism. It has been tested for the prevention of substance-use relapse in clinical trials for a number of substances, with largely negative results. Specifically, treatment with levodopa does not reliably extend periods of drug abstinence. Interestingly though, its most robust effects are in individuals who were not baseline abstinent (Schmitz et al, 2014) indicating that levodopa treatment may reduce active drug consumption rather than sustain abstinence. The current study seeks to provide preliminary data in animals on whether levodopa can reduce drug consumption outside the context of abstinence across classes of abused substances.

Methods: Wistar rats were first exposed to a two-bottle-choice paradigm where one bottle contained water and the other contained an aqueous solution of ethanol or fentanyl. In this paradigm, the animals could freely choose between the water and drug bottles. The amount of water provided exceeded the animals' daily requirements and so they could drink from only the water bottle without experiencing dehydration. For alcohol experiments, ethanol was provided at 100 mg/ml in water (10 % weight by volume) for the first several two-bottle choice sessions and then the concentration was increased to 200 mg/ml (20 % weight by volume) for the remainder of the sessions. For opioid studies, the drug bottle contained 50 µg/ml of fentanyl in water and was present in the home cage for three hours per day. Following this phase, rats were trained to self-administer ethanol (200 mg/ml) or fentanyl (50 µg/ml) solutions in operant chambers. Following a response into a nose-poke port, solutions were delivered to an adjacent receptacle where it could be freely retrieved. Drug delivery was paired to an audio-visual compound stimulus. The amount of solution delivered per delivery was 200 µl of ethanol, or 20 µg/kg (i.e., 200 µl for a 500-g rat) of fentanyl. A subset of the animals were trained on the operant fentanyl self-administration tasks without prior exposure to the two-bottle choice procedure. To test the effects of levodopa on drug consumption, it was administered by intraperitoneal injection (30 mg/kg) with the peripheral decarboxylase inhibitor, benserazide (2 mg/kg) 20 minutes before a test session.

Results: Across multiple cohorts of rats with different training histories (total n = 34), levodopa (30 mg/kg) with benserazide (2 mg/kg) treatment consistently produced a significant reduction of ethanol self-administration compared to within-animal vehicle-

treated control sessions. The magnitude of the effect ranged between groups from 35- to 70-% reduction in consumption ($P = 0.0253 - 0.0011$). For fentanyl, when levodopa and benserazide were administered before a three-hour two-bottle choice session ($n = 5$), there was a significant reduction in fentanyl consumption ($P < 0.05$) compared to vehicle-treated controls ($n = 6$), with no effect on water consumption ($P > 0.05$). Likewise, levodopa/benserazide treatment significantly reduced instrumental responding for fentanyl ($P < 0.01$) as well as total fentanyl consumption ($P < 0.05$) during the operant self-administration task compared to within-animal vehicle-treated sessions ($n = 15$). There were no sex differences observed in fentanyl consumption or the effects of levodopa thereon during the self-administration task ($n = 7$ males, $n = 8$ females, $P > 0.05$).

Conclusions: The current work demonstrates that levodopa treatment reduces voluntary ethanol and opioid consumption in rodents. These findings extend our published work showing such an action on psychostimulant use. While levodopa treatment has been tested in numerous clinical trials for substance-use disorders with chiefly negative results, it is important to recognize that the clinical endpoint of most, if not all, of those studies was sustained abstinence. The current work considers a different endpoint—reduced consumption during active drug taking. This endpoint falls into the category of harm reduction since reducing intake during ongoing drug taking should presumably diminish the risk of adverse consequences, including death, that are a result of drug overdose. Moreover, this approach has potential utility for regaining control of substance use, especially as an adjunctive to behavioral and/or cognitive therapies.

Keywords: L-DOPA, Fentanyl, Ethanol, Cocaine, Alcohol Consumption

Disclosure: Numedii, Employee (Spouse)

M241

Imaging the 18-KDa Translocator Protein in Tobacco Smokers: Comparing Baseline and Endotoxin-Stimulated Levels With Controls

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Background: Altered immune signaling is associated with tobacco smoking, however, these effects are complex. For example, nicotine has immunosuppressive properties, while other constituents in tobacco smoke have pro-inflammatory effects. In the brain, dysregulated immune signaling can contribute to compulsive drug use and associated comorbidities. Therefore, this work aimed to compare neuroimmune function in tobacco smokers and nonsmokers. This was accomplished using [11C]PBR28 positron emission tomography (PET) brain imaging of the 18-kDa translocator protein (TSPO), a marker of microglia. Baseline TSPO levels were acquired in all subjects, while a subset completed a paradigm including a second PET scan acquired after injection of the classic pro-inflammatory stimulus lipopolysaccharide (LPS).

Methods: Baseline [11C]PBR28 PET scans were acquired in 16 tobacco smokers and 19 non-smokers. In a subset of 8 smokers and 9 non-smokers, a second [11C]PBR28 scan was acquired 3 hours after administration of LPS (1 ng/kg, IV). PET data were acquired with a High Resolution Research Tomograph (HRRT) for 120 min following injection of 520 ± 195 MBq [11C]PBR28. Arterial

blood samples were collected to measure the metabolite corrected input function. Multilinear analysis was used to estimate [11C]PBR28 distribution volumes (VT) in 9 brain regions. The percent change from baseline in [11C]PBR28 VT (Δ VT) after LPS administration was used to quantify neuroimmune response. Separate linear mixed models compared baseline [11C]PBR28 VT between smokers and nonsmokers and [11C]PBR28 Δ VT between smokers and nonsmokers.

Results: There was no evidence for differences in baseline [11C]PBR28 VT between smokers and nonsmokers. In contrast, for neuroimmune response a significant interaction of smoking status by region was identified ($p=0.02$), where [11C]PBR28 Δ VT was lower in smokers compared to nonsmokers in areas of striatum and cortex (post-hoc contrasts yielded $p=0.02$ - 0.04 , uncorrected for multiple comparisons). For example, in frontal cortex ($p=0.02$), group Δ VT values were $33.0 \pm 11.7\%$ and $51.4 \pm 15.4\%$ for smokers and nonsmokers, respectively.

Conclusions: No evidence for differences in baseline [11C]PBR28 VT between smokers and nonsmokers were found. This finding informs previous PET studies reporting lower TSPO radiotracer concentrations in brain (measured as standardized uptake value, SUV) of tobacco smokers compared to nonsmokers, highlighting the need to account for radiotracer behavior in plasma for full quantification of [11C]PBR28 VT. In contrast, the magnitude of response to LPS was significantly lower in smokers vs. nonsmokers, but only in regions of cortex and striatum. These results provide evidence for impaired neuroimmune function in tobacco smokers compared to nonsmokers, but in a regionally specific pattern.

Keywords: Immune responses, TSPO and [11C]PBR-28 PET, Tobacco Smoking, Endotoxin

Disclosure: Nothing to disclose.

M242

Adolescent Δ 9-THC Exposure in Adolescence is Associated With Limited Alterations in Adult Rat Brain Structure

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Background: Adolescence is a dynamic period of brain maturation, which underscores the development of higher order cognition and emotional behaviours in which the endocannabinoid system plays a critical role. Hence, chronic exposure to delta-9-tetrahydrocannabinol (Δ 9-THC), the major psychoactive compound in marijuana during adolescence may interfere with the maturation and refinement of neural circuitry, with the potential for long-lasting behavioural consequences. Supporting this view is epidemiological evidence linking early cannabis use to increased risk for psychosis and data from rodent models suggesting long-term disruption of cognitive and emotional behaviours following chronic adolescent Δ 9-THC exposure (Renard et al., *Can. J. Psychiatry*, 2016). We therefore set out to identify the specific neuroanatomical circuits by which adolescent Δ 9-THC exposure may predispose the developing brain for later onset of psychopathology. To do so, we combined a validated rat model of adolescent Δ 9-THC exposure with an assessment of adult rat brain macrostructure using structural magnetic resonance imaging (MRI) combined with voxel-wise computational neuroanatomical analysis tools.

Methods: Sprague-Dawley rats (male) were exposed to escalating (2.5; 5; 10 mg/kg; intraperitoneal [i.p.]; $n=10$) doses

of Δ 9-THC (T2386, Sigma-Aldrich, UK $n=10$) or drug vehicle (a 1:1:18 ratio of poly-ethylene glycol, Tween-80 and sterile saline; $n=10$) during adolescence, defined as post-natal day (P) 35-45 (Rubino et al., *Neuropsychopharmacology*, 2008). To confirm Δ 9-THC exposure, a plasma sample was collected from the tail vein on P44, 30 minutes after injection. High Performance Liquid Chromatography (HPLC) was used to determine the concentrations of Δ 9-THC in rat plasma with tandem mass spectrometry (MS/MS) detection. Animals were left undisturbed until adulthood (P80) and culled by cardiac perfusion (0.9% saline followed by 4% paraformaldehyde) under terminal anaesthesia (sodium pentobarbital, 60 mg/kg i.p.). Perfusion-fixed brain tissues were kept intact in the cranium and post-fixed for 24 hours in 4% PFA followed by 4 weeks in 0.01M phosphate buffer containing 0.05% (w/v) sodium azide at 4°C. T2-weighted ex vivo 3D MR images were then acquired using a 7T small bore MRI scanner (Agilent technologies) and a quadrature volume radiofrequency (RF) coil (39 mm inner diameter, RAPID Biomedical) using the following parameters: TE/TR=60/2000, echo train length = 8, matrix size = $192 \times 128 \times 192$ and field of view (FOV) = $28.8 \times 19.2 \times 28.8$ mm, yielding isotropic voxels of $150 \mu\text{m}^3$. Total scan-time per brain was 1hr 44min. One MR image was excluded from the Δ 9-THC group due to visible dissection damage in the cerebellum, hence the final n -values for group comparisons were vehicle, $n=10$ and Δ 9-THC, $n=9$. MR images were converted to NIFTI format and processed using a combination of FSL, ANTs and in-house C++ software utilizing the ITK library, available from <https://github.com/spiniceist/QUIT>. Data were then analysed for group level differences using voxel-wise tensor-based morphometry (TBM) and automated cortical thickness analysis. Specifically, we performed voxel or vertex-wise t -tests, including total brain volume as a covariate (Vernon et al., *Biological Psychiatry*, 2014). The resulting maps were then corrected for multiple comparisons using the family-wise error rate (FWE $p < 0.05$).

Results: On P44, 30 minutes post-injection, drug plasma levels of Δ 9-THC were 653 ± 140 nM, equivalent to 205.3 ng/ml. After correction for multiple comparisons (FWE $p < 0.05$), there were no statistically significant effects of adolescent Δ 9-THC exposure on either adult rat cortical thickness or local brain volumes at P80. Follow up analyses at an exploratory threshold ($p < 0.01$ uncorrected for multiple comparisons) indicated cortical thickness and local volume decreases in the prefrontal cortex of Δ 9-THC-exposed rats compared to vehicle controls. In contrast, the local volumes of the striatum, globus pallidus, ventral midbrain, ventral thalamus, pontine nuclei and cerebellar grey matter were increased. The magnitude of these apparent volume differences was however small (range 1-4%).

Conclusions: In sum, structural brain metrics were largely similar among adult male rats exposed in adolescence to either Δ 9-THC or drug vehicle. Our data converge with prior longitudinal studies in humans suggesting small or limited associations between adolescent cannabis use and structural brain measures in youth (Koenders et al., *J Psychopharmacology*, 2017). Exploratory follow-up analyses revealed subtle trend-anatomical abnormalities were present in rat brain regions involved in mesocorticolimbic dopamine signalling, including the prefrontal cortex, striatum, globus pallidus and ventral midbrain following adolescent Δ 9-THC exposure. In this context, neuronal hyperactivity of the mesocorticolimbic dopamine pathway has been previously reported in adult rats following a similar adolescent Δ 9-THC exposure regimen (Renard et al., *Cerebral Cortex*, 2017). Prospective longitudinal in vivo MRI studies are now clearly warranted to confirm our findings.

Keywords: Cannabis, Magnetic Resonance Imaging, Adolescence

Disclosure: UCB Biopharma SprL, Grant

M243

Functional Dissection of Basolateral Amygdala Neural Circuits for Alcohol Seeking Behaviors*Junghyup Suh*, Kerry Ressler**Harvard Medical School McLean Hospital, Belmont, Massachusetts, United States*

Background: Alcohol is the most commonly abused substance and alcoholism is one of the leading causes of disease and premature deaths in modern societies. A large body of research has implicated that repeated excessive alcohol use, often triggered by stress, seems to start a vicious cycle to promote escalated alcohol intake. Currently, however, there is substantial gap in our understanding of the neural mechanisms that drive a transition from controlled alcohol consumption to the development of alcohol seeking and dependence. The amygdala, a critical neural substrate of both aversive and appetitive behaviors, is directly affected by a variety of addictive substances. Recently, studies in mice have indicated that distinct subpopulations of neurons within the amygdala are differentially responsible for the activation and inhibition of fear memory. In addition, divergent ensemble activity from these subpopulations seems to mediate positive or negative valence coding. Specifically, we have found that a specific excitatory neuronal population in the basolateral amygdala (BLA), marked by Thy1 expression (Thy1+), serve as 'Fear-Off' neurons. They also heavily project to the nucleus accumbens (NAcc), a core structure for reward-based learning and substance addiction, instead of the central amygdala (CeA), suggesting that these neurons directly inhibit fear and may support appetitive behavior. Understanding the roles of this molecularly identified population in alcohol related behaviors is central to our study.

Methods: First, to determine whether Thy1+ neurons are involved in associative Pavlovian conditioning with alcohol, we employed a conditioned place preference (CPP) paradigm with systemic injection of alcohol (EtOH, 2g/kg BW) in male mice (N = 3; control n = 12, EtOH n = 12). Then, we have mapped EtOH-CPP-induced neuronal activity changes by quantifying the number of c-Fos protein-expressing (c-Fos+) neurons in the BLA of Thy1-eYFP mice. Second, to investigate the roles of BLA Thy1+ neurons and their projections during conditioning or recall phase, we performed inhibitory optogenetic manipulation in Thy1+ neurons using Thy1-Cre driver mice and AAV encoding Cre-dependent halorhodopsin (eNpHR) or eYFP. AAV-eNpHR was injected to the BLA, but optic fibers were bilaterally implanted above the BLA (N = 3; control n = 11, EtOH n = 10), NAcc (N = 3; control n = 9, EtOH n = 12) or prefrontal cortex (PFC) (N = 2; control n = 8, EtOH n = 7).

Results: First, there was no difference in the number of total c-Fos+ neurons and c-Fos+ neurons in Thy1+ neurons in non-conditioned group and EtOH-conditioned group on the first day of conditioning (control, 22.20 ± 10.36 and 3.25 ± 1.44 ; EtOH, 16.80 ± 5.67 and 2.20 ± 0.20 at AP -1.5mm). However, total c-Fos+ neurons and c-Fos+ neurons in Thy1+ neurons were significantly greater in EtOH-conditioned group than non-conditioned group on the last day of conditioning (control, 12.67 ± 6.22 and 1.67 ± 0.42 ; EtOH, 29.00 ± 9.05 and 6.67 ± 1.94 at AP -1.5mm; *P < 0.05).

Second, the preference formation of EtOH-associated compartment was disrupted when optogenetic inhibition was performed at Thy1+ cell bodies in the BLA (eYFP, $69.25 \pm 2.95\%$; eNpHR, $33.63 \pm 6.12\%$; *P < 0.05) or Thy1+ projections in the NAcc (eYFP, $61.69 \pm 4.80\%$; eNpHR, $31.92 \pm 3.40\%$; *P < 0.05) during the conditioning phase. Conversely, the recall of CPP was disrupted when optogenetic inhibition was performed at Thy1+ cell bodies in the BLA (eYFP,

$62.53 \pm 5.19\%$; eNpHR, $40.90 \pm 3.45\%$; *P < 0.05), but not at Thy1+ projections in the NAcc (eYFP, $65.50 \pm 3.48\%$; eNpHR, $69.50 \pm 4.29\%$).

Conclusions: Given the amygdala's roles in fear, anxiety and substance abuse, the findings suggest that alcohol exposure alters the firing activity of subpopulations of amygdala neurons. Interactions between the BLA and other brain areas via its projections are differentially involved in the formation of memory associating alcohol experience and environmental cues, and recall of the memory.

Keywords: Basolateral Amygdala, Alcohol-seeking behavior, Circuit Optogenetics, Conditioned Place Preference, c-Fos-Expressing Ensembles

Disclosure: Nothing to disclose.

M244

Sex Differences in Endocannabinoid Modulation of Cocaine-Memory Strength During Reconsolidation*Rita Fuchs*, Rong Wang, Jennifer Walters, Jobe Ritchie, Jessica Higginbotham**Washington State University, Pullman, Washington, United States*

Background: Cocaine memories become labile upon retrieval and require protein synthesis-dependent reconsolidation into long-term memory stores in order to persist over time. Substance use disorder patients report pathologically salient and intrusive cocaine memories, which perpetuate drug use. Thus, reconsolidation provides an interesting therapeutic target in that interference with memory reconsolidation weakens memories both in rodent models of drug relapse and in human models of cue-induced drug craving. Our laboratory has shown that cannabinoid type 1 receptor (CB1R) stimulation within the basolateral amygdala (BLA) regulates the reconsolidation of cocaine memories in rats, and AM251-mediated CB1R antagonism in the BLA enhances post-reconsolidation memory strength and prolongs memory retrieval-associated rises in plasma corticosterone levels. In the present study, we investigated the contribution of distinct endocannabinoids and potential sex differences to this phenomenon.

Methods: To promote the acquisition of context1-response-cocaine associative memories, adult Sprague-Dawley rats were trained to self-administer IV cocaine infusions by pressing a lever in a distinct context. Rats then received extinction training in a distinctly different context to promote the acquisition of context2-response-no cocaine associations. On post-cocaine day 8, rats were exposed to the cocaine context for 15 min, in order to trigger cocaine memory retrieval, destabilization, and reconsolidation. Immediately after the memory retrieval session ("reactivation groups) or six hours later (no reactivation groups), rats received bilateral intra-BLA microinfusions of the FAAH inhibitor, URB597 (1 µg/side or Veh), the DAGL inhibitor, DO34 (1.67 µg/side or Veh), or the MAGL inhibitor, JZL185 (1 µg/side or Veh), in order to manipulate levels of two endocannabinoids, anandamide and 2-arachydonoyl glycerol (2-AG). The effects of these manipulations were assessed on extinction- and cocaine-memory strength, as indexed by non-reinforced lever presses during test sessions in the extinction context (24 and 48 hours later) and in the cocaine-paired context (72 hours later). Data were analyzed in subjects with accurate cannula placements using analyses of variance and Sidak's posthoc tests, with a set at 0.05.

Results: Intra-BLA URB597 administration immediately after memory reactivation failed to alter cocaine-seeking behavior in either sex and in either test context, relative to Veh. In males, DO34 administration after memory reactivation increased subsequent cocaine-seeking behavior in the cocaine-paired context, but not in the extinction context, relative to Veh. Also in males,

JZL185 administration immediately, but not 6 hours, after memory reactivation attenuated subsequent cocaine-seeking behavior in the cocaine-paired context, relative to Veh. In contrast, DO34 or JZL administration immediately after memory reactivation failed alter cocaine-seeking behavior in either context in females.

Conclusions: Together with the results of our earlier CB1R antagonist study, these new findings indicate that 2-AG-mediated stimulation of CB1Rs in the BLA gates cocaine memory strength during reconsolidation. 2-AG may elicit this effect by interacting with the hypothalamic-pituitary-adrenal axis to diminish memory-retrieval associated HPA axis activation during reconsolidation and thereby promote the faithful maintenance of cocaine-related emotional memories. Furthermore, sex differences in this maladaptive-memory gating mechanism may be responsible for more severe addiction phenotypes in women relative to men.

Keywords: Cocaine Seeking, Cocaine Self-Administration and Reinstatement, Amygdala, Sex Differences, Endocannabinoids

Disclosure: Supernus Pharmaceuticals, Consultant

M245

Effects of a Fentanyl/Heroin Vaccine on the Antinociceptive and Reinforcing Effects of a Fentanyl/Heroin Mixture in Male and Female Rats

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Background: The current opioid crisis remains a significant public health issue and there is a critical need for biomedical research to develop effective and easily deployable candidate treatments. One emerging treatment strategy for opioid use disorder includes immunopharmacotherapies or opioid-targeted vaccines. Recent preclinical research has explored the development of combination immunopharmacotherapy approaches directed at multiple, structurally dissimilar and commonly abused opioids (e.g., fentanyl and heroin). The present study evaluated the effectiveness of a fentanyl/heroin conjugate vaccine on fentanyl/heroin mixture self-administration and antinociception in rats.

