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W1. Efforts to Develop and Validate Models and Endpoints Within the NINDS Preclinical Screening Platform for Pain (PSPP)

Taleen Hanania, Mark Varney, Emer Leahy, David Budac, Elizabeth Dugan, Steven Leiser, Mark Urban, Sarah Woller, Smriti Iyengar*

NINDS/NIH, Carmel, Indiana, United States

Background: The NIH Helping to End Addiction Long-term Initiative, or NIH HEAL Initiative, is a trans-agency effort to provide scientific solutions to the opioid crisis. As part of the NIH HEAL Initiative, the National Institute of Neurological Disorders and Stroke (NINDS) has been charged with enhancing pain management and accelerating the discovery and development of new non-addictive pain therapeutics. Toward this goal, NINDS created the Preclinical Screening Platform for Pain (PSPP) with the aim of accelerating the preclinical development of non-opioid, non-addictive therapeutics for pain. PSPP is working with PsychoGenics to develop and validate preclinical models and endpoints to enable the screening and profiling of assets, including small molecules, biologics, natural products, and devices.

Methods: The PSPP aims to screen and profile candidate therapeutics in a number of preclinical assays. Assets are evaluated in a tiered manner, starting with in vitro functional assays to rule out opioid receptor activity and to assess in vitro abuse liability. In vivo pharmacokinetic studies are then used to measure plasma and brain exposures to guide the dose range and pretreatment times for the side effect profile, in vivo efficacy, and in vivo abuse liability studies. All experiments are blinded, both sexes are included, group sizes are determined by power analysis, and data are reported in accordance with ARRIVE guidelines

Results: A key component of the PSPP is to validate new and existing models and endpoints. In collaboration with key opinion leaders in the field, NINDS and PsychoGenics have been validating existing preclinical models of pain in rodents for inclusion in the PSPP workflow. This includes validation of models of nerve injury, chemotherapy-induced neuropathic pain, post-operative pain, pain associated with osteoarthritis, deep muscle pain, and migraine, as well as validation of existing endpoints, of evoked pain measures but more importantly, validating non-evoked endpoints for use in these models. For example, we aim to understand whether endpoints such as gait, wheel running, guarding, place escape avoidance paradigm (PEAP), and electro-encephalogram (EEG) are useful endpoints in a model of nerve injury. Another exciting feature of the program that will be highlighted in this presentation will be assessment of abuse liability in the context of efficacy in pain endpoints. Advances in understanding these endpoints and representative data will be presented.

Conclusions: This presentation will describe efforts to standardize models and endpoints to enhance rigor and reproducibility while evaluating potential non-opioid, non-addictive therapeutics for pain within the NINDS HEAL Initiative PSPP program.

Keywords: Pain, Preclinical Models and Endpoints, Validation, Heal Initiative

Disclosure: Electrical Engineering, Delphi: Employee (Spouse), Eli Lilly: Retiree, Stock / Equity (Self)

W2. The D1 Positive Allosteric Modulator, DETQ, Improves Cognition in Aged Mice and Enhance Cortical and Hippocampal Acetylcholine Efflux

Herbert Meltzer*, Lakshmi Rajagopal, Mei Huang, Kjell Svensson

Northwestern University, Chicago, Illinois, United States

Background: Decline in memory function during normal aging is one of the major causes of disability. Loss of dopaminergic function is one of the major causes of decline in aging. Numerous studies point towards decrease in the availability of dopamine as a primary cause in the decline in memory, although it may be that downstream signaling after stimulation of D1R in cortex or hippocampus may also contribute. Thus, a prime target for improving age-associated memory impairment has been a D1R agonist. However, chronic administration of orthosteric D1 agonists have proven to be of little value clinically or in animal models when given chronically. Thus, the development of a D1 positive allosteric modulator (Bruns et al) which does not desensitize or show an inverted U-shaped curve is a promising development. We now demonstrated that the tool D1 PAM DETQ is effective to restore novel object recognition and other memory function in aged C57Bl6 mice in which a human D1R has been substituted for the mouse D1R as DETQ is only fully active at the hD1r.

Methods: Methods; hD1RKI mice were tested for novel object recognition at various ages, and with pretreatment with DETQ and various other drugs to clarify mechanism of action. We also did a microdialysis study in freely moving mice to compare neurotransmitter release in mPFC and hippocampus (HIP),

Results: We detected a small but significant decrease in NOR, as measured by the discriminant index (DI) at 10-11 months, a 50% decrease at 16-17 months, and a complete loss, by 19 months.

An acute dose of DETQ, 10 mg/kg po, restored NOR to normal levels. This was blocked by pretreatment with the D1 antagonist, SCH23390. Prolonged treatment with DETQ led to a prolonged recovery of NOR for three weeks at least. The GABA A agonist did not restore NOR in the aged mice, However, gabazine, a GABA

antagonist, blocked the effect of DETQ, while the NKCC1 inhibitor, bumetanide, which restores excitatory GABA to inhibitory GABA in scPCP mice (Kim et al, in press), also rescued NOR in the aged mice. The D1 positive allosteric modulator, DETQ, improves cognition and negative symptoms in aged mice as well as subchronic-PCP treated mice. A single dose of Mg threonate also restored NOR in aged and PCP treated mice.

Conclusions: These preliminary results are promising and suggest D1PAM which is active in man may be of clinical value in treating age-associate memory impairment and related causes of cognitive decline. The effectiveness of D1 PAM in both normal aging and the PCP model of schizophrenia is noteworthy and suggests some common pathophysiology. The efficacy of bumetanide suggests depolarizing GABA may be important for both aging and schizophrenia. The combination of bumetanide and a D1PAM could be of clinical interest.

Keywords: Aging, Dopamine, Gaba, Magnesium, Bumetanide

Disclosure: Eli Lilly: Grant (Self)

W3. Differential Vulnerability and Resilience of Cholinergic Circuits in Aging

Mala Ananth*, David Talmage, Lorna Role

National Institutes of Health, Bethesda, Maryland, United States

Background: Acetylcholine critically modulates cognition. Cholinergic input to both cortical and subcortical regions originate in cell bodies that reside at the base of the forebrain and the brainstem. Although sparsely distributed, these cell bodies send highly branched projections to almost the entire brain with high functional and topographical organization, making a strong case for the functional importance of acetylcholine in cognitive behaviors.

It has been well-established that alterations to the cholinergic system can lead to cognitive dysfunction. For example, loss of cholinergic markers is a hallmark of age-related cognitive decline. In a cross-species investigation, we have previously found a marked reduction of cholinergic terminals in early, mild cognitive impairment in the entorhinal cortex, a brain region known to be vulnerable early in aging. Importantly, we found a relationship between the density of cholinergic terminals in the entorhinal cortex and performance on entorhinal cortex dependent cognitive tasks.

Alterations to entorhinal cortical functions, however, are not the only warning signs of early cognitive impairment. Many temporal lobe functions are also thought to be altered in aging. Given this, we investigated the health of other temporal lobe terminal fields in aging. In this focused regional analysis, we found surprising regional heterogeneity across cholinergic terminal fields in early cognitive impairment. Despite having a common cell body nucleus of origin, some terminal fields were dramatically affected, while others remained largely intact. In addition, the progression of cell body loss differed between cholinergic nuclei, as well as between subsets of cholinergic neurons within the same nuclei. Given these fascinating, heterogenous results, we sought to understand what underlies this differential vulnerability and resilience of cholinergic circuits in aging.

Methods: In order to study the cholinergic system in rodents, we used transgenic mouse models designed to selectively target and visualize the cholinergic system. Both male and female mice were examined at age points classified as young (6-8 weeks) and aged (18-24 months). For whole brain imaging experiments, passive tissue clearing (CUBIC) methods and high-resolution imaging were used. For focused, circuit-specific functional experiments, we combined the strength of chemogenetics with behavioral assays and with Fos immunohistochemistry.

Results: First, in a brain-wide analysis, we used light-sheet microscopy to identify cholinergic terminal fields and cell body clusters across the brain to categorize them as either vulnerable or resilient in aged vs. young mice. Next, to determine the functional relevance of these identified terminal fields, ongoing studies utilize Fos immunohistochemistry, inhibitory DREADDS, and fluorescent biosensors coupled with behavioral assays. To determine whether resilience or vulnerability in aging arose from distinct cholinergic subpopulations defined by function or topography, we layered cell-type specific retrograde tracing methods on to these studies. Future studies will utilize RNAseq for a thorough examination of gene-expression differences across these proposed subpopulations.

Conclusions: Taken together, these studies contribute to the growing body of data highlighting the diversity of cholinergic neurons in the brain. In aging, we find differences in cholinergic terminal field and cell body health both between and within brain regions. Better understanding the organizing principles and underlying diversity in cholinergic neurons will be critical not only for determining what factors contribute to vulnerable vs. resilient cholinergic circuits in the brain, but also how the cholinergic system facilitates higher-order processing across the brain.