Methods: 12 Sprague-Dawley rats (6 male, 6 female) were acquired at 10 weeks of age (Envigo Laboratories, New Jersey, USA) and surgically implanted with custom jugular catheters and vascular access ports (Instech, Plymouth Meeting, PA). Animals were singly housed in a vivarium maintained on a 12-h light/dark cycle (lights off at 6:00 PM). Water and food were provided ad lib in the home cage. Animal maintenance and research were conducted in accordance with the 2011 guidelines of the NIH Committee on Laboratory Animal Resources and protocols were approved by the Institutional Animal Care and Use Committee. Experiment 1 (3 males/3 females) examined heroin/fentanyl conjugate vaccine effects on 1:27 fentanyl/heroin self-administration and experiment 2 (3 males/3 females) examined vaccine effects on heroin alone, fentanyl alone, 1:27 fentanyl/heroin mixture, and methadone (control) in a warm-water tail withdrawal procedure (50 °C). In experiment 1, rats was trained to respond under a concurrent FR5:FR5 "choice" schedule of liquid food and fentanyl/heroin mixture availability. The behavioral session consisted of five 20-min response components each preceded by a 4-min "sample" component. During each response component, both levers were extended, a red stimulus light above the left lever was illuminated to signal liquid food availability and a green stimulus light above the right lever was illuminated to signal fentanyl availability. FR5 completion on the left lever resulted in a 5-s presentation of liquid food; whereas, FR5

completion on the right lever resulted in fentanyl delivery. Responding on one lever reset the ratio requirement for the other lever. A different 1:27 fentanyl/heroin mixture dose was available during each of the five successive response components (0, 0.32, 1.0, 3.2, and 10 µg/kg/inj during components 1-5, respectively based on fentanyl dose). A 1:27 fentanyl/heroin mixture was selected based on the relative individual potencies (ED50) for fentanyl and heroin alone in the warm-water tail withdrawal procedure. Mixture dose varied by changing the infusion duration and visually signaled by the frequency of the flashing right green light above the drug-associated lever in 3-s cycles. Choice training was considered stable when the smallest mixture dose that maintained at least 80% of completed ratio requirements on the mixture-associated lever was non-trending for three consecutive days. In Experiment 2, each session began by gently wrapping the rat with a towel, leaving the tail exposed. The distal five cm of the tail was immersed in heated water (50°C) and the latency to fully remove the tail was recorded with a digital chronograph with a 0.01 s resolution (Sports Timer, Fisher Brand, Hampton, NH). If the rat did not remove its tail by 20 s, the experimenter removed the tail and a latency of 20 s was assigned. Following baseline latency determination, IV saline or fentanyl (1-1000 µg/kg), heroin (32-320 µg/kg), 1:27 fentanyl/heroin mixture, methadone (320-3200 µg/kg) was administered followed by a 0.1 mL saline flush and tail-withdrawal latency was redetermined 2 min later for opioid agonists alone or in a mixture. Four vaccine boosts were administered (weeks 0, 2, 4, and 10) and behavioral effects were tracked over 14 weeks.

Results: Under non-vaccinated conditions, 1:27 fentanyl/heroin mixture maintained a dose-dependent increase in choice over an alternative food reinforcer and a dose-dependent decrease in operant behavior. The fentanyl/heroin conjugate vaccine did not significantly alter fentanyl/heroin self-administration but did significantly attenuate the potency of the 1:27 fentanyl/heroin mixture to produce rate-decreasing effects that was evident at week 6 and sustained until week 14 (mixture dose: F1.375,6.877=353, $p < 0.0001$; treatment: F1.5=48.6, $p = 0.0009$; interaction: F1.387,6.933=40.4, $p = 0.0002$). IV fentanyl alone, heroin alone, 1:27 fentanyl/heroin mixture, and methadone produced dose-dependent antinociception. The fentanyl/heroin vaccine was most effective in shifting the antinociceptive potency of fentanyl (peak effect at week 13: 27-fold) and did not significantly alter the antinociceptive potency of methadone at any time point (Time: F3.16,15.8=4.6, $p = 0.0156$; Drug: F1.15,5.75=38.4, $p = 0.0008$). The vaccine significantly shifted the antinociceptive potency of the 1:27 fentanyl/heroin mixture 7.5-fold at week 8 and then declined over time. Heroin antinociceptive potency was significantly shifted 4.5-fold only at week 12.

Conclusions: Effectiveness of a fentanyl/heroin conjugate vaccine depended upon the experimental endpoint (i.e. antinociception > reinforcement) and the target opioid (fentanyl > heroin).

Keywords: Drug Self-Administration, Medication Development, Antinociception, Fentanyl, Heroin

Disclosure: Nothing to disclose.

M246

Resilience and Alcohol Use Disorder: Association With Addiction Severity and Psychiatric Comorbidity

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Background: In previous work looking at individuals exposed to childhood trauma, we identified several factors associated with a lower risk for the development of alcohol use disorder (AUD). These factors include lower levels of neuroticism, impulsivity, and trait anxiety, and higher levels of conscientiousness, suggesting that these characteristics contribute to a relative resilience against AUD. In the current study, we aim to confirm and expand on these findings using the Connor-Davidson Resilience Scale (CD-RISC), a validated scale that more accurately reflects the multidimensional nature of resilience to adversity. We hypothesized that the proxy measures of resilience identified above would be significantly associated with CD-RISC score, which in turn would be positively associated with measures of quality of life (convergent validity). We further hypothesized that among individuals with AUD, higher resilience scores would be associated with decreased disorder severity and lower risk for psychiatric comorbidity.

Methods: Participants included 189 individuals (105 males, 84 females) ranging from non-drinking healthy volunteers to heavy drinkers diagnosed with AUD ($n = 107$). In addition to the CD-RISC, participants were administered the Structured Clinical Interview for DSM-5 (SCID-5) disorders, and were assessed for recent alcohol consumption (Timeline Follow-back), alcohol use disorder severity (Alcohol Dependence Scale and Alcohol Use Disorders Identification Test), early life and current life stress (Childhood Trauma Questionnaire, Perceived Stress Scale), quality of life (World Health Organization Quality of Life), personality (NEO Personality Inventory), impulsivity (Barratt Impulsiveness Scale), and negative affect (Spielberger Trait Anxiety, Comprehensive Psychopathological Rating Scale). Multiple regression analyses were conducted using SAS 9.4.

Results: Significant negative associations were seen between CD-RISC score and neuroticism, impulsivity, and trait anxiety (all $p < 0.01$), while conscientiousness was positively associated with CD-RISC score ($p = 0.01$). Gender moderated the relationship between CD-RISC score and neuroticism, with a stronger negative association in females compared to males. CD-RISC score was positively associated with quality of life measures (physical health, psychological health, social relationships, and environment, all $p < 0.01$) and negatively associated with current perceived stress ($p = 0.006$). Among individuals with a diagnosis of AUD ($n = 107$), CD-RISC score was lower (mean = 70.8) than that in individuals without AUD (mean = 80.7), and was negatively associated with AUD severity, quantity of alcohol consumed, severity of anxiety and depression symptoms, and risk for lifetime mood disorder. Resilience score was not associated with any of these measures among non-AUD individuals.

Conclusions: The current findings support the notion that lower levels of neuroticism, impulsivity, and trait anxiety, and higher levels of conscientiousness are associated with resilience. Resilience plays a role not only in the development of AUD, but in the severity of the disorder. Among individuals diagnosed with AUD, greater resilience is associated with less severe AUD, reduced depression and anxiety symptoms, and lower risk for comorbid mood disorder. While resilience is to some extent driven by intrinsic factors such as personality, studies suggest that interventions can have positive effects on individual resilience, and that boosting resilience may be associated with greater improvement during treatment in clinical populations such as those with PTSD. Additional research is needed to determine if the same is true for individuals seeking treatment for AUD.

Keywords: Alcohol Use Disorder, Risk and Resilience, Anxiety, Impulsivity

Disclosure: Nothing to disclose.

M247

The Novel N-Methyl-D-Aspartate Receptor Modulator NYX-783 Exhibits Therapeutic Effects in Rodent Models Useful for the Study of Post-Traumatic Stress Disorder and Comorbid Alcohol Use Disorder

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Background: Individuals with post-traumatic stress disorder (PTSD) are at significantly greater risk for developing alcohol use disorder (AUD) than the general population. The N-Methyl-D-aspartate receptor (NMDAR) is critically involved in aberrant plasticity thought to underlie the etiology of both PTSD and AUD. NYX-783 is a novel, orally bioavailable NMDAR modulator, discovered by Aptinyx Inc. and currently in Phase 2 clinical development for PTSD. We have previously demonstrated that NYX-783 facilitates fear extinction and reduces spontaneous recovery of fear in a one-trial fear conditioning model. Here, we sought to expand upon these efforts by testing the hypotheses that NYX-783 would attenuate extinction-resistant fear and stress-exacerbated, alcohol-seeking behavior in rodents.

Methods: Single-trial fear conditioning and extinction. Rats were exposed to a novel context in which 3 inescapable electrostatic footshocks were delivered in a single session. NYX-783 (1 mg/kg, PO), the NMDAR glycine site agonist D-cycloserine (15 mg/kg, SC), or vehicle was given one time only (1 h prior to the first extinction session). Rats underwent 6 daily extinction sessions in the same context without foot shock. Rats were then returned to their home cage for 7 days before being subjected to the fear conditioning context for a single session to evaluate spontaneous recovery of conditioned fear. Partially reinforced fear conditioning and extinction. Mice were placed in a novel context for 6 contiguous days; footshock was delivered on days 1, 4, and 6. This fear conditioning paradigm is known to induce extinction-resistant fear. Next, mice were returned to the same context for 6 daily extinction sessions without footshock. NYX-783 (10 mg/kg, IP), D-cycloserine (15 mg/kg, SC), or vehicle was administered 1 h prior to each extinction session. Alcohol self-administration, extinction, and reinstatement. A separate, treatment-naïve rat cohort underwent restraint stress in the presence of sandalwood odor 3 days prior to voluntary operant alcohol self-administration (fixed-ratio 1 schedule of reinforcement, 10% v/v). Control rats underwent sham stress, which consisted of placing these rats in a novel environment paired with sandalwood odor. After meeting self-administration criteria (stable responding and > 80 mg% blood alcohol concentration), extinction sessions commenced under self-administration conditions, but no alcohol was delivered upon schedule completion. After meeting extinction criteria, reinstatement was precipitated by exposure to sandalwood odor in the operant chamber. NYX-783 was given once (0.1 or 6 mg/kg, IP, 1 hour prior to either the first extinction or reinstatement session).

Results: NYX-783 robustly facilitated extinction of conditioned fear after either single-trial fear conditioning or partially reinforced fear conditioning that is normally extinction-resistant. D-cycloserine was as effective as NYX-783 in facilitating extinction of conditioned fear regardless of the conditioning paradigm. However, D-cycloserine had no effect on spontaneous recovery of fear, whereas the single NYX-783 administration significantly reduced sponta-

n-

eous recovery of previously extinguished conditioned fear. In the alcohol self-administration study, restraint stress increased alcohol self-administration (compared to sham stress) and rendered alcohol-seeking behavior resistant to extinction. NYX-783 significantly facilitated extinction of alcohol-seeking behavior and significantly reduced reinstatement precipitated by the stress-paired odor cue. NYX-783 administered either before extinction or reinstatement was equally effective in blocking reinstatement of alcohol-seeking behavior primed by the stress-conditioned odor cue. Control studies indicate that NYX-783 did not affect locomotion or anxiety-like behavior, suggesting that the effects of NYX-783 on extinction and spontaneous recovery may be attributed to NMDAR-mediated enhancement of learning and memory processes rather than gross motor impairment or anxiolysis.

Conclusions: NYX-783 significantly facilitated extinction of conditioned fear across 2 rodent species and 2 conditioning paradigms. NYX-783 also reduced both alcohol-seeking behavior and relapse-like behavior precipitated by an odor cue previously associated with stress. Together, these data indicate that NYX-783 has potential to be a novel therapeutic for PTSD and comorbid AUD.

Keywords: PTSD, Alcohol, NMDAR

Disclosure: Financial compensation and stock options, Employee

M248

Hierarchical Cue Control of Cocaine Seeking in the Face of Cost

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Background: Drug addiction is characterized by intermittent, persistent drug seeking despite rising costs. Drug-associated cues are a powerful trigger of this behavior, capable of inciting relapse in recovering addicts. We set out to model three key aspects of human drug use in rats: the intermittent, binge-and-stop nature of drug intake, the motivational conflict of drug seeking in the face of escalating negative costs, and the ability of different types of drug cues to modulate seeking and spur relapse. Dopamine release within the nucleus accumbens core (NAc) has been implicated in cue-induced relapse of drug seeking. It is less clear, however, if dopamine signaling may encode hierarchical drug-related learning states where drug cues interact to guide seeking, and so we examined dopamine signaling during this self-administration paradigm, using fiber photometry.

Methods: We used an intermittent access paradigm, wherein rats (male and female Long Evans, $n = 25$) were trained to self-administer cocaine during brief drug available periods that are interspersed with longer epochs of no drug availability, within the same session. The drug available periods were signaled by a change in "global" cues comprising the context of the chamber. During these periods, transient "proximal" cues were presented contingent with a drug seeking response and coincident with each cocaine infusion. Following this, rats underwent a "conflict" phase, wherein they had to overcome a cost (crossing an electrified floor barrier) in order to continue to use cocaine. This cost escalated between sessions until drug seeking was suppressed. Rats then underwent relapse tests where we presented the proximal, drug-delivery associated cue in the presence or absence of the global drug availability cue to assess its ability to evoke relapse. In a separate group of rats, we measured changes in dopamine receptor activity within the NAc core with fiber photometry, using the genetically encoded dopamine sensor

dLight, during intermittent access self-administration and cue relapse tests.

Results: During intermittent access training, rats' behavior quickly came under the control of the drug availability cue, and they exhibited rapid, binge-like drug seeking during the availability period. By comparing intermittent access and conflict behavior, we found that greater cocaine binging history predicted persistence in the face of higher drug seeking cost. Following two weeks of abstinence, we next assessed the ability of the proximal, drug-delivery associated cues to trigger relapse despite the continued presence of cost. Proximal cues were presented noncontingently in the presence or absence of global cues that had signaled drug availability during intermittent access. Critically, we found that the ability of proximal cues to trigger relapse was gated by the presence of global drug available cues ($p < .05$), suggesting that hierarchical cue interactions exert an important modulating influence on drug-seeking motivation. Our preliminary fiber photometry data suggests that the dopaminergic signaling profile in the NAc core changes throughout intermittent access, as rats learned to pattern their intake in response to global signals of drug availability.

Conclusions: Together these results demonstrate hierarchical cue control of drug seeking despite cost, and point to a role for NAc core dopamine in this process.

Keywords: Addiction Circuitry, Dopamine, Cocaine Seeking

Disclosure: Nothing to disclose.

M249

Effects of Sleep Inconsistency Between Weekdays and Weekend on Task-Induced Brain Activation and on Resting State Functional Connectivity

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Background: Sleep deprivation and circadian disruptions impair brain function and cognitive performance. But limited studies have investigated the effect of inconsistent sleep duration and sleep time between weekdays (WD) and weekend (WE) caused by work-related sleep loss and circadian misalignment on WD and compensatory sleep on WE. Studies on sleep inconsistency, which have been mostly done in adolescents reported that it was associated with impaired attention and higher vulnerability to drug use. Here, we aimed to examine sleep inconsistency in adults and how it affects attentional performance, task-induced brain activation and resting-state functional connectivity (RSFC).

Methods: Fifty-six (43.9 ± 13.6 years, 26 male) healthy subjects participated. Differences between weekdays (WD) and weekend (WE) in terms of sleep duration and sleep midpoint were calculated using one-week actigraphy data. All subjects underwent 3Tesla BOLD-fMRI to measure brain activity during a visual attention task (VAT) and in resting-state condition (eyes open; 15 min). We controlled for age and gender effects in all analyses.

Results: WE-WD inconsistency of sleep duration and sleep midpoint were independent of each other ($r = .08$, $p = .58$) and differentially affected behavior, task brain activation and RSFC. In subjects who were experiencing mild work-related sleep restriction (WE-WD: 0.59 hours), larger WE-WD inconsistency of sleep duration (more WE catch-up sleep) predicted higher accuracy for high VA-load (3-ball: $\beta = .30$, $t = 2.35$, $p = .023$; 4-ball: $\beta = .30$, $t = 2.21$, $p = .032$; but not 2-ball: $\beta = .12$, $t = .83$, $p = .408$) and was associated with greater deactivation of the default mode network

(DMN) during the VAT ($p < 0.05$, cluster-corrected), and with stronger RSFC between posterior and parietal DMN ($p < 0.01$, cluster-corrected). In contrast, inconsistent WE-WD sleep midpoint (WE-WD: 1.11 hours), also referred to as social jetlag predicted lower accuracy for high VA-load (4-ball: $\beta = -.33$, $t = -2.42$, $p = .020$) and larger reaction time (2-ball: $\beta = .28$, $t = 2.06$, $p = .045$); and was associated with lower occipital activation during the VAT ($p < 0.05$, cluster-corrected), and with reduced RSFC between superior frontal gyrus and cerebellum ($p < 0.01$, cluster-corrected).

Conclusions: Our findings suggest that WE catch-up sleep helps recover from mild sleep loss. As greater DMN deactivation is associated with better performance in the VAT (Tomi et al., 2009), WE catch-up sleep might contribute to improved attention by enhancing task-induced deactivation of DMN and strengthening its connectivity at rest. In contrast, inconsistent sleep time had significant detrimental effects on behavior and in brain function during task and at rest. Our study provides evidence for the importance of consistent sleep time and of the beneficial effect of WE catch-up sleep in individuals with mild sleep loss.

Keywords: Sleep Homeostasis, Circadian Rhythms, Attention, Resting and Task fMRI, Sleep Inconsistency

Disclosure: Nothing to disclose.

M250

Hippocampal Activity Dynamics During Contextual Reward Association in Virtual Reality Place Conditioning

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Background: Exposure to environmental contexts associated with drug use can induce cravings that promote continued use and/or relapse. Opioid abuse is marked by high relapse rates, suggesting that contextual memories formed during opioid use may be particularly strong. While it is known that reward-seeking behavior is controlled by the mesolimbic reward circuit, little is understood about how contextual memories are altered by drug use. The dorsal hippocampus (dHPC) is necessary for multiple types of contextual learning and the place-specific activity of CA1 place cells map out space in a given environment.

Methods: Here we examined the neuronal representation of context as animals developed a natural reward and morphine-paired environmental associations using a conditioned place preference (CPP) paradigm. We designed a three chamber VR-CPP apparatus, based on the classical CPP apparatus, composed of two conditioning chambers with distinct visual cues. To investigate changes in the hippocampal encoding before, during, and after drug-pairing, we paired our virtual reality morphine CPP (Mor-CPP) paradigm with in vivo two-photon calcium imaging to record the activity of CA1 pyramidal neurons and, more specifically, place cells. Both male and female mice were used in this work.

Results: Here we provide evidence that VR environments are sufficient to establish opioid-induced contextual associations. The behavioral preference observed in the VR-CPP is similar to that generated using classical CPP approaches. We found increased neuronal activity, including more place cells, in water-rewarded contexts following real-time operant conditioning, but not after Mor-CPP training. Our results indicate different neural encoding mechanisms for natural reinforcers and morphine.

Conclusions: Here we present a powerful and flexible virtual environment for associating rewards with context. This platform,

in combination with two-photon imaging, allows us to monitor the activity of large neuronal ensembles during the formation of reward-associated memories. We propose that the long-lasting association between opioid use and environmental context arises, in part, from a fundamental alteration in place coding by pyramidal neurons in the dorsal hippocampus. This information may give rise to new targeted strategies for breaking the cycle of relapse that perpetuates the current opioid epidemic.

Keywords: Virtual Reality, Conditioned Place Preference, Memory Encoding and Retrieval, Dorsal Hippocampus, Opioid Use Disorder

Disclosure: Nothing to disclose.

M251

RCT of Gabapentin for Alcohol Use Disorder: Response Based on Alcohol Withdrawal History

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Background: Pharmacotherapies for Alcohol Use Disorder (AUD) do not work for everyone suggesting a more personalized approach to treatment is needed. Gabapentin has been reported to have mixed results in clinical trials, however past trials suggest that alcohol withdrawal (AW) status might be a crucial variable to consider in evaluating efficacy. We therefore conducted a randomized clinical trial of gabapentin in those meeting DSM-5 AUD criteria with AW. The level of AW was further evaluated as a predictor of treatment response.