Keywords: Cholinergic System, Whole-Brain Rodent Imaging, Aging

Disclosure: Nothing to disclose.

W4. Altered Frontoparietal Beta Coherence Dynamics in Visual Discrimination as an Early Biomarker to Predict Alzheimer's Disease

Vinay Parikh*, Irem Asci, Evan Haley

Temple University, Philadelphia, Pennsylvania, United States

Background: Alzheimer's disease (AD) is a progressive age-related neurodegenerative disorder characterized by loss of synapses and neurons and a continuous decline in cognitive abilities. Currently, there are no effective therapeutic approaches to prevent the disease or halt its progression. The existence of an effective biomarker for AD prediction at the presymptomatic stage will not only improve diagnostic accuracy but also facilitate timely intervention. Functional connectivity between the prefrontal cortex (PFC) and parietal cortex (PC) is critical for attentional control and mnemonic operations and these cognitive processes are affected in AD. Here we hypothesized that desynchronized electroencephalogram (EEG) oscillations in these networks during a task of attention would precede the onset of AD.

Methods: We utilized 3-months old triple transgenic mice (3xTG-AD; N=5), which harbors mutations in three genes (human presenilin-1, human amyloid precursor protein, and human tau), and wild-type (WT; N=7) mice to examine electroencephalographic (EEG) activity during an attention task. No pathological AD features (Aß accumulation and tau pathology) are observed at this age in AD mutants. Animals were trained in an operant visual discrimination task that required animals to discriminate the lever with an activated cue light presented randomly through either the left or the right panel. Mice trained to criterion (≥80% correct responses for 3 consecutive days) were implanted with EEG bone screws and 6-pin connector headstage. Following recovery, EEG activity was recorded from performing animals. EEG data was sampled at 1KHz with a 100 Hz preamplifier gain and analyzed for spectral power and coherence using a custom written program in MATLAB.

Results: No genotypic difference were observed in the trials to criterion ($F(1,10)=0.07$). Spectral analysis revealed that 3xTG-AD mice displayed significantly reduced power in the delta and theta bands in the PFC during hits as compared to the WTs (both

p<0.001). Conversely, the mutants displayed significantly higher power in the gamma band (p=0.02 vs WTs) when responded correctly. Frontoparietal coherence analysis indicated significantly higher coherence in the beta band in 3xTg-AD mice during hits (p=0.02); no significant differences were observed in other oscillations. Interestingly, bivariate correlation indicated a positive association between hit latencies and beta coherence in 3xTg-AD mice (Pearson's r=0.35; p=0.02), but this trend was not observed in WTs.

Conclusions: Although behavioral differences between the genotypes were not visible at 3 months, clear differences in the PFC delta, theta and gamma band power emerged during performance reflecting differential recruitment of this brain region. Higher frontoparietal beta coherence during hits in 3xTG-AD mice is suggestive of increased synchronization in the PFC and PC areas. Given that beta oscillations are more prominent during alert and attentive states, higher PFC-PC network connectivity might reflect an adaptive response to maintain visual discrimination performance in the mutants. Higher reaction times for hits in attentional tasks represent enhanced cognitive load. A significant positive correlation between hit latencies and beta coherence for 3xTG-AD mice suggest that high PFC-PC coherent activity might be reflective of functional compensation in these mutants. Collectively, our findings indicate that neurophysiological changes in the frontoparietal network are evident well before the pathological features of AD emerge in a mouse model. Specifically, a relative increase in the beta frequency band power in this network during a task that requires stimulus discrimination and attention may serve as a predictive biomarker for AD.

Keywords: EEG Biomarkers, Frontoparietal Network, Attention, Transgenic Mice, Alzheimer's Disease

Disclosure: Nothing to disclose.

W5. Excitatory and Inhibitory Synapses in Dorsal CA3 Distinguish Age-Related Cognitive Decline and Individual Differences in Learning and Memory

David Horovitz, Laura Askins, Grace Regnier, Joseph McQuail*

University of South Carolina, Columbia, South Carolina, United States

Background: Aging is characterized by decline of hippocampus-dependent learning and memory as well as accumulating risk for Alzheimer's disease (AD). Anomalous patterns of hippocampal activity manifest in prodromal AD, wherein a shift in the excitatory-inhibitory (E/I) dynamic contributes to memory deficits and signals the transition from normal brain aging to neurological disease. Memory loss in normal aging, as opposed to AD, is not associated with loss of hippocampal neurons. Consequently, attention has shifted to examine the structural and functional integrity of hippocampal synapses in the aging brain and to determine relationship with memory loss in susceptible individuals. E/I disruptions in the dentate gyrus (DG)-CA3 network are proposed to underlie age-related deficits in spatial learning that are ascribed to the dorsal hippocampus (functionally homologous to posterior hippocampus of primates), and not the ventral hippocampus (functionally homologous to primate anterior hippocampus). Collectively, these observations indicate that a neuroanatomically precise characterization of changes to excitatory and inhibitory synapses in the hippocampus of aging individuals could elucidate the circuit-basis of memory changes that confer increased risk for AD. Such information will be vital to the creation of targeted therapeutics designed to rebalance E/I dynamics in the aging brain, reverse later-life memory decline, and prevent the clinical conversion of at-risk elderly to dementia of the Alzheimer's type.

Methods: We conducted neurobehavioral assessments of young adult (6 mo; n=9) and aged (24 mo; n=24) male, Fischer 344 × Brown Norway-F1 hybrid rats using a multi-day, hidden platform/place-learning procedure in the Morris water maze (MWM) that depends on the rodent dorsal hippocampus. After place-learning, all rats were cross-trained on a visible platform/cue-learning version of the water maze that does not tax the hippocampus while still requiring the same visual, physical and motivational abilities as place-learning. Brains from these rats were subsequently prepared for examination by histological analysis. From every rat, a neuroanatomically registered series of sections spanning the dorsal-ventral extent of the hippocampus was labelled using antibodies raised against VGluT1, a marker of glutamatergic synapses, and VGAT, a marker of GABAergic synapses, and visualized with secondary antibodies coupled to fluorescent dyes. Immunofluorescent staining was visualized using a high-resolution (10 μm pixel) fluorescence imaging system. Intensity of staining in each subregion (DG, CA1 and CA3) within the dorsal and ventral hippocampus was quantified using ImageJ software.

Results: Our behavioral data demonstrate that dorsal hippocampus-dependent memory impairments may be ascribed to a subset of aged rats (aged-impaired; n=12) that do not exhibit evidence of a spatially guided search strategy in the MWM relative to young adults and age-matched peers with intact memory (aged-unimpaired; n=12). Immunofluorescence data revealed marginal differences in VGluT1 intensity among cognitive subgroups, but no differences in VGAT. Planned contrasts suggest any differences are attributed to lower level of VGluT1 in dorsal and ventral CA3 of aged-impaired rats relative to young adults. Using a complementary approach that leverages the full range of individual differences in spatial learning, correlational analyses applied across the entire spectrum of aged performance revealed a significant positive relationship between spatial learning and VGAT intensity in the dorsal CA3. This relationship may have significance across the entire lifespan as age-adjusted correlational analyses determined reliable associations between spatial learning and VGAT in dorsal CA3, as well as dorsal CA1, of young adult and aged rats.

Conclusions: These new findings are consistent with our hypothesis that E/I disruption centered on CA3 is of special relevance to individualized decline of memory function in aging. Furthermore, these changes were consistently, though not exclusively, evident in the dorsal hippocampus. From these data, we conclude that the neurochemical/neuroanatomical basis for imbalanced E/I dynamic that associates with memory abilities involves an interaction between an age-dependent loss of glutamatergic CA3 synapses with age-independent individual differences in levels of GABAergic CA3 synapses. Forthcoming analyses from our lab will differentiate discrete CA3 synaptic layers as well as examine relationships to adjacent medial temporal lobe structures (perirhinal/entorhinal cortex, amygdala) to comprehensively characterize differences in memory ability and reorganization of E/I circuitry in the aging brain. Furthermore, these new data direct us to examine methods that can selectively potentiate CA3 synaptic neurotransmission in an activity-dependent manner with the long-term goal to restore memory function that is susceptible to decline with age.

Keywords: Glutamate, Gaba, Hippocampus, Brain Aging, Learning and Memory

Disclosure: Nothing to disclose.