Methods: 96 individuals (mean age 50 + /- 10, 77% male, 94% Caucasian), recruited from the community, meeting DSM-5 criteria for AUD with a history of AW symptoms with no other drug abuse (except nicotine or THC), nor significant current Axis 1 psychiatric disorder, except stable PTSD (26%). Participants met 4.5 SCID criteria for AW, had 83% heavy drinking days, and 11 drinks/day in the 90 days prior to screening. They were assessed for medical stability, alcohol severity, and administered a validated AW symptom self-rating form (Pittman 2007). After a minimum 3 days of abstinence, participants were randomized to gabapentin (total final dose 1200 mg/day in divided doses) (N = 45, 44 evaluable) or matching placebo (N = 50, 46 evaluable) for 16 weeks. During participation they received 9 sessions of medical management (20 min maximum) tailored to motivate compliance, review adverse events, and collect drinking data (TLFB). %dCDT, a sensitive and specific marker of heavy drinking, was obtained at baseline and 4 times during the study. The primary drinking endpoints were number of subjects with no heavy drinking days (NHD) and number with total abstinence (TA) with correction for %dCDT levels and analyzed with logistic regression. These and other drinking variables were evaluated as main effect of medication and interacting with pre-study self-rated level of AW symptoms using chi-square or linear mixed model analyses. Number needed to treat (NNT in favor of gabapentin) or harm (NNH in favor of placebo) were also calculated.

Results: Study completion rate (65%) was similar between medication groups. Overall, gabapentin-treated individuals did not relapse to heavy drinking ($p = 0.028$, OR 3.9, NNT=5.4) and had more TA ($p = 0.053$, OR 4.9, NNT=7) compared to placebo. However, when taking pre-study AW level into account (median split into low vs. high), within the high AW group, gabapentin had a very large positive effect on number of individuals reporting no heavy drinking days ($p < 0.02$, NNT=3) and produced more

individuals with TA ($p < 0.007$, $NNT=3$) compared to placebo, while within the low AW group, neither the number of individuals with no HD nor those with TA differed between gabapentin and placebo-treated individuals ($p = 0.67$, $NNH=25$ and $p = 0.32$, $NNH=23$, respectively). The interaction between medication group and AW symptoms was evident with % heavy drinking days ($p = 0.04$), percent day abstinent ($p = 0.02$) and Drinks per Day ($p = 0.07$) were considered, with gabapentin more efficacious than placebo in the high AWS group but not in the low AWS group. Gabapentin was well-tolerated with more gabapentin-treated individuals reporting dizziness of a mild to moderate nature than placebo ($p = 0.021$) and more overall complaints across study time points of nervousness ($p = 0.001$) and headache ($p = 0.002$) (none severe) while placebo-treated individuals reported more insomnia ($p = 0.001$).

Conclusions: These data add to a growing list of studies evaluating gabapentin for AUD treatment. Compared to past studies which had mixed results (Kranzler 2019) this study showed a positive effect of gabapentin on total abstinence, and for less heavy drinking days - two relatively conservative measures of medication efficacy. Compared to past studies the main difference in this study was that participants were chosen based on their past self-reported AW symptoms. Indeed, when levels of AW symptoms are taken into account, only those with the more intense symptoms benefitted from gabapentin. This finding is consistent with a number of previous reports suggesting gabapentin is useful for AW treatment and perhaps extending into the post-withdrawal period. Its unique pharmacology of altering voltage sensitive calcium channels with secondary effects on brain GABA and glutamate function is also consistent with its effectiveness in treatment-seekers with AW, who, perhaps also have protracted withdrawal states that would lead to early relapse drinking that could be diminished by gabapentin. Future studies should evaluate the role of sleep change, mood, and negative aspects of drinking on gabapentin response.

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Keywords: Alcohol Use Disorder, Pharmacotherapy, Alcohol Withdrawal, Gabapentin, Alcohol Relapse Treatment

Disclosure: Nothing to disclose.

M252

Sex Differences in Cue-Evoked Alcohol Seeking Following the Induction of Dependence in Rats

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Background: Alcohol dependence can drive escalated alcohol consumption and alcohol seeking, but the impact of dependence on neural and behavioral responses to alcohol cues is less well understood. We have previously found that voluntary, intermittent access, can potentiate neural responses to cues predicting sucrose availability (Ottenheimer et al., 2019). Here, we assessed the effects of chronic intermittent exposure to ethanol vapor on alcohol-seeking elicited by cues predicting alcohol availability, and the brain networks recruited by these cues.

Methods: Male and female Long-Evans rats ($n = 35$) were pre-exposed to 15% ethanol and then trained in a discriminative stimulus task. In this task, one auditory cue (the discriminative stimulus; DS) signals ethanol availability; if the rat enters the reward delivery port during the cue it receives ethanol. A control

cue (the neutral stimulus; NS) signals nothing. After learning to discriminate between these cues, half of the rats underwent chronic, intermittent exposure to ethanol vapor 14 hours a day, 4 days a week, for 3 weeks to induce alcohol dependence. Two weeks after the induction of dependence, rats underwent a cue probe test, in which the DS and NS cues under extinction conditions, to determine whether they would alter their responses to these cues. Rats then underwent a series of reacquisition sessions identical to training, in which ethanol was delivered when rats entered the port during the DS cue. Finally, rats underwent a final cue probe test, and their brains were processed to visualize Fos immunoreactivity to identify neurons activated by cues.

Results: As expected, we found that port entry likelihood was significantly greater following the DS than the NS, and when port entries during the DS were reinforced (in comparison to extinction conditions). The impact of vapor exposure on port entry probability depended on whether ethanol was available and differed by sex. When vapor-exposed rats were tested under extinction conditions, they showed disrupted cue discrimination, consisting of suppressed responses to the DS and potentiated responses to the NS ($F(1,31) = 4.25$, $p = 0.048$). In contrast, when port entries were reinforced with ethanol delivery, vapor-exposed animals did not differ from controls. Disrupted cue discrimination following vapor exposure was most robust in female rats ($F(1,31) = 4.24$, $p = 0.048$).

Conclusions: Together our results indicate that ethanol vapor exposure alters cue discrimination but does not potentiate the motivational value of ethanol cues. Deficits in cue discrimination appear strongest in vapor-exposed female rats, suggesting that this population may be more vulnerable to some alcohol-induced behavioral impairments. While cues elicited similar alcohol seeking behavior in vapor-exposed rats and controls, the neural networks recruited by these cues may be distinct.

Keywords: Alcohol, Dependence, Cues, Sex Differences

Disclosure: Nothing to disclose.

M253

Modeling Motivation for Alcohol in Humans Using Traditional and Machine Learning Approaches

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Background: Chronic use of alcohol can result in alcohol use disorder (AUD), a chronic relapsing disorder that is often untreated. Only a subset of alcohol users develop AUD. Individual variability in the development of AUD likely reflects the interaction between chronic alcohol use, as well as biological, psychosocial, and environmental risk factors. Recently, family history, male sex, and higher delay discounting impulsivity were found to be significant risk factors for high rates of binge drinking during an alcohol self-administration challenge. However, it remains unknown if other clinical variables are associated with alcohol self-administration phenotypes, which themselves may reflect risk factors for the development of AUD. This study aimed to examine clinical predictors of alcohol self-administration phenotypes in heavy drinking individuals.

Methods: Non-treatment-seeking heavy drinkers ($n = 67$; 36M/31F; age = 29.09 ± 6.56) completed an intravenous alcohol administration paradigm combining an alcohol challenge (target BrAC = 0.06 g/dl) and a progressive ratio alcohol self-administration (maximum BrAC = 0.12 g/dl). Growth curve analysis was conducted on the self-administration data to identify

self-administration phenotypes. Two follow-up analyses were conducted to characterize clinical variables that predicted cluster membership. First, a logistic regression was conducted using previously identified risk factors for AUD (sex, family history, and delay discounting impulsivity). Second, a series of random forest models, a machine learning approach, were run to identify significant clinical predictors.

Results: Two self-administration phenotypes were identified: (1) “motivated”, in which participants continued to work for alcohol throughout the self-administration session ($n = 41$); and (2) “unmotivated”, in which participants exhibited limited motivation to work for alcohol during the session ($n = 26$). In the logistic regression model, only delay discounting impulsivity significantly predicted self-administration phenotype ($B = -0.54$, $SE = 0.23$, $\chi^2 = 5.50$, $p = 0.02$). The three most important variables identified by the random forests for predicting alcohol self-administration phenotype cluster membership were phasic craving for alcohol, current AUD severity, and delay discounting impulsivity.

Conclusions: Clinical characteristics associated with risk for developing an AUD can predict alcohol self-administration phenotypes in non-treatment-seeking heavy drinkers. Specifically, higher delay discounting impulsivity, indicating a preference for smaller, sooner rewards over larger, later rewards, was predictive of a high motivation to work for intravenous alcohol in both the traditional and machine-learning models. The data-driven approach identified two additional variables which predicted group membership: phasic craving for alcohol and current AUD severity, such that greater phasic alcohol craving and less severe AUD diagnoses was predictive of the “motivated” phenotype. Together these results indicate that using data-driven approaches to investigate alcohol motivation represents a promising new tool to identify individual vulnerability for the development of AUD.

Keywords: Alcohol Self-Administration, Machine Learning Classification, Delay Discounting, Motivation, Alcohol Use Disorder

Disclosure: Nothing to disclose.

M254

Decreased Striatal Dopamine D2Rs Underlie Acute and Anxiolytic Responses to Ethanol in Mice

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Background: Several behavioral factors have been associated with the propensity to develop alcohol use disorder (AUD). In the clinical and animal literature, both the acute stimulatory and anxiolytic responses to alcohol are known to confer vulnerability for AUD. However, the underlying neural circuitry is unknown. The mesolimbic dopamine system has been implicated in AUD, with both humans and rodents showing low levels of dopamine D2 receptors (D2Rs) in the striatum. We hypothesize that this decrease in striatal D2R availability is a critical mechanism underlying the stimulatory and anxiolytic responses, ultimately leading to compulsive-like drinking.

Methods: To first identify the specific population of D2Rs involved in disordered ethanol consumption, we implemented genetically engineered mice lacking D2Rs on medium spiny neurons (MSNs) in the striatum or D2 autoreceptors on midbrain dopamine neurons. To explore the rewarding and reinforcing aspects of ethanol in these transgenic mice, we examined intake parameters. With evidence of aberrant ethanol intake in mice selectively lacking D2Rs on striatal MSNs, we examined the acute stimulatory and sedative effects of ethanol in these mice;

stimulatory effects were operationalized using ethanol-induced locomotion, while sedative effects were examined using the Loss of Righting Reflex (LORR) task. The anxiolytic effects of ethanol were examined in the transgenic mice and littermate controls using the elevated zero maze, light-dark box and open field. Finally, we used molecular and pharmacological approaches to understand the D2R-associated circuitry underlying the stimulatory and anxiolytic effects of ethanol.

Results: Mice lacking D2Rs on MSNs demonstrated higher preference for ethanol than littermate controls in a two-bottle choice test, increased relapse in a self-administration paradigm, resistance to adulteration with a bitter tastant. Together, these data indicate compulsive-like ethanol intake. Further, these mice show a significantly increased locomotor response to ethanol and more resilience to the sedative effects. Mice lacking striatal D2Rs also showed enhanced basal anxiety and a heightened anxiolytic response to ethanol. Thus, striatal D2Rs seem to be a common mechanism underlying both the acute stimulatory and anxiolytic responses to ethanol. However, we identified distinct circuitry underlying each behavior. We provide evidence of a mechanism in which low striatal D2Rs triggers D1R hypersensitivity, leading to a heightened acute stimulatory response to ethanol. However, this does not appear to be the mechanism by which D2Rs mediate the anxiolytic effects of ethanol, as a selective knockdown of dopamine D1Rs in the dorsal striatum attenuates the ethanol-stimulatory response but not the anxiolytic response.

Conclusions: Taken together, these data begin to describe neural circuitry underlying and perpetuating two behavioral responses known to confer vulnerability for AUD. While both behaviors share a common underlying neurobiological phenotype, we propose this is mediated by divergent circuitry. Finally, these data suggest that changes in striatal D2Rs predispose rodents to a sensitivity to the stimulatory and anxiolytic properties of ethanol, potentially ultimately driving compulsive-like intake.

Keywords: Ethanol, Alcohol, D2 Dopamine Receptor, Striatum, Dopamine

Disclosure: Nothing to disclose.

M255

Brain Wide Neural Networks Associated With Alcohol Abstinence in a Mouse Model of Alcohol Dependence

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Background: An unresolved issue is whether addiction is a brain disorder that is associated with pervasive neurobiological changes or a psychological failure that is associated with preserved brain function. A central feature of brain disorders, including dementia and traumatic brain injury, is a change in brain structure and function, including a decrease in modular functional architecture of the brain. However, it is currently unclear whether or not abstinence from alcohol dependence produces widespread changes to brain structure and organization. Furthermore, identification and quantification of the neuronal networks that are activated during alcohol abstinence is a critical step in the understanding of alcohol use disorder. Several brain regions have been identified as being recruited during withdrawal from alcohol drinking, however, previous work used manual cell counting and often a priori selection of region of interests, biasing and dramatically limiting the number of brain regions analyzed. These

issues can be addressed in a preclinical model of alcohol use disorder using novel techniques for whole brain imaging (iDISCO+) and graph theory to analyze network properties. Overall the goal of this study was to identify changes in neuronal structure.

Methods: We used a mouse model of alcohol dependence (two-bottle choice/chronic intermittent ethanol vapor) to induce alcohol dependence (measured by increased drinking and withdrawal symptoms). We collected brains from alcohol dependent ($n = 4$), nondependent ($n = 5$) and naive ($n = 5$) mice. Brains from alcohol dependent mice were collected 1 week into protracted abstinence from alcohol vapor. Brains wide neuronal activity was assessed using the whole brain clearing pipeline (iDISCO+ /ClearMap) to immunostain for the immediate early gene *c-Fos* and detect localization of *Fos* in individual brain regions. Network properties (e.g. functional connectivity, modular organization and hub brain regions of network function) were examined using Pearson correlations, hierarchical clustering, and graph theory.

Results: Abstinence in alcohol dependent mice resulted in increased drinking and signs of withdrawal (increased irritability-like and anxiety-like behavior). When examining brain wide neural function, we found that alcohol abstinence in alcohol dependent mice produced a major structural reorganization of the brain that was evident by the increased functional connectivity between brain regions throughout the brain. Further, a major decrease in modularity, when compared to controls, was found in the neural network of alcohol abstinent animals. The alcohol abstinent network contained 3 modules (a cortico-hippocampo-thalamic module, a midbrain striatal module, and an extended amygdala module), which closely resembled the hypothesized organization of brain regions proposed to be involved in addiction based on the three-stage theory of addiction. When we characterized the specific contributions of individual brain regions to the network associated with alcohol abstinence we found that a subset of brain regions, associated with the extended amygdala, are likely to drive network activity. These brain regions included several novel brain regions that have previously not been examined in addiction such as the intercalated amygdala, parasubthalamic nucleus, and tuberal nucleus.

Conclusions: The present study demonstrates that alcohol dependence and abstinence significantly decrease modularity and remodels the functional architecture of the brain into three major groups (i.e., a cortico-hippocampo-thalamic module, a midbrain striatal module, and an extended amygdala module), thus matching well the neurobiological three-stage theory of addiction better than any single addiction theory (incentive salience, hedonic allostasis, habit) for the organization of brain regions. Graph theory analyses identified existing and novel hub regions that may drive network dysfunction during alcohol abstinence. Altogether, these results provide a unique brain map of alcohol dependence and demonstrate that alcohol dependence, but not casual drinking completely remodels functional architecture of the brain. Such neuroadaptations strongly reinforce the brain disease model and may explain why addiction is such a pervasive disease and why relapse is so common.

Keywords: Addiction, Network-Analysis, Alcohol and Substance Use Disorders

Disclosure: Nothing to disclose.

M256

Sex Differences in Hippocampal CA3 Delta Opioid Receptors in Adult Rats are Differentially Altered by Acute delta9-Tetrahydrocannabinol Administration

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Background: Following oxycodone conditioned place preference (CPP), redistribution of opioid receptors principally mu (MOR) and delta (DOR) opioid receptors and changes in level of the endogenous opioid ligand Leu-enkephalin (LENK) in hippocampal circuits occurs to promote opioid-associative learning processes, particularly in females. The endocannabinoid (ECB) system and its cognate receptors (CB1R and CB2R) have previously been shown to interact with the opioid system to mediate the rewarding effect of MOR agonists such as oxycodone. Here we examined if acute exposure to the ECB agonist delta9-tetrahydrocannabinol (THC) results in a "priming" of hippocampal circuits for oxycodone associative learning.

Methods: All experiments and procedures were approved by the Rockefeller University IACUC and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Adult male and female Sprague-Dawley rats (Charles River, 10 weeks old on arrival, $n = 6$ /group) were group housed (2-3/cage) in a stress minimized animal facility with ad libitum access to food and water on a standard 12h:12h light cycle (lights on at 7a). Animals received either 5 mg/kg THC or vehicle (7.75% Tween20 in saline; i.p.) 1h prior to perfusion done with acrolein + paraformaldehyde. Dorsal hippocampal sections ($n = 3$ /group) were then processed for dual label electron microscopy of DOR [silver-intensified gold (SIG)] and GABA (ABC). Dendritic morphometry was determined using MCID. DOR-SIG particles were counted based on three locations [on plasmalemma, near plasmalemma (within one average particle distance away from plasmalemma), or cytoplasmic]. Statistical analyses were performed using JMP.

Results: In CA3 stratum radiatum (SR), a baseline sex difference was observed. Vehicle injected female rats had increased total DOR-SIG particle density ($p = 0.04$) in large dendrites ($1.0 < x < 2.5 \mu\text{m}$) compared to vehicle injected males. Following acute THC administration, females showed a significant reduction in total ($p = 0.01$) and cytoplasmic ($p = 0.02$) DOR-SIG particle densities in large CA3 SR dendrites compared to vehicle injected females. In contrast, following acute THC administration, DOR-SIG density ($p = 0.05$) and partitioning ratio ($p = 0.01$) of near plasmalemma particles in large CA3 SR dendrites was significantly increased in male rats compared to vehicle injected males and not significantly different from vehicle injected females. Moreover, when THC is administered females had no change in DORs in CA3 dendritic spines contacted by mossy fibers while THC males had reduced spines compared to vehicle injected males ($p = 0.05$).

Conclusions: Consistent with our prior studies, drug-naïve female rats' hippocampal DORs are positioned in hippocampal circuits that promote opioid dependent long-term potentiation and are "primed" for opioid agonist associative learning. Following acute THC administration in females, THC reduced CA3 SR DOR densities potentially due to trafficking of cytoplasmic DOR to the plasma membrane to maintain synaptic DOR density. Conversely, following THC, DOR appears to be trafficked from CA3 mossy fiber spines to SR dendrites near the plasmalemma where they could enhance plasticity processes. This redistribution of hippocampal opioid receptors in THC injected-males suggesting that acute THC may "prime" males for opioid associative learning. Overall, acute exposure of THC was still able to effect hippocampal DOR trafficking in both males

and females but DOR redistribution only in males occurred to promote plasticity processes.

Keywords: Sex Differences, delta9-tetrahydrocannabinol, Delta Opioid Receptor, Hippocampal CA3

Disclosure: Nothing to disclose.

M257

Contributions of Mesoaccumbal GABA Projections to Adaptive Reward Learning and Incubation of Cocaine Craving

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Background: The ventral tegmental area (VTA) contains GABAergic interneurons and projection neurons, the latter of which constitute approximately one third of mesoaccumbal projections. Yet, the functional significance of these GABA projections in cue processing is relatively unknown. Recently, we used a combinatorial viral approach to target activating designer receptors exclusively activated by designer drugs (DREADDs, hM3d) to glutamate decarboxylase 1 (GAD1)-positive neurons in the VTA of rats. Global chemogenetic activation of VTA GABA neurons decreases motivation for reward-predictive cues in an operant model reinforced by a natural reward (e.g. sucrose). Surprisingly, activation of the dense VTA GABA projections to the nucleus accumbens (NAc) alone, by microinfusion of CNO directly into the NAc, did not change motivation for the cues. In the current study, we sought to determine if VTA GABA neurons projecting to the NAc regulate how reward predictive cues influence learning new reward contingencies, as well as the ability of cocaine-paired cues to drive incubated cocaine seeking after extended forced abstinence.

Methods: To examine adaptive reward learning, we used a cue-dependent operant task where well-trained rats were probed in a session in which the magnitude of the natural reward (i.e. sucrose) was unexpectedly altered within session ($n = 23$). We chemogenetically activated VTA GABA terminals in the NAc of male rats (300 nL of 1 μ M of clozapine n-oxide, CNO), and compared this to saline pretreated and hM3D-free (mCherry) controls. For incubation of cocaine craving, a cohort of male rats ($n = 17$) were trained to self-administer cocaine (0.5 mg/kg/inf) for 6 hours/day for 14 days, and then re-exposed to the operant chambers and cocaine-paired cues after early (1-3 days) and late (30-32 days) forced abstinence. We microinfused CNO or vehicle at either early or late forced abstinence test days, in both hM3D containing and mCherry control rats.