W6. 3,4-Methylenedioxymethamphetamine Stimulates RhoA Activation and Transporter Internalization Through the Trace Amine Associated Receptor in Serotonin Neurons

Suzanne Underhill*, Susan Amara

NIMH, NIH, Bethesda, Maryland, United States

Background: The use of 3,4-methylenedioxymethamphetamine (MDMA) has recently re-emerged as a therapeutic tool for the treatment of post-traumatic stress disorder (PTSD). However, the mechanism of action of this drug has not yet been fully elucidated. Here we demonstrate that MDMA acts by targeting the intracellular trace amine associated receptor 1 (TAAR1), a GPCR expressed in neurons throughout the brain, including serotonergic neurons. In previous studies, we have shown that the closely related compound, amphetamine (AMPH), enters neurons through the dopamine and norepinephrine transporters (DAT and NET) and activates TAAR1 which couples to both GS and G13 signaling pathways. G13 signaling through TAAR1 increases the activity of RhoA, a small GTPase that increases internalization of the DAT and NET resulting in an increase in extracellular DA and NE concentrations. Like AMPH, MDMA is a potent agonist for TAAR1. In this study, we show that MDMA enters serotonin neurons through the 5-HT transporters (SERT) and activates similar G-protein coupled signaling cascades through TAAR1 in serotonergic neurons.

Methods: Serotonin (5-HT) neurons in culture were derived from Raphe nuclei of E15 Swiss-Webster mice. Confirmation of 5-HT (+) neurons was assessed with immunocytochemistry for 5-HT. 3H-5-HT uptake was measured in these cells in order to assess functional SERT at the plasma membrane. We also used total internal reflection fluorescence (TIRF) microscopy in HEK293 cells transiently transfected with a GFP-tagged SERT in order to study the membrane localization of SERT and its response to MDMA. The role of the TAAR1 receptor in these assays were determined with parallel experiments in HEK293 cells lacking the TAAR1 receptor. Acute coronal murine brain slices that included the Raphe nucleus were assessed for SERT cell surface expression with a cell-impermeable biotin reagent, used to isolate and assess proteins at the cell surface.

Results: Cells isolated from the Raphe grew robustly in mixed cultures of astrocytes and neurons. While only a small proportion of the neurons in these cultures stained positive for 5-HT, they exhibited elaborate neurites throughout the cultures. We measured 3H-5-HT uptake in these cultures that was blocked by the SERT inhibitor citalopram (100 nM), indicating SERT-mediated transport activity. Pre-treatment of Raphe cultures for thirty minutes with 1 μM MDMA resulted in a decrease of SERT activity ($n=6$; $P<0.001$). This MDMA-induced decrease in SERT activity was blocked by an inhibitor of RhoA ($n=9$), indicating a similar biochemical cascade of that which we observed for DAT and NET in response to AMPH. By total internal reflection fluorescence (TIRF) microscopy in HEK293 cells we found that loss of SERT activity was due to internalization of a GFP-tagged SERT in response to MDMA ($n=9$). This event was absent in HEK293 cells in which the TAAR1 gene was deleted by CRISPR-Cas9 ($n=36$). In order to assess SERT trafficking in mature, more intact systems, we made acute coronal brain slices from mice. Slices containing the Raphe nucleus were treated with either vehicle control or MDMA (1 μ M) and subsequently treated with a cell-impermeable biotin reagent that would label all surface proteins. By western blot of biotinylated proteins from these tissue lysates, we were able to assess SERT internalization in response to the MDMA treatment ($n=4$; $p<0.01$)

Conclusions: These data indicate that pharmacological agents can regulate TAAR1 signaling in serotonergic neurons and that the selectivity of TAAR1 agonists depends not only on affinity for the GPCR but also on their transport efficiency. These findings suggest that the activation of TAAR1 in serotonin neurons and the subsequent internalization of surface SERT is likely contribute to the modulation of serotonin signaling observed in response to MDMA. These results suggest potential mechanisms to target for the development of novel drugs to treat PTSD.

Keywords: MDMA, Serotonin, TAAR1, SERT

Disclosure: Nothing to disclose.

W7. Targeted Neuroepigenetic Editing Regulates Stress Phenotype

Ajinkya Sase*, Brandon Santhumayor, Sonia Lombroso, Kyle Czarnecki, Michael Murphy, Mia Dittrich, Elizabeth Heller

Perelman School of Medicine University of Pennsylvania, Philadelphia, Pennsylvania, Unites States

Background: Sex-differences in the expression and prevalence of trauma- and stressor-related disorders have led to a growing interest in the sex-specific molecular and epigenetic mechanisms underlying these diseases. In the adult rodent brain, Cyclin-dependent kinase 5 (Cdk5) modulates learning and memory, anxiety, and depressive phenotype. Cdk5 regulates such behaviors and the magnitude of these behaviors by phosphorylating downstream target proteins. Because dysregulation in Cdk5 activity leads to synaptic and neuronal loss, binding partners such as p25 tightly regulate Cdk5 activity. Alternatively, very little is known about the epigenetic regulation of Cdk5 expression in stress-related disorders. Most studies of Cdk5 have used only male rodents, while human data shows that women are at a greater risk for stress-related psychiatric disorders, such as depression & post-traumatic stress disorder (PTSD). We hypothesized that histone modifications at the Cdk5 promoter are sufficient to regulate its expression, and influence sexually dimorphic behavioral responses to chronic stress.