Results: All data were analyzed by repeated measures ANOVA. Activation of VTA GABA terminals in the NAc enhanced adapting to when the reward value was unexpectedly decreased, an effect characterized by a significant decrease in responding over time within the session. Whereas responding was maintained after saline pretreatment and in mCherry control rats. Likewise, in the incubation of cocaine craving experiment, mCherry controls exhibited a significant increase in responding between early and late forced abstinence periods. While microinfusion of CNO into the NAc did not change responding during early forced abstinence (1-3 days), it significantly attenuated the enhanced responding with extended forced abstinence at day 30-32. Under these conditions, rats emitted the same number of responses as during the 1-3 days of forced abstinence period after vehicle and in mCherry control rats. CNO alone had no effect in mCherry control rats in either experiment.

Conclusions: Our data indicate that mesoaccumbal GABA neurotransmission causally contributes to reward learning, independently from reward-seeking mediated by cue salience. Further, activation of these projections prevents the expression of incubation of cocaine craving, indicating that mesoaccumbal GABA projections regulates cocaine induced adaptations in cue processing.

Keywords: Adaptive Behavior, Incubation of Cocaine Craving, Ventral Tegmental Area (VTA), Nucleus Accumbens, GABA

Disclosure: Nothing to disclose.

M258

Examining Partial Versus Full mGlu5 Negative Allosteric Modulators on Cocaine-Related Behaviors and Brain Function Using Electroencephalography in Rats

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Background: There remains no FDA-approved medications for Cocaine Use Disorder (CUD). Glutamate-mediated synaptic plasticity changes within the mesocorticolimbic circuit contribute to hyper-responsiveness to drug-related cues that contribute to relapse. Moreover, glutamate is involved in regulating other behaviors associated with abstinence that are associated with relapse potential including anhedonia, anxiety, and sleep disturbances. Receptor function and localization suggest that antagonism of the metabotropic glutamate receptor subtype 5 (mGlu5) is a promising potential treatment for multiple aspects of CUD. mGlu5 negative allosteric modulators (NAMs) can attenuate cocaine self-administration (SA) and cue- or drug-induced increases in previously extinguished responses following SA (reinstatement model of relapse) in animals. However, full mGlu5 NAMs may also engender dose-limiting adverse effects. Partial mGlu5 NAMs block less than 100% of the effects assessed in vitro when compared to a full mGlu5 NAM, at concentrations that fully occupy the allosteric binding site on mGlu5. Partial mGlu5 NAMs, represented by M-5MPEP, may still produce positive effects in preclinical models of CUD without the adverse effects observed with full mGlu5 NAMs. Here, we describe studies comparing behavioral effects of the partial mGlu5 NAM M-5MPEP with the full mGlu5 NAM, VU0424238 in rodent models of CUD. Further, using electroencephalography (EEG) studies in freely moving rats, we are evaluating effects of these full and partial mGlu5 NAMs on sleep architecture and arousal alone, and to reverse cocaine-induced changes to understand dose-effect relationships between behavior and brain function. Examining effects of mGlu5 NAMs on cocaine-maintained behaviors and cocaine-induced brain changes will provide valuable insight into the pharmacotherapeutic potential of partial versus full mGlu5 NAMs for treating CUD.

Methods: Male ($n = 55$) and female ($n = 27$) Sprague-Dawley rats implanted with indwelling intravenous catheters were used for behavioral studies. Rats were trained to lever press to SA 0.5 mg/kg/infusion of cocaine under a 5-response, fixed ratio (FR5) schedule, during daily 2-h sessions followed by 10 days of 6-hr extended access SA. Each SA session was paired with a vanilla odor cue and each infusion was paired with a 10-sec light cue presentation. Following extended access SA, responding was extinguished during daily 2-hr sessions in which saline was substituted for cocaine, the odor cue was absent, and the light cue

was not presented following completion of each ratio. Once responding was reduced by $\geq 80\%$ compared to the first day of extinction training, the light and odor cues were reintroduced in a single cue-induced reinstatement (CIR) test, followed 3 days later by a cocaine-induced reinstatement session (10 mg/kg, IP administered immediately prior to the session). To examine the ability of the mGlu5 NAMs to attenuate reinstatement, 18-56.6 mg/kg M-5MPEP, 3-30 mg/kg VU0424238 or vehicle (10% Tween 80) was administered (IP) 30 min prior to each reinstatement session. Following cue and cocaine-induced reinstatement tests, cocaine SA was continued under a progressive ratio (PR) schedule of reinforcement. Lastly, rats responded under the same PR schedule when sucrose pellets were available as the reinforcer and effects of M-5MPEP and VU0424238 were evaluated.

For electroencephalography studies, rats are implanted with surface electrodes in contact with the dura above the frontal and contralateral occipital cortex. EEG is recorded for 24 consecutive hours in freely moving rats within their homecage. Effects of 18-56.6 mg/kg M-5MPEP, 3-30 mg/kg VU0424238 or vehicle (10% Tween 80) will be evaluated on sleep duration (REM, NREM, and Wake time) and brain function using quantitative EEG (qEEG). Based on behavioral data, effects of 56.6 mg/kg M-5MPEP and 30 mg/kg VU0424238 will be evaluated to attenuate 10 mg/kg (IP) cocaine. All experiments were approved by the Vanderbilt and Wake Forest University Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. For all studies, ANOVAs were used for statistical analysis to examine differences from respective vehicle-treated control groups and, when appropriate, followed by post-hoc Bonferroni t-tests.

Results: In behavioral studies, M-5MPEP and VU0424238 dose-dependently attenuated cue-induced reinstatement. VU0424238 but not M-5MPEP attenuated 10 mg/kg cocaine-induced reinstatement. VU0424238 dose-dependently attenuated the reinforcing strength of cocaine across several unit doses of cocaine, although the highest dose tested (30 mg/kg) significantly decreased food-maintained responding. Only the highest dose of M-5MPEP (56.6 mg/kg) attenuated the reinforcing strength of a low dose of cocaine. Ongoing EEG studies will help establish a relationship between behavioral outcomes and brain function.

Conclusions: These results indicate that partial negative allosteric modulation of the mGlu5 receptor may be sufficient to attenuate some cocaine-related behaviors modeling multiple aspects of substance use disorder without adverse effects associated with full mGlu5 NAMs. Together, behavior and EEG data will further our understanding regarding the degree of functional antagonism of mGlu5 required for potential therapeutic effects without dose-limiting side effects and the utility of mGlu5 NAMs for the treatment of CUD.

Keywords: Cocaine Self-Administration and Reinstatement, Electroencephalography, Metabotropic Glutamate Receptor 5 (mglu5)

Disclosure: Nothing to disclose.

M259

In Vivo Studies of the Role of ERK1/2 Phosphatase MKP3 in Dopaminergic Neurons on Cocaine-Associated Dopamine Signaling, Gene Expression and Behavior

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Background: Abundant evidence indicates that repeated exposure to cocaine results in cellular and molecular changes in the mesolimbic dopamine system, reorienting behavior towards drug seeking and drug use. Despite this knowledge, there are no FDA-approved pharmacotherapies for cocaine use disorder, suggesting that we need a more detailed understanding of the neurobiology that underlies cocaine addiction. Cocaine exerts its addictive effects by blocking the dopamine transporter (DAT), leading to excess dopamine (DA) in the synaptic cleft, resulting in the euphoric "high" that is often sought-after during addiction. The ERK1/2 Map Kinase signaling pathway has been implicated in the locomotor-stimulant effects of psychostimulants such as cocaine, as well as the sensitization effects induced by repeated cocaine administration. It is imperative to identify specific downstream targets of this pathway to reveal novel therapeutic targets for treating cocaine use disorders. In this study we describe the modulation of the ERK1/2 pathway in vivo by specifically expressing the ERK1/2 phosphatase MKP3 only in dopaminergic neurons of the ventral tegmental area (VTA).

Methods: We have generated adeno-associated viral (AAV) vectors with Cre recombinase (Cre)-dependent expression of MKP3 resulting in a decrease of the ERK1/2 signaling specifically in DA cells of the ventral tegmental area (VTA). This construct was stereotactically injected into the VTA of male Long Evans rats expressing Cre in tyrosine hydroxylase positive cells (TH-Cre rats). A cre-dependent AAV expressing green-fluorescent protein was used as an experimental control. A cocaine conditioned place paradigm was used to assess the drug-seeking behaviors of MKP3-overexpressing rats. For CPP, a three-chamber apparatus with visually and tactilely distinguished contexts, separated by a removable partition, was used following an unbiased design. To examine the effects of inhibiting intracellular ERK1/2 signaling on the risk of engaging in behaviors associated with cocaine addiction, we performed self-administration. Rats were given access to cocaine-paired levers for limited access FR1 sessions (twenty 0.75 mg/kg cocaine injections max per session). Rats were then trained to self-administer on a PR schedule of reinforcement in which single cocaine (0.75 mg/kg) injections were contingent on a progressively increasing number of lever responses. Fast Scan Cyclic Voltammetry (FSCV) was performed in vivo to examine the effect of modulating intracellular ERK1/2 signaling on DA neurotransmission. TH-cre rats were anesthetized and placed into a stereotaxic apparatus. Recording and stimulating electrodes were lowered into the NAC core and VTA respectively, until stable DA release was achieved. Following 30 min of baseline collection, an i.v. injection of cocaine (1.5 mg/kg) was delivered and resulting changes in DA signaling were monitored. Viral Translating Ribosome Affinity Purification (vTRAP) and RNA-Seq utilized to characterize the translating mRNAs in DA neurons within the VTA to study cell-specific molecular phenotypes after modulating ERK1/2 signaling.

Results: We have demonstrated that inhibiting ERK1/2 signaling in DA neurons of the VTA decreases cocaine-seeking behaviors in a model of conditioned placed preference as well as the motivation to obtain cocaine via a progressive-ratio schedule of reinforcement during self-administration studies. FSCV determines that inhibition of ERK1/2 signaling in DA neurons influences the function of the dopamine transporter (DAT) in the nucleus accumbens (NAC). Also, studies utilizing vTRAP and resulting RNA-Seq data demonstrate upregulation of dopaminergic genes, such as tyrosine hydroxylase and the dopamine transporter, which has been confirmed via biochemistry experiments.

Conclusions: These studies indicate that intracellular ERK1/2 signaling in DA neurons influences behaviors associated with cocaine use, as well as DA neurotransmission and gene expression. We hypothesize that ERK1/2's role in these events may

underlie the long-term molecular changes that characterize the behavioral manifestations of addiction. These studies will enable the identification of novel targets that may have therapeutic potential.

Keywords: ERK, Cocaine Addiction, Drug Abuse, Phosphatase

Disclosure: Nothing to disclose.

M260

Recapitulating Phenotypes of Alcohol Dependence via Oprk1 Overexpression in Non-Dependent Rats

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Background: The kappa-opioid receptor (KOR) that has dynorphin (DYN) as an endogenous ligand is an important regulator of dopamine (DA) neurotransmission implicated in motivation, emotion and executive function. Our laboratory and others have identified that alcohol dependence dysregulates DYN and the KOR in a manner that promotes multiple symptoms of dependence including escalated alcohol self-administration. Based on our assessment of Oprk1 (gene for the KOR) mRNA expression and DYN A-stimulated GTPyS data in non-dependent and alcohol dependent rats, neuroadaptations involving mesolimbocortical DA projections originating in the ventral tegmental area (VTA) were implicated as a basis for escalated alcohol self-administration during acute withdrawal.

Methods: We utilized transgenic TH::Cre rats that have Cre recombinase under the control of the tyrosine hydroxylase (TH) promoter in conjunction with a floxed Oprk1 viral construct to test the hypothesis that overexpression of Oprk1 in the VTA of male and female alcohol non-dependent TH::Cre rats would increase operant alcohol self-administration. Initial phenotyping of male and female TH::Cre rats for operant alcohol self-administration confirmed normal non-dependent alcohol self-administration ($n = 7$ / sex), as well as normal dependence-induced escalation of self-administration ($n = 7$ / sex) during acute withdrawal following intermittent alcohol vapor exposure. Following optimization of viral infusions involving confirmation of VTA TH+ immunostaining / viral EYFP overlap via fluorescent microscopy and, importantly, RT-qPCR confirmation of viral construct-induced increases in Oprk1 mRNA expression in the VTA of both male and female TH::Cre rats ($n = 8$ / sex), the effect of inducible overexpression of Oprk1 in non-dependent TH::Cre rats, conditionally in VTA DAergic neurons, on operant alcohol self-administration was assessed. Male and female TH::Cre rats ($n = 20$ / sex) were trained for operant alcohol self-administration until stable responding during 30-min limited-access sessions was achieved, separated into groups ($n = 10$ / grp) matched for alcohol self-administration and site-specifically infused with an active floxed Oprk1 viral construct (AAV5-Ef1a-OPRK1-DIO-EYFP) or a control viral construct (AAV5-Ef1a-DIO-EYFP). Beginning four weeks after viral infusions, the non-dependent TH::Cre rats were again allowed to self-administer alcohol and water in 30-min limited access sessions.

Results: The results indicated that both male and female non-dependent TH::Cre rats infused with the active floxed Oprk1 viral construct in the VTA demonstrated escalated operant alcohol self-administration over a two-week period of testing when compared to TH::Cre rats infused with the control construct or those infused with active viral construct outside of the VTA (main effect of condition, $p < 0.01$). Furthermore, RT-qPCR assessment showed that VTA Oprk1 mRNA expression in the active viral infusion

animals that displayed escalated alcohol self-administration was significantly increased compared to the control infusion animals ($p < 0.001$). Lastly, water self-administration was equivalent ($p > 0.05$) for both the active and control viral infusion groups.

Conclusions: These data support the hypothesis that dysregulation of KOR signaling within the mesolimbocortical DA system is an important contributor to symptoms of alcohol dependence. Importantly, these data also posit that differential levels of Oprk1 expression could be an important contributor to maladaptive behavioral regulation in alcohol non-dependent organisms. Collectively, these data identify that understanding Oprk1-mediated contributions to alcohol use disorder should be an important future goal.

Keywords: Ventral Tegmental Area (VTA), Dopaminergic System, Oprk1 Gene Expression, Kappa Opioid Receptor, Alcohol Self-Administration

Disclosure: Nothing to disclose.

M261

A Superiority Trial of Varenicline Plus Naltrexone: Preliminary Drinking Outcomes

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Background: Individually, both naltrexone and varenicline have shown to reduce alcohol intake in clinical trials, albeit with moderate effects. To advance medications development and harness the effects of combination pharmacotherapy, the present clinical trial tested the combination of varenicline (VAR, 2mg) and naltrexone (NTX, 50 mg) compared to varenicline plus matched placebo in a treatment-seeking sample of heavy drinking smokers. It was hypothesized that the combination pharmacotherapy would be superior to monotherapy in reducing alcohol consumption during the trial (NCT02698215).

Methods: Treatment-seeking daily smokers (5+ cigarettes per day) who were also heavy drinkers (7/14 drinks per week for females, respectively) enrolled in the trial. Participants were required to express a desire to quit smoking and to reduce or quit drinking. A total of 450 individuals were screening for eligibility and 165 were randomized to either (1) VAR plus NTX or (2) VAR plus matched placebo. Participants were assessed at baseline, 4, 8, 12, 16, and 26 weeks post randomization. Active medication was provided for the initial 12 weeks. Initial analyses considered drinking days and drinks per drinking day at the 12-week endpoint, controlling for baseline drinking.

Results: For the outcome of drinks per drinking day, participants in the varenicline plus placebo group reported 3.29 drinks (SD = 3.72) at baseline and that number was reduced to 2.75 (SD = 3.01) at 12-week ($t = -1.44$, $p = .15$). The varenicline plus naltrexone group reduced drinks per drinking day from 3.17 (SD = 3.40) at baseline to 2.54 (2.91) at 12-week ($t = -1.39$, $p = .17$). Results for drinking days suggests a reduction from 2.44 (SD = 2.31) to 2.06 (2.23) drinking days per week in the varenicline plus placebo group ($t = -2.22$, $p < .05$), while the varenicline plus naltrexone group reported virtually no change in drinking days (baseline = 2.34 and 12-week = 2.30) ($t = 0.12$, $p = .90$). Analyses are ongoing and include multivariate models, intent-to-treat, and additional outcomes such as percent heavy drinking days.

Conclusions: Initial results comparing these two active treatments do not show a clear benefit of the combination of varenicline plus naltrexone versus naltrexone alone for the drinking reduction in this sample of heavy drinking smokers.

Ongoing analyses using more complex models as well as models that integrate smoking cessation outcomes will provide a more complete understanding of the combined effects of these pharmacotherapies used for both the treatment of smoking cessation and drinking reduction.

Keywords: Smoking Cessation, Alcohol Drinking, Clinical Trial

Disclosure: Nothing to disclose.

M262

Optogenetic Stimulation of the Prelimbic Cortex to Nucleus Accumbens Core Pathway Reverses Cocaine-Induced Deficits in Behavioral Flexibility

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Background: Substance use disorders (SUDs) are characterized by the inability to stop reward-seeking behaviors despite maladaptive consequences. Indeed, prior history of cocaine leads to impaired behavioral flexibility in rats. We have previously shown that neural encoding in the nucleus accumbens (NAc) core and in the prelimbic cortex (PrL), during learning predicted subsequent behavioral flexibility in a reinforcer devaluation task (West and Carelli 2016). PrL sends dense, glutamatergic projections to the NAc core, and coherent activity across these regions, particularly at high gamma frequencies, during learning also predicts subsequent behavioral flexibility. In addition, specifically suppressing transmission in PrL-NAc core neurons during learning (using retrograde Cre virus in the NAc core and Cre-on halorhodopsin virus in the PrL) is sufficient to induce deficits in the ability to shift behavior post-devaluation. Finally, prior exposure to cocaine, followed by a month of experimentally imposed abstinence, suppresses coherent activity in the PrL-NAc core connection and induced deficits in flexibility compared to controls. Here, we used a reinforcer devaluation task following a history of cocaine to determine if optogenetic stimulation of the PrL-NAc core pathway is sufficient to reverse behavioral flexibility deficits following prior drug exposure.

Methods: We used Long-Evans male rats ($n = 14$) and all experimental protocols in animal were approved by the IACUC at the University of North Carolina and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. First, we used a viral paradigm to transfect channelrhodopsin (ChR2 or mCherry) in PrL neurons that directly project to NAc core (retrograde Cre virus in NAc core and Cre-on ChR2 virus in the PrL). In a second surgery, 4 weeks later, we implanted optical ferrules into PrL and intrajugular catheters for i.v. administration. Rats ($n = 8$ ChR2 and $n = 6$ mCherry control) self-administered cocaine (cocaine (14 days; 2-hr self-administration; 0.33 mg/inf, ~25 mg/kg), followed by 18 days of abstinence. High frequency (83Hz) blue laser was delivered via optical ferrules into the PrL for 3 days (10s on/10s off, 20 cycles/day; days 18-20 of abstinence) to excite the PrL-NAc core pathway. Next, rats underwent Pavlovian conditioning over 10 days. On each daily session, one conditioned stimulus (CS + 1) predicted food, while a different conditioned stimulus (CS + 2) predicted sugar, while two other stimuli did not predict anything (CS-1 and CS-2). Next rats underwent a conditioned taste aversion paradigm in which sugar pellets were paired with Lithium Chloride (0.3 M), while food pellets were paired with saline. Finally, on a post-devaluation test session, we measured the rats' ability to avoid the cue paired with sugar pellets (i.e., CS + 2) under extinction.

Results: Both m-Cherry and ChR2 rats self-administered the same amount of cocaine during self-administration ($t = 0.98$, $p > 0.10$). In addition, rats both groups spent more time in the foodcup during the CS + (both CS +) compared to the CS - (both CS-) after 10 days of Pavlovian conditioning (mCherry. Day vs CS interaction $F(3,15) = 58.96$, $p < 0.0001$, ChR2, $F(3,21) = 28.28$, $p < 0.0001$). Critically, there was a significant impairment in behavioral flexibility (measured as a devaluation index, $ND-D/ND + D$, West and Carelli 2016) in mCherry rats compared to ChR2 rats suggesting that optical stimulation prior to learning and testing restored cocaine-induced behavior flexibility deficits as measured by reinforcer devaluation (unpaired t-test, $t = 2.789$, * represents $p = 0.0164$).