Methods: We used chronic unpredictable stress (CUMS) in male and female mice, as it is a robust translational paradigm to elucidate molecular mechanisms of chronic stressor exposure. Male or female mice (6-10 per group) were subject to CUMS that included a combination of one day and one overnight stressor for 7, 14, 21 or 28 days. Day stressors included 1-hour (h) home cage orbital shaking (100 r.p.m.), 30-min immobilization in a 50ml tube, 1-h white noise with overcrowding (4-5 mice/round plastic container 8"Dx7"H with ventilation holes). Overnight stressors: wet bedding; tilted cage (45°); 24-h lights on (no dark period). Following CUMS or home cage (control), stress was assayed by sucrose preference test, marble burying test, light/dark transition test and forced swim test to measure anhedonia, anxiety and depression-like behavior. We calculated a composite Z-score for each mouse of responses to all four stress phenotyping behavioral tests, normalized to male home-cage controls and corrected for the direction of effect for each behavioral test. We also analyzed blood corticosterone by radioimmunoassay at 0, 7, 14, 21 and 28 days. In a separate cohort of animals, we analyzed Cdk5 mRNA and protein expression, and Cdk5 histone modifications by chromatin immunoprecipitation (ChIP) in stress-related brain areas: nucleus accumbens (NAc), prefrontal cortex (PFC). Next, we applied intra-NAc CRISPR-mediated epigenetic editing at the Cdk5 promoter prior to CUMS, and assayed stress phenotype, expression of Cdk5, and enrichment of histone modifications at the Cdk5 promoter. Epigenetic editing was carried out by intra-NAc transfection of a single-guide RNA targeting the Cdk5 promoter and a catalytically dead, Cas9 protein fused to either histone deacetylase 3 (dCas9-HDAC3) or histone acetyltransferase (dCas9-p300), expressed neuronally under human synapsin 1 (hSyn) promoter. Data were analyzed using 2-way ANOVA with sex and composite stress score as factors followed by Bonferroni's post hoc analysis for multiple comparisons.

Results: We found that 21 or 28 but not 7 & 14 days of CUMS increased blood corticosterone levels in male and female mice (main effects of sex: $F(3,108) = 10.15$ $p < 0.0001$ and day $F(3,108) = 6.238$ $p = 0.006$). CUMS gradually decreased body weight in both male and female mice over 28 days (main effect of stress $F(80,320) = 42.04$ $p < 0.0001$ main effect of days $F(4,320) = 10.65$ $p < 0.0001$). We found sex-specific behavioral responses to 14, 21 and 28 days of CUMS. Specifically at 14 days CUMS did not show

main effect of stress $F(1,36) = 0.1484$ $p = 0.7023$ but showed main effect of sex $F(1,36) = 14.53$ $p = 0.0005$. Next, at 21 days CUMS increased the composite stress score in female but not in male (main effect of stress $F(1,36) = 8.193$ $p = 0.0070$, main effect of sex $F(1,36) = 20.90$ $p < 0.0001$ post hoc female $p = 0.0172$). At 28 days CUMS increased composite score in both male and female (main effect of stress $F(1,36) = 23.62$ $p < 0.0001$, main effect of sex $F(1,36) = 4.135$ $p = 0.0494$ post hoc male $p = 0.0061$ and female $p = 0.0133$). We also found that 14 and 21 days of CUMS activated Cdk5 expression in the NAc of male, but not female mice (main effect of sex, 14 days $F(1,25) = 3.079$ $p = 0.04$. post hoc male $p = 0.03$. 21 days $F(1,16) = 1.587$ $p = 0.02$. post hoc male $p = 0.01$. Similarly we found that histone H3 lysine27 (H3K27ac) was enriched at Cdk5 promoter at 14 and 21 days but not 28 days of CUMS (main effect of sex, 14 days $F(1, 16) = 12.69$ $p = 0.0026$, main effect of stress $F(1, 16) = 5.765$ $p = 0.0289$ post hoc 14 days male $p = 0.0135$ and 21 days male $p = 0.0390$). Alternatively, 14 but not 21 or 28 days of CUMS decreased Cdk5 mRNA in both male and female PFC (main effect of CUMS, $F(1,26) = 3.297$ $p = 0.040$, post-hoc male $p = 0.031$, female $p = 0.043$). In NAc, CRISPR mediated Cdk5 activation using dCas9-p300 increased baseline composite stress score in both male and female mice in the absence of CUMS (main effect of activation $F(1,9) = 32.25$ $p = 0.0003$.) On the other hand in NAc, CRISPR mediated Cdk5 repression using dCas9-HDAC3 had no effect on composite stress score in either sex $F(1,9) = 3.230$ $p = 0.10$).

Conclusions: In conclusion, our work provides a model of stress evoked sex-specific chromatin remodeling at Cdk5 promoter, and reveal the causal relevance of Cdk5 histone acetylation to stress-induced behavior. Future work will evaluate whether CRISPR mediated epigenetic editing in combination with CUMS is sufficient to rescue the stress phenotype.

Keywords: Crispr/Dcas9, Chronic Unpredictable Mild Stress, Sex Difference, Cdk5, Neuroepigenetic Editing

Disclosure: Nothing to disclose.

W8. Molecular Roadmap of the Healthy Stress Response in the Mouse Hippocampus

Amalia Floriou Servou*, Lukas von Ziegler, Pierre-Luc Germain, Rebecca das Gupta, Oliver Sturman, Christina Maat, Tobias Kockmann, Han-Yu Lin, Sian Duss, Rebecca Waag, Hanns U. Zeilhofer, Johannes Bohacek

ETH Zurich, Zurich, Switzerland

Background: In biomedical research, the stress response is typically studied in light of maladaptive coping and psychiatric disease. A healthy stress response, however, peaks rapidly and terminates efficiently, without permanent consequences for the organism. Although the series of physiological events accompanying exposure to stress are well-characterized (Joëls and Baram 2009), the time course and intensity of molecular changes in the brain, as well as the cell types affected, remain elusive.

Here, we investigated the time course of the molecular stress response in the dorsal and ventral hippocampus (dHC and vHC), since they have been shown to be particularly responsive

to acute stress, based on transcriptomic and proteomic data (Floriou-Servou et al., 2018).

References (*equal contribution):

- Floriou-Servou A.*, von Ziegler L.*, Stalder L., Sturman O., Privitera M., Rassi A., Cremonesi A., Thöny B., Bohacek J. Distinct Proteomic, Transcriptomic, and Epigenetic Stress Responses in Dorsal and Ventral Hippocampus. Biol. Psychiatry 84, 531–541 (2018).

- Joëls, M. & Baram, T. Z. The neuro-symphony of stress. Nat. Rev. Neurosci. 10, 459–66 (2009)

Methods: First, we use phosphoproteomics to capture the initial signaling events that take place immediately after exposure to stress. Then, we use next generation sequencing (NGS) to detect gene expression changes at the transcriptional level at different time points after acute stress. To test which genes likely become translated, we employ translating ribosome affinity purification (TRAP) and investigate the effects of stress on the translatome of excitatory and inhibitory neurons.

Forced swim stress was used for acute stress: male or female mice (N=5-8) were placed in 18°C cold water for 6 minutes. Mice were sacrificed either immediately after acute stress or at different time-points after onset. The vHC and dHC were dissected, and RNA or proteins were extracted for analysis.

For statistical analysis we used R version 3.6.2. For two group comparisons a genewise exact test was used, for more complex designs we used a generalized linear model (GLM) with empirical Bayes quasi-likelihood F-tests. For multiple testing correction the Benjamini-Hochberg false discovery rate (FDR) method was used.

Results: - Acute stress induces rapid and short-lived changes on the phosphoproteome: Immediately after stress, we detected hundreds of significantly (FDR adjusted pvalue < 0.05) altered modified peptides of which ~93% were phosphopeptides. The number of significantly changed phosphopeptides dropped at 15 minutes after stress, while we could not detect any more significant changes at the 30 and 45 minutes time points, indicating that protein phosphorylation changes after acute stress occur rapidly and seem to be tightly regulated and short-lived.

- Stress-induced transcriptomic changes evolve over time: We observed highly dynamic gene expression changes over time in response to swim stress exposure. Using 5% FDR-corrected p-values, we observe the highest number of genes changing at 45 and 90 minutes after stress, and then a gradual decline in both dHC and vHC. At 4 hrs, no significant changes were detected anymore, suggesting that gene expression is tightly regulated after acute stress exposure and that the transcriptional machinery quickly re-establishes equilibrium.