Conclusions: These findings suggest that the PrL connection with the NAc may serve as a putative target for drug-induced deficits in behavioral flexibility. Ongoing studies will test the utility of non-invasive, translatable methods to target this region and restore behavioral flexibility deficits post-cocaine.

Keywords: Cocaine, Nucleus Accumbens Core, Prelimbic Cortex

Disclosure: Nothing to disclose.

M263

Real Time fMRI Neurofeedback for Downregulation of Food and Alcohol Craving

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Background: Real time fMRI neurofeedback (rt-fMRI-NF) guided neuromodulation has in recent years demonstrated encouraging ability in modulating neural substrates associated with depression and substance use disorders. In a proof-of-concept study of patients with depression (Linden et al. 2012) participants were able to upregulate the ventrolateral prefrontal cortex and insula which significantly improved their clinical symptoms. Real time fMRI-NF has also enabled cocaine users to downregulate the ventral tegmental area, VTA (Kirschner et al., 2017). In smokers, nicotine craving was reduced via downregulation of ventral anterior cingulate, vACC (Brady et al., 2015). Social heavy alcohol drinkers have been able to downregulate their ventral striatum, VS, responses to alcohol cues (Kirsch et al., 2016). Also, individuals with AUD modulated neural response to alcohol cues in anterior cingulate, ACC, and dorsolateral prefrontal cortex, dlPFC, or anterior insula, AI, which corresponded to reduced craving (Karch et al., 2015). We have piloted a multi-ROI approach in an effort to allow inter-subject variability not only in the localization of the modulated region but also the selection of regions themselves.

Methods: Preliminary analysis was conducted in adult volunteers (13 controls and 8 AUD, 12 females) who completed a magnetic resonance imaging protocol. Four runs of rt-fMRI neurofeedback craving control training were conducted using the realtime plug-in for AFNI. For each run, baseline measures of craving and non-craving were assessed using a cue-reactivity test. Two cue types were used: food pictures for healthy control volunteers and alcohol pictures for participants with alcohol use disorder. BOLD fMRI data was processed in real-time using AFNI to measure and compute the signal change in each of the 5 pairs of ROIs: right and left VTA/substantia nigra (SN), AI, VS, dlPFC, and ACC/mPFC, which established the neurofeedback stimulus display. The stimuli display for rt-fMRI-NF consisted of craving cue images alongside a brain activity "thermometer". A composite index was calculated from the ROIs as part of the computation in

real time to provide feedback to the participant on a 100 point "thermometer" scale.

Results: In examining the signal changes across the four runs in each of the ROIs offline, we found that all participants improved their ability to volitionally control cue reactivity related neural activity bilaterally in ACC, AI, VS, and dIPFC. However, activity in VTA/SN did not seem responsive to volitional control.

Both alcohol and healthy control groups were able to down-regulate craving related activity. Moreover, we observed AUD hyperactivation compared to controls when contrasting successful downregulation versus unsuccessful downregulation trials. The hyperactive regions included fusiform gyrus and right anterior insula.

When comparing the craving for food and alcohol in healthy controls and AUD subjects, respectively we found activation in right hippocampus for alcohol craving in contrast to neutral stimuli. Healthy control volunteers were not able to up-regulate food craving.

Conclusions: Results from this pilot sample indicate multi-ROI rt-fMRI-NF did enable subjects to reduce craving related neural signal over 4 runs. This corresponded to additional engagement in the fusiform and right anterior insula. Though implicated in face and color recognition, the fusiform gyrus has been shown in some studies to be activated in response to dopamine release. A rich body of literature has shown the involvement of dopamine in reward system and its association with substance use disorders. Previous studies have also implicated anterior insula in the development and maintenance of AUD. The anterior insula change across runs suggests that neurofeedback training for craving may work by affecting the salience of alcohol cues.

The involvement of hippocampus in alcohol craving might be pointing to these participants reminiscing about alcohol consumption. We are currently implementing a support vector machine version of this approach which enables us to utilize the signal in the entire brain to provide feedback to the participants.

Keywords: Real-Time fMRI neurofeedback, Alcohol, Craving, Multi-ROI

Disclosure: Nothing to disclose.

M264

Amperometric Analysis of Nucleus Accumbens Core Glutamate and Nitric Oxide Levels During Cued Cocaine Seeking

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Background: Drug addiction is a neuropsychiatric disorder characterized by high relapse rates despite extended periods of abstinence and profound negative consequences. Preclinical models of drug self-administration and reinstated seeking demonstrate that dysfunctional glutamate transmission between the prelimbic (PL) cortex and nucleus accumbens core (NAcore) is directly linked to relapse. Specifically, cue-induced reinstatement of cocaine seeking relies on increased extracellular glutamate in the NAcore. Recently, activation of nNOS interneurons has been shown to be critical for the induction of the synaptic plasticity in the NAcore mediating relapse. However, it has yet to be demonstrated that cue-induced cocaine seeking in rats elevates extracellular nitric oxide (NO). Further, it is currently unknown what glutamate inputs are required to engage NO release and cued relapse.

Methods: Head mounted second-by-second amperometric recordings of glutamate and NO in freely-moving rats were performed during cue-induced reinstatement of cocaine seeking. Rats self-administered cocaine in two-hour daily sessions, were given head caps and implanted with cannula, then went through extinction training and cued reinstatement testing. Recordings were also combined with pathway-specific DREADD-based manipulation using a combinatorial viral vector approach. In this design, Cre-dependent viruses (mCherry or DREADD) were infused into the PL, and a retrogradely transported viral vector expressing Cre was infused into the NAcore, prior to cocaine SA. To observe the impact of pathway manipulation on glutamate, NO and drug seeking behavior, CNO was administered 30 mins prior to the reinstatement session. DREADD testing prior to reinstatement was conducted with ip injections of 1 mg/kg CNO.

Results: Amperometric measurements of cue-induced glutamate in the NAcore demonstrate an increase of ~1µM in cocaine SA rats (effect size 3.0). Animals self-administering saline also show a discernible rise of ~250 nM during cue-induced reinstatement, but only during the first 10 minutes, even though saline animals showed no clear motivation (i.e. lack of reinstatement). Temporally, we observed a significant increase in glutamate relative to baseline in as little as one minute during cue-induced reinstatement but, interestingly, this is evident in both cocaine and saline SA rats; with no significant divergence until ~10 minutes into reinstatement. Amperometric measurements of cue-induced NO in the NAcore demonstrate an increase of ~8 nM in the NAcore (effect size 2.8). Animals self-administering saline also show a discernible rise of ~4 nM NO during cue-induced reinstatement, yet unlike glutamate recordings do not return to baseline levels during the session. Here we observed a significant increase in NO relative to baseline two minutes into cue-induced reinstatement. Again, this was evident in both cocaine and saline SA rats; with no divergence until ~12 minutes into reinstatement for NO recordings. Our preliminary data also indicates that inhibition of PL cortex-NAcore neurons prevents cue-induced glutamate and cocaine seeking, and experiments are underway to examine pathway-specific regulation of cue-induced NO release.

Conclusions: Here we reproduce previous microdialysis data demonstrating cue-induced glutamate release in the NAcore during relapse testing, on a second-by-second sample rate, in awake animals. We also extend these findings to NO, demonstrating for the first time that conditioned cue exposure and reinstated cocaine seeking engages NO release in the NAcore. Taken together, our data indicate that cue-induced NO release likely occurs temporally second, as a consequence of increased glutamate levels in the NAcore. Finally, our preliminary data suggests that the PL cortex is likely the primary driver of this effect.

Keywords: Drug Relapse, Nitric Oxide, DREADDs, Glutamate, Cocaine

Disclosure: Nothing to disclose.

M265

A Dorsal Raphe to Nucleus Accumbens Medial Shell Circuit Underlies Mu-Opioid Receptor Control of Motivation

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Background: Overdose deaths involving opioids have skyrocketed nationally over the last 5 years. Most highly addictive opioids preferentially act on mu opioid receptors (MORs), which are found throughout the central and peripheral nervous system. One major site of MOR action is nucleus accumbens medial shell (NAc). Here, MOR activation has been shown to enhance the motivation for both natural and drug rewards. Despite the powerful effects of MOR activation in NAc on motivated behaviors, the mechanisms underlying their effects are largely unknown. Here, we sought to determine where, when, and how MORs mediate motivated behaviors to better understand how they may become modified in addictive states.

Methods: We used a multidimensional approach to pinpoint the mechanisms of MORs within NAc medial shell. Specifically, we used localized and systemic pharmacology, constitutive, conditional and cell-type specific genetic knockout/knockin models, fluorescent in situ hybridization (FISH), viral and fluorescent dye tracers, opto and chemogenetic modulation, selective CRISPR-Cas9 guided deletion, and in vivo imaging. Behaviorally, mice were tested on a food intake task in which they were allowed to freely consume sucrose pellets for one hour on two different test days. On one test day, mice were tested ad libitum (baseline motivation), and on the second, they were tested after 24hrs of food deprivation (enhanced motivation). MORs systems were modulated during each test session to determine whether they were necessary or sufficient for baseline or enhanced motivated states.

Results: We found that MOR constitutive knockout or local microinjections of the MOR antagonist CTAP in NAc prevented 24hr food deprived enhancement of intake, but did not affect ad libitum intake. FISH experiments showed that MORs are predominantly expressed on dynorphin/D1 and enkephalin/D2 medium spiny neurons. To determine whether MORs preferentially act on one population, we crossed *Oprm1*fl/fl mice with dynorphin or enkephalin-cre mouse lines to selectively delete receptors from that particular cell type. We found that the loss of MORs on enkephalin, but not dynorphin, neurons resulted in decreased hunger-enhanced intake. To verify the NAc localization, we deleted MORs from NAc neurons via local viral microinjections. Surprisingly, we did not observe a decrease in hunger-enhanced intake. In contrast, retrograde deletion of MORs from neurons that project NAc resulted in reduced hunger-enhanced intake. Cumulatively, these results suggest that NAc MORs are on presynaptic enkephalinergic NAc afferents. Retroviral tracing in enkephalin-cre mice showed robust labeling in lateral dorsal raphe nucleus/ventrolateral periaqueductal gray (IDRN/vIPAG), indicating that this may be a site of interest. FISH analyses revealed that MORs were expressed on more than 50% of IDRN enkephalin neurons, that enkephalin and serotonin neurons are largely separate populations, and that enkephalin and vGAT are highly colocalized, indicating that the enkephalinergic projections to NAc are long range GABA projection neurons. To test the functionality of this pathway, we infused a cre-dependent MOR rescue virus directly into DRN of MOR knockout crossed with enkephalin-cre mice. We found that selective rescue of MORs was sufficient to restore hunger-enhanced intake. To further determine whether MORs were specifically acting on DRN-NAc terminals, we injected the calcium indicator GCaMP6s into DRN and placed a photometry optic fiber into NAc to measure changes in fluorescence during ad libitum or food deprived intake. Preliminary results show that DRN enkephalin terminals increase their activity as mice begin to eat, and peak as mice finish eating. During food deprived states, this increase is delayed. This delay may be related to MOR activity, as it can be prevented by preadministration of naloxone. To test the functional role of MORs on the terminal, we used the light-activated opto-XR oMOR. Initial tests show that activation of oMOR on DRN-NAc terminals was sufficient to drive intake. Finally, we used of combination of DREADD inhibition/excitation, cell-type

specific caspase deletion, and virally mediated peptidergic deletion of dynorphin or enkephalin to demonstrate that local NAc D2/enkephalin neurons use enkephalin to activate MORs on DRN-NAc terminals to increase food intake during food deprived states.

Conclusions: These results indicate that a novel GABAergic, enkephalinergic, non-serotonergic projection from IDRN to NAc express MORs on its terminal afferents which, when activated, enhance motivated behaviors. Additionally, these terminal MORs are engaged by locally released NAc enkephalin. Collectively, these experiments pinpoint one of the principal means through which MORs in NAc can powerfully modulate motivated behaviors. Future studies could evaluate how this system changes in response to abused opioids (e.g., fentanyl), or how motivated systems change in response to chronic pain.

Keywords: Opioid system, Nucleus Accumbens Shell, Molecular Genetics, Neural Circuit and Animal Behavior

Disclosure: Nothing to disclose.

M266

Topiramate Attenuates Neural Responses to Alcohol Cues: A Randomized, Double-Blind, Placebo-Controlled Pharmacogenetic Study in European-American Heavy Drinkers

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Background: Globally, alcohol consumption contributes to over 3 million deaths, 132.6 million disability-adjusted life years, and 5% of the burden of disease and injury annually. In the United States, over 15.1 million adults aged 18 and older have an alcohol use disorder (AUD). Despite these concerning statistics and the availability of FDA-approved medication to treat AUD, few patients are treated with medications. In part, the reluctance of physicians to prescribe alcohol treatment medications and of patient to take them stem from the medications' limited efficacy. Thus, several medications have been prescribed off label and have shown promise in reducing alcohol consumption. Topiramate, a GABA/glutamate modulator, has shown promise in reducing alcohol consumption. Both preclinical and clinical studies show that topiramate is effective in reducing heavy drinking. In the current study, we integrated genetic, neuroimaging, and pharmacological approaches to evaluate the potential therapeutic benefits of topiramate for reducing heavy drinking by examining topiramate's effects on heavy drinking and neural responses to alcohol cues.

Methods: Twenty-two patients (13 topiramate, 9 placebo) participating in a larger, randomized, double-blind, placebo-controlled pharmacogenetic study (NCT02074904) completed two functional magnetic resonance imaging sessions (one prior to randomized, one following six weeks of study medication). Using linear mixed models, the primary model evaluated neural responses to alcohol cues compared to nonalcohol cues with a within-subject factor for time and between-subject factor for medication and the interaction (FWE-corrected at $p < 0.05$).

Results: Analyses revealed a significant medication X time interaction in two clusters located in the bilateral striatum with individuals who received topiramate showing significant reductions in cerebral blood flow in the bilateral striatum during exposure to alcohol cues (versus nonalcohol cues) following 6 weeks of topiramate treatment (max dose 200 mg/day).

Reductions in striatal blood flow correlated with reductions in heavy drinking days ($r = 0.59$, $p = 0.05$).

Conclusions: This is the first study to explore topiramate's effects on neural responses to alcohol cues and heavy drinking. Relative to placebo, topiramate reduced alcohol-cue elicited neural responses in the bilateral striatum, and this reduction correlated with reductions in heavy drinking days. These findings provide additional support for topiramate's efficacy in reducing heavy drinking and provide a potential mechanism for topiramate's effects on alcohol consumption.

Keywords: Functional MRI (fMRI), Cue Reactivity, Topiramate

Disclosure: Nothing to disclose.

M267

DREADDed to the Core: Opposite Effects of DREADDs on Binge-Like Drinking in Male and Female Mice

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Background: The nucleus accumbens (NAc) is important for regulating a number of behaviors, including alcohol and substance use. Promising clinical trials have shown that deep brain stimulation of the NAc decreases alcohol craving and relapse in alcohol dependent male subjects. Much of what we know about the NAc is based on studies carried out in males. We studied the effects of excitatory and inhibitory DREADDs in the NAc on binge-like drinking in female mice. We found that increasing activity specifically in the NAc core reduced binge-like drinking, and decreasing activity in the NAc core increased drinking in female C57BL/6J mice. Here, we investigated whether manipulating NAc core activity would produce similar results on binge drinking in male C57BL/6J mice.

Methods: Mice were stereotactically injected with AAV2 hSyn-HA hM3Dq (excitatory), -hM4Di (inhibitory), or -eGFP (negative control) bilaterally into NAc core and allowed to recover for 3 weeks. We first tested the effects of altering NAc activity on binge-like ethanol intake ("Drinking in the Dark", DID). We subsequently measured intake of sucrose, quinine, and water using a serial drinking in the dark ($n = 11-15/\text{group}$). Procedures were carried out as described in Purohit et al., 2018. During the first week of ethanol DID, mice were pre-treated with vehicle to establish baseline levels of DID intake, and in week 2, mice were treated with CNO (1 mg/kg) 15-30 minutes prior to DID to determine the effects of changing NAc core activity on drinking. Two weeks later, serial testing was carried out to measure the effects of CNO on intake of other fluids. Mice were treated with CNO (1 mg/kg) 15-30 minutes prior to being offered limited access to quinine (1st week), sucrose (2nd week), and water (3rd week; each solution was offered for 2 hour/day, 4 days/week).

Results: Increasing NAc core activity via CNO/hM3Dq significantly increased binge-like ethanol drinking ($p < 0.01$), whereas decreasing activity via CNO/hM4Di significantly decreased binge-like drinking in male mice ($p < 0.01$). For serial tastant testing, we found that increasing NAc core activity increased quinine intake ($p < 0.01$), and increased water intake over the 4 days of testing ($p < 0.01$). We did not observe significant effects on inhibitory DREADDs on fluid intake.

Conclusions: We find that it is possible to bi-directionally modulate binge-like drinking by manipulating NAc core activity, and note opposing effects in male and female mice. These observations are fascinating and suggest there exist sex differences in NAc core contributions to binge-like alcohol drinking.

Keywords: Binge Drinking, DREADDs, Nucleus Accumbens Core, Sex Differences

Disclosure: Nothing to disclose.

M268

Cannabis Use Disorder Prevalence Among Medical and/or Recreational Cannabis Users: Initial Estimates for United States Adults, 2015-2017

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Background: For cannabis-only users in the United States (US), the estimated cumulative (lifetime) occurrence of drug dependence syndromes with social maladaptation is modest (~2%), versus ~16% when cannabis use has broadened to use of other drugs (e.g., cocaine). While numbers of medical cannabis users are growing, little is known about consequences of such use, with or without other drug use. This study aims to fill a current gap in evidence about the occurrence of cannabis use syndromes, via contrasts of adults with medical-only use, recreational-only user, and adult users with both medical and recreational use.

Methods: The US study population of community male and female residents was sampled for the 2015-2017 National Surveys on Drug Use and Health (NSDUH). Among interviewed adults (18+ years), standardized self-interviews identified 1,318 with repeated medical-only use, 17,767 with repeated recreational-only use, and 929 repeatedly using for medical and/or recreational motives. We used survey analysis weights and Taylor series calculus to estimate prevalence and 95% confidence intervals (CI) for active DSM-IV cannabis use disorder (CUD) occurrence in these subgroups, and used multiple logistic regression to account for cannabis onset age.

Results: Overall, recently active CUD was tangibly more prevalent among adults who had used 6+ times for both medical and recreational motives (16%), with a 95% CI not overlapping with CI for medical-only and recreational-only users, for whom CUD prevalence was 9%-10% ($p < 0.001$). Age of cannabis onset (elapsed time since 1st use) does not account for this variation, but with stratification to focus on cannabis-only users and to exclude use of Internationally Regulated Drugs (IRD) other than cannabis, the estimates show no prevalence differences across these subgroups. Extending the regressions, we now are exploring estimates for medical + recreational users also have used IRD other than cannabis (e.g., cocaine, opioids), which might account for the originally observed 16% versus 9%-10% difference.

Conclusions: Among adults with recent repeated cannabis use (6+ uses), a marker of greater prevalence (and odds) of active CUD is using this drug for both recreational and medical motives. This novel but still superficial finding requires attention to confounding influences other than age-of-onset (controlled here via regression), especially use of IRD other than cannabis, which is now being studied for its potential influence on cannabis outcomes. If confirmed, an evidence-based approach for cannabis prescribing will include careful assessment of patients with cannabis and CUD histories, and may need to consider emerging evidence on histories with IRD other than cannabis. Biases (e.g., self-report) deserve attention in continuing research on CUD and other potential hazards (or benefits) of medical and/or recreational cannabis use.

Keywords: Cannabis, Cannabis Use Disorder, Polydrug Use, Medical Marijuana

Disclosure: Nothing to disclose.

M269

Behavioral and Neural Indices of Inhibitory Control are Related to Alcohol-Induced Stimulation and Sedation

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Background: Poor inhibitory control is a risk factor for drug and alcohol use disorders. Evidence from both animal and human studies suggests that this increased risk could be due in part to greater sensitivity to drug reinforcement. In line with this, we recently showed that individuals with poor inhibitory control and less right frontal brain engagement during motor inhibition report greater euphoria and stimulation following a single dose of amphetamine. Here we tested the degree to which these findings generalize to another drug of abuse, alcohol.

Methods: In the behavioral phase of the study, participants ($n = 69$ men and women) performed the stop signal task, a behavioral measure of inhibitory control, and then participated in four experimental sessions in which they received alcohol (0.8 g/kg, oral) or placebo, in alternating order. They completed the Biphasic Alcohol Effects Scale to assess alcohol-induced stimulation and sedation at 30-minute intervals over 2.5 hours. Then in the imaging phase, subjects completed an fMRI scan to assess neural correlates of inhibitory control using the same stop signal task, adapted for use during fMRI.

Results: In the behavioral phase, linear mixed effects models showed that longer stop signal reaction time (indicating poor inhibitory control) was associated with greater stimulation ($t = 2.19$, $p = 0.029$) and less sedation ($t = 2.55$, $p = 0.011$) following alcohol compared to placebo. In the fMRI phase, correlational analyses showed that less brain engagement in the right middle frontal gyrus during response inhibition [stop > go] was associated with greater stimulation following alcohol ($r = -0.255$, $p = 0.037$). Additionally, less brain engagement in the supplementary motor area during inhibition was associated with less sedation following alcohol ($r = 0.298$, $p = 0.014$).

Conclusions: These findings show that poor inhibitory control at the behavioral level and reduced pre-frontal and pre-motor engagement during inhibition at the neural level are associated with a risky alcohol response profile (i.e., greater positive, stimulant-like effects of alcohol and less negative sedative-like effects). This extends our previous findings showing a similar association between poor inhibitory control and less right frontal brain engagement during inhibition and greater sensitivity to amphetamine reward. Together, the findings show that inhibition and reward are closely linked. Further, they provide novel behavioral and neural targets for prevention and treatment of substance use disorders aimed at simultaneously improving inhibitory control and dampening subjective responses to drugs of abuse.

Keywords: Alcohol, Brain Imaging, fMRI, Inhibitory Control

Disclosure: Nothing to disclose.

M270

Effects of Exenatide and Cocaine on Plasma Levels of GLP-1, Insulin, and Amylin Among Human Cocaine Users

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Background: Several peptides which modulate food intake and satiety also regulate behavioral responses to drugs of abuse. One of these is glucagon-like peptide 1 (GLP-1). Preclinical studies show that synthetic analogs of GLP-1 (i.e., exenatide – 4) decrease cocaine self-administration, reinstatement of cocaine seeking, and cocaine-induced reward. In addition, clinical studies demonstrated their capacity to facilitate weight loss. However, the mechanism of action underlying such effects on food intake/drug reward is not well understood. One possibility is that GLP-1 may mediate its effects, in whole or in part, through actions on other peptides, including insulin and amylin (i.e., GLP-1 promotes insulin release and insulin is co-secreted with amylin).

Preclinical studies on the relationship between cocaine administration and plasma levels of these hormones have been mixed. Clinical studies showed that a single intravenous (IV) injection of cocaine reduced both GLP-1 and insulin levels (and trend reductions were observed for amylin as well). However; to date, no studies have examined the effects of binge self-administration of cocaine. The current study explored relationships between self-administered cocaine, treatment with the GLP-1 receptor agonist exenatide, and plasma levels of GLP-1, Insulin, and Amylin.

Methods: Subjects with Cocaine Use Disorder (CUD) underwent three inpatient human laboratory sessions using a cocaine self-administration paradigm that included a single training/habituation session and two experimental sessions following acute pre-treatment with exenatide (5 mcg) and placebo (in randomized order). Each experimental session began with a drug-drug interaction (DDI) phase consisting of 3 serial, fixed-order, nurse initiated bolus cocaine injections (4, 8, 16 mg/70kg) followed by 1.5 hours of ad libitum self-administered intravenous (IV) boluses (16 mg/70 kg) of cocaine according to a fixed-ratio 1 schedule. Outcomes consisted of plasma levels of GLP-1, insulin, and amylin at baseline (T1), after the first bolus of cocaine (T2), and at the end of ad libitum cocaine self-administration (T3). Peptide levels were analyzed with commercially-available ELISA kits and statistical analyses were performed with SAS using mixed models testing for treatment effect (i.e., exenatide vs. placebo) and time effect (i.e., T1 vs. T2. Vs. T3).

Results: Of the 13 individuals who enrolled in the study, data was collected from 12 (1 female, 10 African American (AA), 1 White Hispanic (WH); 45 ± 7 yrs). Significant effects of treatment were observed for GLP-1 (Den DF = 55, $F = 4.65$, $P = 0.03$) and insulin (Den DF = 55, $F = 5.69$, $P = 0.02$) levels, which were lower following exenatide as compared to placebo. A significant effect of time was also found for GLP-1 (Den DF = 55, $F = 18.43$, $P < 0.0001$), insulin (Den DF = 55, $F = 18.43$, $P < 0.0001$), and amylin (Den DF = 50, $F = 14.82$, $P < 0.0001$), in which levels were lower after cocaine administration.

Conclusions: To our knowledge, this is the first human study to examine the effects of exenatide on binge cocaine-induced changes in GLP-1, insulin, and amylin levels. Results show that exenatide and binge cocaine independently reduce levels of GLP-1 and insulin and that binge cocaine also reduces amylin levels. These results expand our knowledge about how cocaine affects peripheral metabolic hormones using a naturalistic pattern of binge self-administration. Future work is required to understand how amylin/insulin and/or their interactions with GLP-1 are involved in the neurobiology of drug intake.

Keywords: Cocaine, GLP-1, Amylin, Insulin

Disclosure: Nothing to disclose.

M271

NOS1 in the Interpeduncular Nucleus Regulates Behavioral Tolerance to Drugs of Abuse**Jessica Ables*, Zainab Oketokoun, Purva Bali, Molly Heyer, Paul Kenny***Icahn School of Medicine at Mount Sinai, New York, New York, United States*

Background: Previously we demonstrated that nitric oxide synthetase 1 (NOS1) in ChRNA5-expressing cells in the IPN is upregulated by chronic nicotine exposure and disrupting Nos1 in the IPN abolishes place preference for nicotine. Our data suggests that nitric oxide inhibits glutamate release from the Hb, thereby reducing the strength of aversion mediated by the Hb and leading to the development of tolerance to the aversive effects of nicotine. Others have demonstrated that systemic administration of a NOS1 inhibitor can prevent the development of analgesic tolerance to opioids, reverse established tolerance, as well as prevent and reverse withdrawal to chronic opioids. Our studies are now focusing on whether development of tolerance to other drugs, including alcohol and opioids, coincides with an increase in Nos1 in the IPN. We are investigating whether forced versus voluntary administration affects induction of Nos1 in the IPN by nicotine. Further, we are developing tools to directly visualize NO in behaving animals to determine if the release of NO from the IPN may affect the function of VTA neurons to promote tolerance to the rewarding aspect of drugs in addition to the aversive aspects.

Methods: All experimental protocols were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. To assess NOS1 levels after forced chronic nicotine, C57Bl6J mice were given 2% saccharin or 2% saccharin + 500mg/L nicotine tartrate in the drinking water for 6 weeks ($n = 5/\text{group}$). To assess NOS1 levels after voluntary chronic nicotine, mice underwent IV self-administration of escalating doses of nicotine over a period of about 8 weeks. Similarly, to assess NOS1 levels after forced or voluntary chronic opioids, C57Bl6J mice were implanted with minipumps containing either saline or oxycodone (3 mg/kg/d) for 10 days, or assessed after IV self-administration of escalating doses of oxycodone. Mice were perfused with 4% PFA and sections were stained with anti-NOS1 antibody and fluorescent intensity measured. To determine baseline behavioral effects of NOS1, C57Bl6J mice were injected with control virus ($n = 10$) or virus expressing shRNA against Nos1 ($n = 10$) in the IPN. Two weeks after viral injection, animals underwent a behavioral battery, including progressive ratio for a natural reward. To visualize NO in behaving animals, we acquired a plasmid containing a fluorescent NO sensor and cloned the sensor into both constitutive and Cre-dependent AAV vectors.

Results: Chronic exposure to nicotine in the drinking water increases both transcript ($p_{\text{adj}}=0$, DSeq2) and protein levels ($p = 0.04$, t-test, pixel intensity, $n = 5/\text{group}$) of NOS1 in the IPN. Preliminary data from IV self-administration of nicotine suggests that increased NOS1 in the IPN is independent of route. Studies with oxycodone are underway. At baseline, knock-down of Nos1 in the IPN results in a modest increase in anxiety measures ($p = 0.06$, t-test, open field; $p = 0.07$, t-test, light/dark, $n = 5/\text{group}$), but does not significantly affect motivation for natural reward ($p = 0.2$, t-test, progressive ratio, $n = 7/\text{group}$). The genetically encoded NO sensor expresses well and is responsive to a NO donor in N2A cells. The Cre-dependent vector is not leaky.

Conclusions: Development of addiction proceeds through several stages, including development of tolerance and escalation

of dose. Here we provide evidence that NO production in the IPN can regulate behavioral tolerance to multiple drugs of abuse.

Keywords: Nitric Oxide, Interpeduncular, Substance Abuse Disorders

Disclosure: Nothing to disclose.

M272

Behavioral and Transcriptomic Adaptations Induced by Neonatal Opioid Exposure in Outbred Mice**Emily Yao, Kristyn Borrelli, Will Yen, Qiu Ruan, Melanie Chen, Julia Kelliher, Julia Scotellaro, Richard Babbs, Jacob Beierle, William Johnson, Elisha Wachman, Alberto Cruz-Martin, Camron Bryant****Boston University School of Medicine, Boston, Massachusetts, United States*

Background: Opioid Use disorder (OUD) is an epidemic in the United States and has led to a dramatic increase in opioid-dependent mothers giving birth to neonates exhibiting opioid withdrawal – referred to as Neonatal Opioid Withdrawal syndrome (NOWS). NOWS is characterized by a set of hallmark features, including weight loss, irritability, inconsolability, insomnia, and increased pain sensitivity. The neurobiological basis of NOWS is largely unknown but is thought to comprise neurobiological adaptations that are distinct from opioid withdrawal exhibited in adolescence and adulthood. While several rat models for NOWS have been reported, there are very few NOWS models reported in mice. Because mice remain the premiere model organism for mammalian genetic studies, the development of NOWS models for mice will facilitate mechanistic and potentially treatment discovery.

Methods: We treated neonatal outbred mice from the Cartworth Farms White (CFW; Swiss Webster stock) with either saline (SAL; 20 ml/kg; s.c.) or morphine sulfate (MOR; 15 mg/kg, s.c.) twice daily from postnatal day (P)1 through P14 which is the approximate third trimester-equivalent in humans. Weight loss was monitored and behavioral symptoms associated with opioid withdrawal were measured on P7 and P14 at 16 h post-MOR, including locomotor activity, ultrasonic vocalizations, sensitivity to the thermal nociception on the hot plate assay (52.5°C) and the tail withdrawal assay (48.5°C). Additionally, we assessed self-righting as a developmental milestone on P4 and P7. A minimum sample size of $n = 12$ per Treatment was employed based on preliminary data in the hot plate assay indicating an effect size of Cohen's $d = 1.4$ which gave us 80% power to detect differences ($p < 0.05$). Behavioral data were analyzed using mixed-model ANOVAs with Treatment and Sex as factors and Day or Time as a repeated measure. Post-hoc t-tests were employed to detect the source of main effects and interactions and Bonferroni-adjusted p-values were employed to report significant differences in behavior. Brainstem tissue from 12 mice (6 SAL, 6 MOR; sex-balanced) was collected 16 h post-final injection on P15 and processed for transcriptome analysis via mRNA sequencing (RNA-seq) using oligo-dT-selected libraries, a single lane on a NovaSEQ6000 two-lane flow cell, and 100 bp single-end reads yielding an average of 37 million clusters per sample. Differential gene expression analysis was conducted using limma and edgeR ($p < 0.05$). "Sex" was included as a covariate in the combined analysis. Additionally, females and males were analyzed separately. "Family" was always included as a covariate to account for variance across cages. Enrichment and network analyses were conducted using Enrichr and Ingenuity Pathway Analysis (IPA).

Results: Repeated MOR induced profound weight loss from P1 to P14 that persisted at P21 and P50. Additionally, MOR mice exhibited a delayed latency to self-right at P4 and a robust, female-specific delay at P14. MOR females but not males showed a marked increase in USVs per min on P7 during the first 5 min of assessment. Furthermore, both MOR-treated sexes showed a profound increase in USVs per min on P14 relative to SAL mice, supporting a developmental delay. With regard to nociception, MOR mice exhibited thermal hyperalgesia at P7 and P14, with females showing greater sensitivity to hyperalgesia earlier on at P7. MOR mice also exhibited greater anxiety-like behavior at P21 as indicated by reduced locomotor activity and time spent in the center arena of the open field. For brainstem transcriptome analysis of the sex-collapsed data, enrichment analysis identified “morphine addiction” as the top pathway that included GABA-A receptor subunit genes (*Gabra6*, *Gabrae*), protein kinase C genes (*Prkcg*), adenylate cyclase 1 (*Adcy1*), and phosphodiesterase genes (*Pde4c*, *Pde4d*). Top downregulated genes included the methyltransferase gene *Prdm6*, the protocadherin gene *Pcdha9*, the protein kinase C gene *Prkcg*, *Serpina3m*, *Chil3*, *S100a8*, *Krt25*, *Hoxc6*, and *Kremen2*. MOR also induced a downregulation of the locus coeruleus transcription factor *Onecut3* and the norepinephrine transporter (*Scl6a2*). Top upregulated genes included *Melk*, *Cdc45*, *Samd3*, *Nlrc5*, *Tdgf1*, *Iitm2a*, *Eomes*, *Gabra6*, and *Cnpy1*. For female-only analysis, the top five enrichment terms were Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, thermogenesis, and oxidative phosphorylation which comprised genes largely coding for mitochondrial proteins. Notably, for females only, one of the top upregulated genes was the dopamine transporter (*Slc6a3*). For male-only analysis, the top five enrichment terms were insulin secretion, circadian entrainment, retrograde endocannabinoid signaling, GABAergic synapse, and cholinergic synapse which comprised opioid signaling genes (ryanodine receptors, calcium channels, potassium channels, and G-protein subunits).

Conclusions: This study describes a drug regimen and behavioral battery for examining the neural substrates of NOWS-like behavioral symptoms in mice induced by third trimester-equivalent morphine exposure and support the hypothesis that the neurobiological mechanisms of NOWS may be in part distinct from adult opioid withdrawal and importantly, that the neurobiological mechanisms of NOWS may differ between females and males.

Keywords: Neonatal Abstinence Syndrome, Heroin, Buprenorphine, Methadone, Sex Differences

Disclosure: Nothing to disclose.

M273

Role of Type-2 Corticotrophin-Releasing Factor Receptor in the Regulation of Dopamine and Glutamate Extracellular Levels in Nucleus Accumbens and Prefrontal Cortex by Stimulation of the Rat Basolateral Amygdala

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Background: The basolateral amygdala (BLA) innervates nucleus accumbens (Nac) and prefrontal cortex (PFC). Interestingly, McDonald (1991) showed that at least 75% of BLA neurons project to both Nac and PFC. However, most studies have evaluated the impact of BLA stimulation in either Nac or PFC.

Methods: We decided to study the role of type-2 corticotrophin-releasing factor receptors (CRF2) in the regulation of glutamate (GLU) and dopamine (DA) extracellular levels in both

PFC and Nac induced by BLA stimulation, using in vivo microdialysis. In addition, we have done anatomical experiments to evaluate whether CRF2 are expressed in PFC and Nac nerve terminals by immunofluorescence in synaptosomes devoid of postsynaptic elements.

Results: The local infusion of antisauvagine 30 (aSv30), CRF2 antagonist, in the Nac induced a significant increase in DA and GLU extracellular levels induced by the stimulation of BLA. Similarly, infusion of aSv30 in PFC also increased DA and GLU extracellular levels induced by the stimulation of BLA. We observed a different temporal pattern for the response in both studied nuclei. In the case of PFC, the increase in DA and GLU was observed in the same 10 min of BLA stimulation. Instead, in the Nac, the increase in DA and GLU was observed 10 minutes after BLA stimulation. The anatomical data show that CRF2 is present in Nac and PFC synaptosomes devoid of presynaptic elements.

Conclusions: Our results suggest that CRF2 exerts a differential control over GLU and DA extracellular levels induced by BLA stimulation in NAC and PFC. Our results reveal the complex role of CRF2 in regulating the neurotransmission of Nac and PFC by the BLA.

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Keywords: Corticotropin-Releasing Factor (CRF), Dopamine, Glutamate

Disclosure: Nothing to disclose.

M274

Optical Stimulation of Basolateral Amygdala Glutamate and/or VTA Dopamine Inputs to the Nucleus Accumbens Shell Differentially Restore Motivation-Related Encoding in Cocaine-Experienced Rats

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Background: Repeated experience with drugs of abuse like cocaine can induce lasting deficits in the function of motivation-related neural circuits, and the resulting behavioral deficits from these neural disorders likely contribute to cycles of addiction and relapse. In rat models of drug-induced motivational impairments, we and others have repeatedly shown that cocaine self-administration induces a variety of deficits in motivation-related signals in the nucleus accumbens (NAC). For example, neurons in the NAC core and shell fail to develop phasic neural responses to associative reward-paired cues over several days of learning, an observation that was coupled with these rats’ inability to appropriately use learned motivational information in new settings. Likewise, cocaine-experienced rats displayed abnormal phasic dopamine release in the NAC to both associative cues and reward discrimination. However, it is not known the extent to which dopamine signaling deficits contribute to downstream miscoding in NAC target neurons. Indeed, multiple limbic targets – including the basolateral amygdala (BLA) – appear to also be impaired following cocaine experience, and glutamatergic inputs from this region to the NAC may also significantly contribute to impairments in NAC motivational encoding. We hypothesized that stimulation of these impaired pathways to the NAC shell during associative learning could restore encoding and thus rescue motivated behavior.

Methods: To test this, we injected channelrhodopsin (ChR2) into the VTA only (AAV5-Ef1a-DIO-ChR2-EYFP), BLA only (AAV5-CAMK2-ChR2-mCherry) or both VTA and BLA. An optrode (optical fiber surrounded by electrophysiological wires) was then implanted in the NAC shell to allow for stimulation of afferents

and recording of NAc neural activity. TH::Cre and cre-negative littermate control rats in a Pavlovian-to-Instrumental Transfer (PIT) task. Following two weeks of either cocaine (1 mg/kg/inf; 2hr/d) or water self-administration and period of abstinence (30d), rats then learned a Pavlovian discrimination where one food-paired cue (CS+) was paired with optical stimulation (473nm light, 20mW, 20Hz), while a second cue (CS-) was presented without stimulation or food. Following this, rats then received instrumental training for several days on a variable interval schedule (up to VI60). On the PIT test day, rats were allowed to press under extinction and received presentations of the CS+ and CS-. Following PIT test days, rats were given the opportunity to optically self-administer light to the NAc via an instrumental response for five consecutive 30min sessions.

Results: Cocaine-experienced rats that did not express any ChR2 showed significant impairments in PIT relative to drug-naïve controls, failing to press more during CS+ than the pre-cue baseline. In contrast, cocaine-experienced rats that received stimulated VTA-ChR2 dopamine displayed a restoration of PIT behavior to normal levels. However, neither BLA-ChR2 stimulation nor BLA + VTA-ChR2 stimulation was effective at restoring PIT behavior. Consistent with this, we saw significantly restored phasic neural signals in the NAc shell of rats who received VTA-ChR2 stimulation, but not in BLA-ChR2 or BLA + VTA-ChR2. Finally, in the subsequent optical self-administration sessions, VTA-ChR2 rats vigorously self-administered light, but this was reduced in BLA + VTA-ChR2 rats and absent in BLA-ChR2 rats.

Conclusions: These data suggest dopamine, but not BLA glutamate, is sufficient to rescue motivation-related encoding and related behaviors following drug experience, and may provide insights into potential treatments for cognitive and affective disorders in the human addiction condition.

Keywords: Ventral Tegmental Area (VTA), Associative Learning, Dopamine, Basolateral Amygdala, Electrophysiology

Disclosure: Nothing to disclose.

M275

How Dopamine Release Alters Human Brain Networks: Insights From Simultaneous PET-fMRI

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Background: Methylphenidate (MPH), a stimulant medication used for treatment of attention-deficit hyperactivity disorder, exerts therapeutic effects by increasing dopaminergic/noradrenergic signaling, thereby altering downstream functional brain networks. However, imaging methods used to probe these processes in humans, including PET and fMRI, each have distinct limitations in the type of information they can provide. Simultaneous PET-fMRI has the potential to overcome the limitations of each method and give novel insights underlying MPH's therapeutic efficacy. Here we present preliminary evidence from simultaneous PET-fMRI with oral and IV MPH administration.

Methods: Eight healthy adults (five males, three females) completed three sessions of simultaneous PET-fMRI with [11-C] Raclopride to assess Dopamine D2/3 receptor availability in the following conditions: 1) oral MPH (60 mg) and IV placebo, 2) oral placebo and IV MPH (0.25 mg/kg) and 3) oral placebo and IV placebo, in a counterbalanced, double-blind design. We correlated MPH-induced dynamic changes in striatal receptor availability

("dopamine release") with dynamic changes in brain network activity (amplitude of low frequency fluctuations, or ALFF) and functional connectivity of 13 large-scale brain networks.

Results: MPH increased subjective ratings of "feeling high" and decreased striatal receptor availability (indicating dopamine release) and these effects were greater for IV vs. oral MPH, replicating prior work. Dynamic changes in striatal dopamine release were associated with changes in brain functional activity depending on baseline activity: regions with high ALFF at baseline showed decreases in ALFF with increases in striatal dopamine release, and vice versa ($R^2 = 0.42$, $p < .05$). Further, dynamic changes in dopamine release from IV (but not oral) MPH correlated with increases in the 'segregation' of the default mode network: that is, it strengthened the ratio of within-network to between-network connectivity.

Conclusions: Striatal dopamine release systematically altered regional brain activity, dampening the activity of regions with high baseline ALFF, and increasing the activity of regions with low baseline ALFF. It also altered the relationship of the default mode network with other brain networks. These preliminary results demonstrate how drug-induced changes in striatal dopamine receptor binding affect human functional brain networks in real time.

Keywords: Resting State, Pharmacology, Catecholamine, Positron Emission Tomography (PET), Drug Abuse

Disclosure: Nothing to disclose.

M276

A Study of the mGluR5 Modulator Get 73 on Alcohol Pharmacokinetics and Pharmacodynamics in Non-Treatment Seeking Alcohol Dependent Individuals

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Background: The development of alcohol use disorder (AUD) is associated with dysfunctional glutamatergic neuroadaptations to chronic and heavy alcohol use. Medications that normalize this hyperglutamatergic state may have efficacy in the treatment of AUD. GET 73 is a small molecule negative allosteric modulator at the mGluR5 receptor that can decrease brain glutamate activity. In rodent studies with alcohol-preferring Sp rats, GET 73 reduced free choice alcohol consumption and the alcohol deprivation effect. In Phase 1 clinical studies, in healthy human volunteers, GET 73 demonstrated a positive safety and tolerability profile.

Methods: We conducted a randomized, double-blind placebo controlled cross-over 14-day study to evaluate the safety/tolerability of GET 73, its pharmacokinetic and pharmacodynamic interactions with alcohol, and its effects on craving and alcohol self-administration in participants with AUD (NCT01842503). Thirty-seven male and female non-treatment seeking, DSM IV alcohol-dependent participants were screened for eligibility and safety and 24 enrolled in the study. They were randomized to receive either 300mg tid of GET 73 for 3 days or placebo tid for 3 days, followed by a 7 day outpatient washout, followed by crossover to receive either 300mg tid of GET 73 for 3 days or placebo for 3 days. Under each drug condition, participants received an oral alcohol challenge to raise peak BAC to 0.12 g/dL (Day 2 or 12) and a laboratory cue-craving and alcohol self-administration session (Day 3 or 13). Participants were assessed for changes in alcohol pharmacokinetics and pharmacodynamics, craving and alcohol self-administration when taking GET 73

compared to placebo, as well as potential moderators of the effects.

Results: The safety analysis was conducted for the 24 enrolled participants. GET 73 had an excellent safety profile in these AUD participants, with no differences in treatment emergent adverse events (TEAEs) from placebo (9 participants with a global 14 TEAEs for placebo versus 10 participants with a global of 20 TEAEs for GET73); All AEs associated with GET 73 were classified as mild; no serious adverse events were registered. GET 73 did not alter alcohol pharmacokinetics compared to placebo (no differences in C_{max}, T_{max}, AUC, elimination). GET 73, compared to placebo, significantly increased alcohol sedation measured by the biphasic alcohol effects scale ($P=0.04$), but did not affect stimulation. However, there was not effect on alcohol performance impairments, as measured by the Connor's Continuous performance or digit-symbol substitution tasks.

Conclusions: GET 73 showed an excellent safety profile in these non-treatment seeking, alcohol-dependent participants, with no effects on alcohol pharmacokinetics during co-administration. GET 73 increased the sedation subscale of the biphasic alcohol effects scale, but did not adversely affect cognitive/motor performance. Increased BAES sedation is observed in other medications that reduce alcohol consumption, including naltrexone, topiramate and baclofen. Data on GET 73 effects on craving and alcohol self-administration are still being analyzed.

Keywords: Alcohol, mGluR5 Negative Allosteric Modulator, Pharmacotherapy

Disclosure: Nothing to disclose.

M277

Does Insomnia at Treatment Entry Predict Retention in Patients on Medication Assisted Therapy (Buprenorphine/Naloxone)?

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Background: Studies have shown that opioids can adversely affect sleep, by disrupting REM sleep, altering sleep breathing patterns, and increasing the risk of developing sleep apnea. Buprenorphine, a partial mu opioid receptor agonist and kappa opioid receptor antagonist has been observed to be associated with sleep disruption specifically, an increase in time spent awake and latency to sleep onset, and a decrease in REM sleep. Also, sleep disturbances may impact recovery in individuals with opioid use disorder. We evaluated whether baseline insomnia symptoms would predict retention in patients receiving medication assisted therapy (buprenorphine/naloxone).

Methods: Participants were 50 outpatients who were enrolled in a study evaluating employment outcomes among people dually diagnosed with opioid use disorder (OUD) and serious mental illness. Complete data for insomnia diagnosis and one-year retention to treatment was available for 44 participants. The participants received medication assisted treatment (buprenorphine/naloxone) for opioid use disorder. We assessed insomnia by reviewing medical records utilizing clinical interviews and self-report surveys (Insomnia Sleep Index and the Clinically useful depression outcome scale) at the beginning of the primary study. Retention was assessed as participation in treatment after one year. SPSS Version 25 was used to calculate a chi-squared test to assess the association between baseline insomnia status and retention to treatment.

Results: Of the 44 participants included in analysis, 30 (68%) had insomnia. Of the participants with insomnia, 19 (73%) were

retained in treatment for a year. Of the 14 participants without insomnia, 7 (50%) were in treatment a year later ($\chi^2(1) = 0.702$ and $p = 0.402$).

Conclusions: Though not statistically significant, numerically more people with insomnia stayed in treatment. Insomnia at treatment entry does not appear to be an impediment to retention in buprenorphine maintenance. Implications of these findings will be discussed.

Keywords: Sleep Disturbance, Clinical Predictors, Opioid Use Disorder, Buprenorphine

Disclosure: Nothing to disclose.

M278

Camkii-Dependent Phosphorylation of HOMER2 Gates the Motivational Valence of High-Dose Cocaine via Regulation of Binding to the MGLU5 Glutamate Receptor

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Background: Homer2 is a post-synaptic scaffolding protein regulating the trafficking, cellular localization and function of Group 1 metabotropic and NMDA glutamate receptor subtypes. Repeated cocaine experience increases and decreases, respectively, Homer2 protein expression within the prefrontal cortex and nucleus accumbens of rodents and these cocaine-induced changes in Homer2 expression are functionally relevant for cocaine-induced changes in behavior. For example, relative to wild-type mice, Homer2 null mutant mice exhibit greater sensitivity to the locomotor-activating effects of acute cocaine, as well as a shift to the left in the dose-response function for cocaine-conditioned place-preference. Further, these phenotypes can be reversed by virus-mediated restoration of Homer2 expression within the nucleus accumbens. Recent studies indicate that Homer2 is phosphorylated on S117/S216 in response to neuronal activity and calcium-calmodulin kinase II alpha (CaMKII) activation. This phosphorylation causes a rapid dissociation of mGluR5-Homer2 binding. Interestingly, (S117/216)Homer2 is hyper-phosphorylated in the Fmr1 knock-out mouse model of Fragile X Syndrome, arguing a potentially important role for Homer2 phosphorylation in not only regulating behavioral sensitivity to cocaine, but also in gating normal sensorimotor, cognitive, emotional and motivational processing.

Methods: To test this hypothesis, mice with alanine point mutations at S117/216 were generated and back-crossed to a C57BL/6J background. Subsets of mice were analyzed by immunoblotting for the effects of an acute injection of 20 mg/kg cocaine upon Homer2 phosphorylation, CaMKII activation and mGluR5-Homer2 binding. Other subsets of mice were subjected to a behavioral test battery to examine for the effects of the phospho-mutation upon measures of negative affect (light-dark box, forced swim test, novel object test, elevated plus-maze), spatial learning and memory (Morris maze), sensorimotor gating (prepulse inhibition of acoustic startle), and motor co-ordination (rotarod). A final subset of mice were assayed for cocaine-induced changes in behavior using a cocaine-induced place-conditioning regimen (4 pairings of either 3 or 30 mg/kg cocaine), coupled with locomotor activity monitoring.

Results: An acute cocaine injection (20 mg/kg) elicited hyper-phosphorylation of Homer2 and phosphorylation of (T286)CaMKII in striatal lysates that was absent in Homer2 phospho-mutants. Co-immunoprecipitation assays also confirmed a dissociation of

mGlu5-Homer2 binding by acute cocaine in wild-type animals. When assayed for negative affect, Homer2 phospho-mutants spent more time in, and entered more frequently, the open arms of an elevated plus maze and exhibited a shorter latency to enter the light-side of a light-dark box, than wild-type animals. While these latter findings suggest an anxiolytic effect of the mutation, no genotypic differences were observed in the novel object or forced swim tests. Absolutely no genotypic differences were observed in either acoustic startle across a range of tone intensities (0-110 dB) or in prepulse inhibition of acoustic startle. Similarly, no genotypic differences were observed regarding the acquisition or 24-h recall of the fixed platform location in the Morris Water Maze, nor were genotypic differences observed with respect to the acquisition of a new platform location on a reversal learning session. No genotypic differences were detected in performance on a rotarod and no differences in locomotor activity were noted in response to a novel environment, to acute or repeated saline injections or to acute or repeated cocaine injections. Although the 3 mg/kg cocaine dose elicited a comparable conditioned place-preference in both genotypes, only the Homer2 phospho-mutants exhibited a place-preference in response to conditioning with 30 mg/kg cocaine.

Conclusions: These findings provide new evidence that CaMKII alpha-dependent phosphorylation of Homer2 is necessary for remodeling Homer scaffolds in response to cocaine. Further, the behavioral results argue that this phosphorylation gates the negative motivational valence of high-dose cocaine, while sparing the positive motivational valence of lower cocaine doses. If relevant to humans, these data implicate Homer2-mGlu5 binding in gating the perception of high-dose cocaine effects as aversive, which has implications for abuse liability and addiction vulnerability.

Keywords: Cocaine Addiction, fragile X syndrome, mGluR5 receptors, Homer, Negative Affect

Disclosure: Nothing to disclose.

M279

Cross-Disorder Analyses of Immune-Related Gene Expressions in Brains of Major Neuropsychiatric Disorders

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Background: Accumulating evidence suggests that inflammation involves in neuropsychiatric disorders. Brain transcriptomic changes of immune-related genes remain puzzling. To understand the role of immunologic changes to these disorders, we assessed gene expression changes related to neuroimmunogenic systems in post-mortem brain samples of individuals with schizophrenia (SCZ), bipolar disorder (BD), autism spectrum disorder (ASD), major depressive disorder (MDD), Alzheimer's disease (AD) and Parkinson's disease (PD).

Methods: We collected RNA-Seq data of 2,540 brain samples of the six major neuropsychiatric disorders, and processed them using a unified process including quality control, filter and removing known and unknown confounding variables. We used the linear-mixed model to detect differentially expressed genes (DEGs), focusing on a curated list of 1,256 immune-related genes (IRGs) collected from databases. We used FDR < 0.05 to define significant changes. We further performed Weighted Gene Co-Expression Network Analysis (WGCNA), placing the 1,256 genes into each coexpression modules, and identified differentially expression modules (DEMs) enriched with the IRGs. Lastly, we

performed pathway and gene ontology analyses for DEMs that are also enriched for IRGs.

Results: We identified various differentially expressed immune genes in each of the six disorders (AD: 631, ASD: 273, BD: 56, MDD: 26, PD: 95, SCZ: 216). The fold changes of these IRGs showed significant sex differences in ASD (FDR $q < 0.05$) and MDD (FDR $q < 0.05$). Pathway analyses revealed specific immune pathways for those differentially expressed IRGs in each disorder. In one instance, we found highly enriched virus-response related genes only in the AD brains (GO:0009615 Response to virus, FDR $q = 1.88e-20$).

To test for similarities among disorders, we tested for correlations of changes of IRGs between disorder pairs. Results showed significant positive correlation between BD and SCZ ($\rho = 0.77$, FDR $q < 0.05$). Negative correlation was observed between AD and other disorders: SCZ ($\rho = -0.27$, FDR $q < 0.05$), BD ($\rho = -0.33$, FDR $q < 0.05$), ASD ($\rho = -0.37$, FDR $q < 0.05$). Single nucleotide polymorphism (SNP) co-heritability, calculated by Brainstorm consortium (2018), was significantly correlated with IRG fold change across the same disease pairs (Pearson's $r = 0.54$, $p = 0.014$). WGCNA identified eleven co-expression modules. Three of the modules were enriched for IRGs. These three modules were also enriched for neuronal development functions, with one enriched for SCZ and intelligence GWAS signals.

Conclusions: Our study identified shared changes and disease-specific IRG changes across different neuropsychiatric disorders in the brain. Different neuropsychiatric disorders have their unique IRG changes that are correlated with their genetic contributors. AD carries the most distinct IRG change pattern, with the largest amount of DEGs, enriched in virus-response related genes.

Keywords: Immune, Gene Expression, Gene Co-Expression Networks, Neuropsychiatric Disorders

Disclosure: Nothing to disclose.

M280

Genome-Wide Association Study of Suicide Death and Polygenic Prediction of Clinical Antecedents

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Background: Suicide death is a preventable yet growing worldwide health crisis. Despite significant heritability of suicide, genetic discovery efforts for the extreme phenotype of suicide death have been virtually nonexistent as no sizable cohorts have been available for study.

Methods: We conducted a large genome-wide association study (GWAS) of suicide death, with 3,413 male and female population-ascertained cases of European ancestry and 14,810 matched controls. Case samples were obtained from the Utah Office of the Medical Examiner and matched control cohorts were provided by Generation Scotland and UK10k collaborators. Genomic data were handled using PLINK. SNPs with ambiguous strand orientation, >5% missing calls, or Hardy-Weinberg equilibrium $p < 0.001$ were excluded. SNPs with minor allele frequency below 0.01 or imputation $R^2 < 0.5$ were also excluded. Final analysis was performed on 7,519,308 imputed variants passing quality control. A Linear Mixed Model (LMM) algorithm tested variant association with suicide death, with follow-up examination of significant hits for linkage disequilibrium and gene set enrichment. GWAS were performed using GEMMA, a computationally efficient and open-source LMM algorithm for GWAS that models population stratification remaining after PCA by use of genomic relatedness matrices. PRS for suicide death was

derived using PRSice 2.0 and summary statistics from a 10-fold cross validation procedure to avoid overfitting. Using summary statistics from the GWAS, we then cross-validated prediction of case-control status, accounting for ancestry, across independent training and test sets using polygenic risk scores for suicide death. We then leveraged genome-wide data and medical record (EHR) data on all suicide cases to examine 1) case-control differences in polygenic risks for 35 other psychiatric and medical phenotypes, and 2) mode of death associations with all polygenic risks and with all psychiatric and medical ICD codes. All statistical analyses were well-powered and corrected for multiple testing.

Results: Genome-wide tests implicate two loci and 10 genes on chromosomes 13, 15, 16, 17, and 19. Eleven of 22 associated genes overlap with schizophrenia results from the GWAS Catalogue, and two of these 11 genes have prior associations with risk of suicidal behavior. Successful prediction of suicide death case-control status was cross-validated. Suicide death cases had significantly increased phenotypic (ICD-9/10) and polygenic risk for several disorders relative to controls. These included (among others) autism spectrum disorder, major depressive disorder, schizophrenia, and alcohol use disorders. Suicide death by violent trauma was significantly associated with both a clinical diagnosis of schizophrenia or schizoaffective disorder and with genome-wide polygenic risk for schizophrenia.

Conclusions: Results suggest significant genome-wide hits for suicide death, and positive genetic associations of suicide death with several risk phenotypes, most notably autism spectrum disorder, major depressive disorder, schizophrenia, and alcohol use disorders. Genome-wide results allowed for direct testing and validation of several established epidemiological risk factors, and of specific clinical and pharmacological targets. Increasing our efforts toward genome-wide examination of suicide death may lead to improvements in early detection of risk and clinical prevention.

Keywords: Molecular Genetics, Suicide, Psychosis, Autism, Risk

Disclosure: Nothing to disclose.

M281

Beneficial Effects of Propranolol and Synergistic Effects With Nicotine on Cognitive Performance in Human Non-Smokers

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Background: Nicotine administration increases the output of every major neurotransmitter in the brain. In previous studies aimed at identifying the secondary neurotransmitter system(s) mediating the attention-enhancing effects of nicotine, the β -adrenoceptor antagonist propranolol blocked the performance-enhancing effects of nicotine in a rat model (Hahn and Stoleran 2005, *Psychopharmacology* 177:438-47). The aim of the present study was to test whether this mechanism would hold true in humans and could potentially guide drug development efforts for cognitive-enhancing compounds.

Methods: Twenty-six adult non-smokers (21-53 years of age, 9 male) completed a nicotine (7 mg/24 hrs) x propranolol (40 mg p.o.) interaction study employing a within-subject design. Each participant completed four test sessions. In each session, participants received a skin patch five hours before cognitive testing and a capsule two hours before testing. The study followed a 2 x 2 factorial design: in one session, both patch and capsule were a placebo; in another, the patch contained nicotine and the

capsule was a placebo; in another, the patch was a placebo and the capsule contained propranolol; and in another, the patch contained nicotine and the capsule propranolol. The sequence of drug conditions was counterbalanced across participants. Vital signs and side effects were assessed hourly, and the Profile of Mood States (POMS) was completed at baseline, just before cognitive testing, and 1.5 h later upon completion of testing. Cognitive testing was computerized and consisted of the Spatial Attentional Resource Allocation Task (SARAT; measuring omission errors and reaction time (RT)), the Rapid Visual Information Processing Task (RVIPT; measuring hit rate and RT) performed in two 15-min blocks separated by a short break, and a change detection task (CDT; measuring accuracy and RT) testing visual short-term memory for one or five color-items.

Results: In the SARAT, no significant drug effects were seen. In the RVIPT, nicotine and propranolol did not affect performance when given in isolation, but acted synergistically when given in combination to significantly shorten reaction time [nicotine x propranolol interaction $F(1,25) = 4.57$, $P = 0.043$]. Drug effects were explored as a function of time on task by adding the factors block (first vs. second 15-min block) and period (three 5-min periods within each block). On both measures, there was a significant performance decrement over periods within each block. The decrease in hit rate was less pronounced over the course of the second block, in which performance was lower overall. In isolation, neither nicotine nor propranolol had any effects on the performance decrement over time, but when given in combination, the two drugs again acted synergistically, alleviating the decrease in hit rate over time in the first block [nicotine x propranolol x block x period $F(2,50) = 6.16$, $P = 0.004$]. In the CDT, propranolol enhanced accuracy and reduced RT independent of set size and the presence or absence of nicotine.

The POMS revealed significant worsening of mood states over time on the tension, vigor, fatigue, and total mood disturbance scales, especially from before to after testing. Nicotine had no effect on mood, but propranolol significantly enhanced vigor and reduced confusion and total mood disturbance, independent of the presence or absence of nicotine. Furthermore, propranolol alleviated the increase in "tension" from pre- to post-testing.

Vital signs: Nicotine significantly increased and propranolol significantly decreased blood pressure and heart rate. On blood pressure, these effects tended to cancel each other out when both drugs were combined, while heart rate was reduced by propranolol equally in the presence and absence of nicotine.

Conclusions: The results suggest that performance effects of changes in β -adrenoceptor tone greatly depend on context. The β antagonist propranolol helped unveil performance-enhancing effects of nicotine in the RVIPT, a task marked by intense and fast processing demands. We suggest that downstream β -adrenoceptor activation by nicotine in the context of the high arousal created by the RVIPT may have limited performance-enhancing effects of nicotine via other mechanisms, which were unmasked by β antagonism. Propranolol also benefited performance of an unspeeded short-term memory task, which was always completed last. Effects of propranolol on mood states, including enhanced vigor and reduced testing-induced tension, are consistent with an overall beneficial effects profile of propranolol on cognition, especially on the maintenance of performance ability with prolonged testing. In contrast, in a rodent paradigm of attention that requires behavioral activation, propranolol had impaired performance and antagonized performance-enhancing effects of nicotine, implying benefit from greater β -adrenoceptor tone. The present results suggest poor translatability of drug effects from rodent paradigms of cognition, which depend on behavioral activation, to human paradigms,

which depend on keeping behavioral activation at bay while dealing with sustained processing demands.

Keywords: Nicotine, Propranolol, Beta-Adrenergic Signalling, Cognition, Attention

Disclosure: Nothing to disclose.

M282

Connectivity Guided Dimensions of Psychopathology in Youth

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Background: Despite a vast body of literature, magnetic resonance imaging research has not yet delivered biomarkers for psychiatry. This is due, in part, to the high comorbidity of mental disorders, calling for a dimensional approach and the computational quantification of these dimensions in both clinical and imaging data. In this study, we use canonical correlation analysis (CCA) to investigate associations between anxiety, irritability, and disruptive behavior, three of the most common, impactful psychiatric problems in youth with each other and intrinsic brain connectivity. To increase treatment relevance, we test the generalizability of our results comparing data across two independent samples.

Methods: We analyzed two samples, one (N = 184) primarily containing youth referred for treatment for emotional problems and another (N = 328) primarily comprised of youth referred for treatment for behavior problems. In both samples, symptoms of irritability, anxiety and disruptive behavior were measured with the Affective Reactivity Index (ARI), the Screen for Child Anxiety Related Disorders (SCARED) and the attention-deficit/hyperactivity disorder subscale from the Child Behavior Checklist (CBCL), respectively. Further, all participants had 10 minutes of resting-state functional magnetic resonance imaging (rsfMRI) data. Functional connectivity was assessed using partial correlations between the 200 parcels (here network nodes) of a common cortical parcellation scheme, plus 16 subcortical structures. Partial correlations offer an estimate of direct (as opposed to indirect or shared) connectivity between each pair of nodes. PCA was used for dimensionality reduction such that only the 20 principal components that represented the largest amount of between-subject variance among the network edges and among the clinical variables were retained. Statistical significance of each canonical variate was assessed using a permutation test, correcting for multiple testing at the level $\alpha_{FWE} < .05$.

Results: In both datasets, three dimensions of psychopathology were identified. The brain-behavior correlations were generally higher in the discovery ($r = .58-.66$) compared to the replication dataset ($r = .43-.46$). The first two dimensions of psychopathology both described a dichotomy between temper outbursts and angry mood. However, temper outbursts on the first dimension appeared to be more related to social and separation anxiety, whereas temper outbursts on the second dimension are more associated with behavioral inhibition deficits. Similarly, the angry mood on the first dimension appeared in the context of socially inappropriate behavior (excessive talking, being loud), whereas angry mood on the second dimension related to inhibition deficits (i.e., distractibility). For the first dimension, temper outbursts were associated with high connectivity between amygdala, default mode, somatomotor, and control networks, whereas mood related to high connectivity between default mode, control, and attention

networks. For the second dimension, temper outbursts were associated with high connectivity between somatomotor, attention, and control networks, whereas mood related to high connectivity between default mode and control networks. The third dimension ran from inattentive symptoms, which were associated with higher connectivity between somatomotor, visual, attention, and control networks, to hyperactive symptoms, which related to higher connectivity of limbic, attention, and somatomotor networks. In the replication data set, the first two canonical variates resembled the first two dimensions from the discovery dataset, with correlations of $r = .38$ and $r = .37$ between the clinical loadings, but we failed to replicate the third dimension.

Conclusions: Our results indicate two dimensions of irritability along an outburst-mood axis: one more associated with the social context and the second related to inhibitory control deficits. Although we could replicate similar components in an independent dataset, it must be noted that CCA - as other latent variable approaches - appears to be highly sensitive to sample characteristics.

Keywords: Canonical Correlation Analysis (CCA), Transdiagnostic, fMRI Resting State, Youth

Disclosure: Nothing to disclose.

M283

Neanderthal-Derived Genetic Variation Affects Functional Connectivity Between Visual and Social Brain Networks in Living Humans

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Background: Recent genetic evidence has shown that Neanderthals and ancestors of modern humans interbred prior to Neanderthals disappearing from the fossil record (Greene 2010). The legacy of this coupling lives on today, accounting for about 2% of the human genome (Prüfer 2014). We previously showed that living humans with relatively larger percentages of Neanderthal ancestry have (1) skull shapes with greater resemblance to Neanderthal fossils, and (2) alterations in underlying brain morphology (Gregory 2017). Though this inheritance affects brain structure, the neurofunctional implications have remained unclear. Here, we tested whether network connectivity was related to Neanderthal-derived genetic variation.

Methods: We collected genetic information and fMRI data on 304 healthy Caucasian adults (30.9 $\pm$ 9.5 years, 145 males). Genetic data were collected on all participants with Illumina SNP chips and imputed using Shapeit and Impute2. After imputation, we calculated the polygenic-load of Neanderthal-derived genetic variants ("NeanderScore") for each participant, as previously reported (Gregory 2017). For the fMRI data, pseudo-resting time series (e.g., Sheffield 2016) were created for each participant from four task-based functional scans, all with the same acquisition parameters (TR=2.0s, TE=28ms, flip angle 90deg, 3.75x3.75x6 mm voxels, 26:08 total minutes of scanning). Functional scans were co-registered, motion-corrected, and normalized to MNI space. Data were concatenated, bandpass filtered, spatially smoothed (6mm FWHM), and effects of no interest were regressed out (including those related to motion and task design). Multivariate-Distance Matrix Regression (MDMR; Shehzad 2014) was used to identify voxels where whole-brain network connectivity associated with NeanderScore, controlling for age, sex and ancestry-related genetic components at $p = 0.005$. Follow-up voxel-wise analysis using the results from the MDMR

analysis as a seed region were carried out to clarify the underlying functional connectivity patterns driving the association.

Results: Using MDMR, NeanderScore was significantly associated with the connectivity patterns of the right intraparietal sulcus (IPS), a region directly underlying the skull changes identified in our prior study and implicated in visuospatial functioning by Neurosynth and a number of previous studies (e.g., Ungerleider 1982; Goodale 1992). No other regions showed associations between whole-brain functional connectivity patterns and NeanderScore. Follow-up investigations using this IPS region as a seed in a functional connectivity analysis showed that NeanderScore was significantly associated with increased connectivity between the IPS and regions associated with visual processing, including occipital cortex and fusiform gyrus. In contrast, NeanderScore was associated with decreased connectivity between the IPS and regions involved in social processing, including posterior cingulate, temporoparietal junction, medial prefrontal cortex, superior temporal sulcus, and superior frontal gyrus (all p 's < 0.05, FWE-corrected).

Conclusions: Though we and others have previously shown that Neanderthal genetic variation impacts skull and brain shape, little work has examined the neurofunctional implications of this inheritance. In addition to skull and brain shape, we now show that the whole-brain functional connectivity patterns of the IPS are related to degree of Neanderthal ancestry in living humans. This IPS region is remarkably similar to the left IPS region identified in our previous report relating NeanderScore to skull shape and brain structure. The IPS is known to be a hub for visuospatial processing and the directionality of these findings are consistent with the hypothesis that Neanderthal brains had greater resources devoted to visual networks at the expense of social networks (Pearce 2018). Our results further support the contention that that Neanderthal-derived genetic variation is functional in the human brain, potentially influencing an evolutionary balance between visuospatial and social functioning.

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Keywords: Evolution, Functional Connectivity, Neanderthal-Derived Admixture, Intraparietal Sulcus, MDMR

Disclosure: Nothing to disclose.

M284

Correlation of Impulsivity With Sign- and Goal-Tracking Behavior in Healthy Human Subjects

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Background: Sign-tracking is a form of Pavlovian conditioned approach to reward-associated cues. Sign-tracking is often contrasted with goal-tracking, which is cue-triggered approach directed toward the location of reward delivery. Interest in sign-tracking has increased in recent years because individual variation in sign-tracking has been shown to predict addiction-like behaviors in animals. Though sign-tracking has been observed in a wide variety of species, including rodents, primates, fish, cephalopods, and insects, there have been very few attempts to document sign-tracking in humans.

Methods: So far 36 healthy men and women aged 18-25 have been recruited for this study. We directly translated a rat PCA task for human subjects, using a retractable lever as a conditioned stimulus that predicts reward delivery into a different physical location (reward magazine). Physical contacts as well as eye-gaze directed toward the lever or magazine were recorded as outcome measures. Subjects also completed questionnaire-based measurements of trait impulsivity.

Results: There was significant inter-individual variation in the extent to which subjects interacted with the "sign" (lever) or the "goal" (magazine) during lever presentation. Overall responses were skewed toward sign-tracking, with 58% of subjects primarily sign-tracking, 28% primarily goal-tracking, and 14% equally engaging in both behaviors. Analysis of impulsivity revealed a negative correlation between motor impulsivity and goal-tracking behavior ($r = 0.35$, $p = 0.032$).

Conclusions: These experiments demonstrate that sign- and goal-tracking behavior can be measured in humans. Furthermore, inter-individual variation in goal-tracking behavior appears to correlate with motor impulsivity, which is a known risk factor for addiction and other psychiatric disorders.

Keywords: Pavlovian Conditioning, Reward Learning, Individual Differences, Incentive Salience, Vulnerability Traits

Disclosure: Nothing to disclose.

M285

Post-Weaning Social Isolation Alters Reward-Seeking Behavior in Male Mice

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Background: Social isolation (SI) is a risk factor for multiple neuropsychiatric disorders. Although SI during all portions of the lifespan is detrimental to health, SI during childhood and adolescence is particularly disruptive due to the concurrent neurodevelopmental processes occurring during this time period. Unfortunately, SI during adolescence is increasing and this increase is thought to contribute to the rising rates of psychiatric disorders in this age group. SI interacts with genetic and other environmental risk factors to induce many neurobiological changes that exert long-term effects on behavior. In this study, we found significant changes in reward-seeking behavior in male mice exposed to post-weaning SI. These data suggest that changes in reward-seeking behavior may contribute to the neuropsychiatric risk associated with SI.

Methods: Mice (C57BL/6J) were weaned on postnatal day 21 (PD21) and separated into group-housed (GH) or SI cohorts. GH mice were housed in cages of three mice for the entire experiment. SI mice were singly-housed from PD21 to PD35. On PD35, SI mice were paired with previously isolated littermates and pair-housed for the remainder of the experiments. Behavioral testing began at PD63. All mice were trained on touchscreen-based fixed and progressive ratio operant tasks that utilized strawberry milk as the reinforcer. We tested the effect of SI on performance on FR1, FR5, and PR4 schedules of reinforcement. For these experiments, we started with nine GH male mice and twelve SI male mice. One SI mouse was removed from the study because it did not complete all of the test sessions. We analyzed total rewards received on the FR schedules and breakpoint on the PR4 schedule using unpaired t-tests. The PR breakpoint was defined as the last ratio completed in the session. PR sessions

were terminated when mice did not make a nose poke response within the previous five minutes.

Results: Post-weaning SI altered reward-seeking behavior. SI mice demonstrated significantly higher breakpoints on a PR4 schedule [$t(18) = 2.744, p = 0.0133$] and more total responses on an FR1 schedule [$t(18) = 2.166, p = 0.0440$]. The SI group also had a higher mean number of total responses on an FR5 schedule, but the difference did not reach our significance threshold [$t(18) = 1.951, p = 0.0668$].

Conclusions: These results provide evidence that reward-seeking behavior is modulated by SI and this effect is present weeks after the termination of the SI stress. These potentially long-lasting effects of SI on reward processing may contribute to pathological effects of SI on behavior in adulthood. Future studies will investigate the neurobiological changes associated with SI during the post-weaning period.

Keywords: Social Isolation Stress, Reward Processing, Touchscreen

Disclosure: SoBran Inc., Consultant

M286

Convergent and Divergent Patterns of Atypical Visual Processing Underlying Face Emotion Recognition in Schizophrenia and ASD

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Background: Deficits in social cognition are core features of many neuropsychiatric disorders including autism spectrum disorder (ASD) and schizophrenia (SZ). One important component of social functioning is the ability to recognize and respond to the emotional content of faces. Face emotion recognition (FER) depends upon low-level and higher-level cognitive processes and, in the visual system, upon coordinated functioning of subcortical and cortical regions including the pulvinar nucleus of the thalamus, for processing of both static and moving facial features. Impaired FER has been reported numerous times in SZ and ASD and, in both cases, linked to atypical neuronal activity within visual cortex. Despite overlaps, however, only a few studies to date have directly explored convergent and divergent neural mechanisms of visual processing in ASD and SZ. In this study we used fMRI and task-based functional connectivity to evaluate impaired FER in relation to activation of subcortical and cortical visual regions.

Methods: Participants were 20 high-functioning adults with ASD (mean age 29 years), 28 patients diagnosed with SZ (mean age 37 years) and 30 neurotypical control (NT) volunteers (mean age 34 years). Psychiatric symptoms in SZ were evaluated using the Positive and Negative Syndrome Scale. ASD diagnosis was ascertained based on the Autism Diagnostic Observation Schedule. FER was evaluated using both static and dynamic faces. Dynamic FER utilized brief video clips of faces dynamically expressing unique emotions selected from the University of Cambridge Mind Reading Emotions Library. Single frames that best represented the emotion were extracted from each video and used as corresponding static stimuli. The cortical and subcortical neural correlates of impaired FER in SZ and ASD were explored with functional MRI during passive viewing of the dynamic and static emotional faces. Functional connectivity analyses (psycho-physiological interactions) were used to estimate the functional coupling between face-emotion sensitive brain regions.

Results: As expected, SZ and ASD participants showed equivalent deficits in FER which correlated with reduced activation

of the posterior superior temporal sulcus (pSTS). Deficits in pSTS activation were correlated with reduced activation of early visual areas in SZ patients but with hyperactivity of these regions in ASD participants. Additionally, compared to control subjects, patients with SZ, showed significantly lower functional connectivity and activation of the pulvinar nucleus which predicted their impaired pSTS activity.

Conclusions: Despite convergent deficits in social cognition, individuals with SZ and ASD show different profiles of physiological dysfunction during face-emotion processing, suggesting differential underlying pathophysiological mechanisms. This investigation sheds light on the relative pathophysiology of FER deficits in SZ and ASD and highlights the role of pulvinar and low-level visual processing abnormalities in dynamic face emotion perception.

Keywords: cortical circuit function, schizophrenia, ASD, Visual Processing, Social Processing, fMRI

Disclosure: NeuroRx, Stock / Equity, Glytech, Inc/LLC, Stock / Equity, NeuroRx, Board Member, Biogen, Advisory Board, Phytechs, Advisory Board, Promentis, Advisory Board, Autifony, Consultant, SK Life Sci, Consultant, Cadence, Consultant, Pfizer, Consultant, Cerevance, Grant

M287

Trait Irritability is Associated With Greater Levels of Negative and Lower Levels of Positive Mood Assessed via Ecological Momentary Assessment Technology in Youth

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Background: Irritability is a trans-diagnostic symptom associated with multiple psychiatric disorders and the most common reason why children are brought for psychiatric evaluation. Childhood irritability is linked to substantial impairment in adulthood, including psychopathology and suicide. Advancing what is known about irritability is a top NIMH priority. To address this need, we sought to improve what is known about the assessment of irritability, by testing the relationship between a cross-sectional parent and child reports of irritability via the Affective Irritability Index (ARI) and longitudinal daily mood assessments via ecological momentary assessment (EMA) technology.

Methods: Forty-two children recruited from the community, outpatient, and inpatient settings participated in this IRB-approved study. Average age of children was 10.11 y.o. (SD = 1.47) and the majority was male (61.9%) and Caucasian (81.0%). Children (about self) and parents (about child) responded to time-synchronized EMA assessments drawn from the Positive and Negative Affect Schedule (PANAS). We used linear mixed models to examine the link between parent and child ARI reports of child irritability at baseline and child and parent EMA assessed 3 times a day over a 2-week period.

Results: We found that child report of higher irritability via ARI at baseline was associated with experiencing greater irritability ($\beta = 0.08, p = 0.004$), frustration ($\beta = 0.09, p = 0.001$), and sadness ($\beta = 0.07, p = 0.006$), but not happiness or relaxation (all $ps > 0.05$) across the two-week EMA period. Parent ARI report of child irritability at baseline was associated with higher child irritability ($\beta = 0.08, p = 0.001$), frustration ($\beta = 0.05, p = 0.05$), but not sadness ($p > 0.05$), and lower levels of happiness ($\beta = -0.04, p = 0.04$), and relaxation ($\beta = -0.05, p = 0.04$). We also found a

significant association between parent and child EMA reports of child's positive and negative mood (all $p < 0.001$).

Conclusions: Conclusion: Pending replication, these preliminary findings suggest that higher levels of cross-sectionally assessed trait irritability are linked to greater levels of negative and lower levels of positive mood longitudinally assessed in children's real-world environment. Interestingly, although parent and child assessments of externalizing behavior frequently differ, we found high agreement between parent and child reports of child's mood in our sample.

Keywords: Irritability, Mood, Ecological Momentary Assessment, Children

Disclosure: Nothing to disclose.

M288

Exploring the Genetic Architecture of Mood Symptoms in Postpartum Depression and Postpartum Psychosis Using Polygenic Risk Scores

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Background: Perinatal psychiatric disorders – including postpartum depression and psychosis – result from an interplay of environmental, psychological, and genetic risk factors. Despite evidence that genetic variation influences susceptibility to postpartum psychiatric disorders, the genetic variants contributing to this risk are not yet clear. In recent years, large genome-wide association studies (GWASs) have successfully identified more than 100 genetic risk loci for general clinical depression (Howard et al. 2019) and psychosis (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). A large GWAS of postpartum psychiatric disorders is under development, led by the Postpartum Depression: Action Towards Causes and Treatment (PACT) Consortium. Here, we used polygenic risk scores (PRSs) from the depression mega-analysis of Howard et al. to explore the genetic architecture of symptom profiles among 322 European ancestry women with postpartum depression and/or psychosis recruited by the PACT Consortium in Canada. Our approach is motivated by recent successes in illuminating the genetic architecture of symptom profiles in non-perinatal psychiatric disorders.

Methods: Women with current or past postpartum depression (identified by a validated algorithm) or past self-reported postpartum psychosis were recruited, and mood symptoms were measured using the Edinburgh Postnatal Depression Scale (EPDS). Samples were genotyped using the Infinium Global Screening

Array-24 v2.0 (GSA) BeadChip. As a non-perinatal depression discovery dataset, we used the largest available GWAS from the Psychiatric Genomics Consortium ($n = 246,363$ cases and 561,190 controls, Howard et al. 2019). We used our postpartum depression and postpartum psychosis cases as the target dataset, analyzing total EPDS score as the phenotype. PRSs for non-perinatal depression were constructed for each sample in the postpartum target dataset using SNPs extracted from the non-perinatal depression discovery dataset. SNPs associated with non-perinatal depression at a range of p-value thresholds (pT 5×10^{-8} , 1×10^{-4} , 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5) were used to construct PRSs by summing the number of risk alleles weighted by the beta for each SNP. To evaluate whether the PRSs for non-perinatal depression predicted mood symptom severity (EPDS) among women with postpartum depression or psychosis, we used linear regression adjusting for age and 10 ancestry-informative dimensions accounting for population substructure.

Results: After quality control for individuals and variants, 319 Canadians of European ancestry and 407,451 variants were available for analysis in the target dataset. Mood symptom severity was significantly greater among women with postpartum psychosis with or without postpartum depression compared to women with postpartum depression alone (mean EPDS = 24.58 ± 3.29 and 22.25 ± 3.61 respectively, $p < 0.001$). We merged the target and discovery datasets and performed quality control including linkage disequilibrium (LD) clumping, resulting in 92,126 independent variants available for analysis. In preliminary analyses, we did not observe evidence that PRSs for non-perinatal depression predicted mood symptom severity measured by EPDS among women with postpartum depression or psychosis ($p > 0.05$ at all pT).

Conclusions: To our knowledge, this is the first study evaluating the genetic architecture of mood symptom severity in postpartum depression and psychosis. Our preliminary PRS analyses did not reveal evidence of shared genetic liability between postpartum mood symptom severity and non-perinatal depression. Interestingly, a previous study applying PRSs to postpartum depression found shared genetic liability with non-perinatal bipolar disorder, but not non-perinatal depression (Byrne et al. 2014). Limitations of our study include reliance on self-reported diagnosis of postpartum depression and psychosis, lack of data on additional psychiatric comorbidities, and the limited overlap in variants between the target and discovery dataset resulting in incomplete genomic coverage of our PRSs. Genotype imputation of the target dataset is underway, in order to maximize overlapping variants with the discovery dataset to address the latter limitation. Future work constructing PRSs from other non-perinatal psychiatric disorders and biomarkers of interest is ongoing, in order to further explore the genetic architecture of mood symptoms in postpartum psychiatric disorders.

Keywords: Postpartum, Psychosis, Depression, Polygenic Risk Score, Depressive Symptoms

Disclosure: Nothing to disclose.