- Stress-induced effects on the translatome of excitatory and inhibitory neurons: After stress, the expression levels of a small number of genes were significantly changed in both excitatory and inhibitory neurons. Most differentially expressed genes were detected in the inhibitory neurons of the vHC, and were mainly immediately early genes.

Conclusions: We find that stress strongly and rapidly triggers widespread changes in intracellular signaling and gene transcription. In a series of dynamic yet tightly regulated molecular events, the stress response terminates efficiently within 4 hours after the initiation of stress. In addition, we find immediate early gene expression signatures specific for inhibitory and excitatory neurons, indicating that the molecular stress response affects the different brain cell types in variable ways.

This work represents the most extensive molecular characterization of the acute stress response to date. It can serve as a roadmap in the endeavor to understand how various stressors can trigger similar or divergent, possibly maladaptive changes in different individuals.

Keywords: Multi-omics, RNA-seq, Transcriptomics, Phosphoproteomics, Translatomics

Disclosure: Nothing to disclose.

W9. Differential Associations Between White Matter Connectivity and Inflammation: Acute Versus Chronic PTSD

Negar Fani*, Jennifer Stevens, Kerry Ressler, Vasiliki Michopoulos

Emory University School of Medicine, Atlanta, Georgia, United States

Background: Post-traumatic stress disorder has been frequently associated with increased immune activation, as shown by higher level of inflammatory markers (such as C-reactive protein, CRP) and abnormal functional connectivity of fronto-limbic pathways. However, little is known about how inflammation is associated with the integrity of white matter within these pathways, as well as the ways in which duration of illness may affect this relationship. As such, we assessed the relationship between CRP, PTSD and white matter structural integrity within two samples: a highly-traumatized population with chronic PTSD (Grady Trauma Project, GTP) and a population of acutely traumatized individuals recruited from the emergency department (ED).

Methods: Clinical assessments, serum CRP, and diffusion tensor imaging (DTI) data were collected from both a cross-sectional study of chronic trauma (GTP) and as part of a longitudinal study of acutely traumatized people (recruited from the ED). With respect to the GTP sample, fifty-seven women aged 19-62 (Mean=38.7, SD=12.2) were recruited from primary care clinics of an inner-city hospital in Atlanta, Georgia; approximately half of the sample had clinically significant PTSD. Separately, twenty-eight men and women aged 19-58 (Mean=33.1, SD=11.5) were recruited from the ED of a level 1 trauma center as part of an NIH-funded study of PTSD biomarkers and scanned at approximately 1 month post-trauma; 12 participants had clinically significant PTSD at this timepoint. In both samples, probabilistic tractography was used to examine structural integrity of white matter connections between the hippocampus and anterior cingulate cortex (cingulum bundle) and the amygdala and vmPFC (uncinate fasciculus). Mean fractional anisotropy (FA) values of these tracts were entered into statistical models.

Results: In the GTP sample, Serum CRP was positively correlated with FA in the cingulum bundle in participants with clinically significant PTSD symptoms (r=.42, p=.03), but not in the low/no symptom group. In the ED sample, FA in the uncinate fasciculus was negatively associated with serum CRP in the high PTSD symptom group (r=-.7, p=.01), but no significant associations were observed in the low/no symptom group.

Conclusions: These findings indicate distinct associations between white matter connectivity and CRP that may be moderated by chronicity of PTSD. Whereas higher CRP levels were associated with lower FA in fronto-limbic tracts in acutely traumatized people with PTSD symptoms, this association was reversed in people with more chronic PTSD and greater trauma exposure. Trauma exposure and symptom chronicity appears to be a moderator of associations between inflammatory markers and PTSD (Michopoulos et al., 2020, American Journal of Psychiatry; Bhatt et al., 2020, Nature Communications). These data suggest that symptom chronicity may have a moderating effect on immune activation and its relationship with white matter connections in fronto-limbic pathways. We will discuss the implications of these findings for acute versus chronic PTSD, elaborating on how immune dysregulation may differentially affect white matter connectivity at earlier vs later stages of PTSD symptom development.

Keywords: Inflammation, PTSD, White Matter Integrity

Disclosure: Nothing to disclose.

W10. Individualizing Transcranial Direct Current Stimulation for Clinical Trials on Posttraumatic Stress Disorder

Mascha van 't Wout*, Alexis Harle, David Sorensen, Noah Philip

Alpert Medical School, Brown University; Center for Neurorestoration and Neurotechnology, Providence VA Medical Center, Providence, Rhode Island, United States

Background: Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that shows promise in modulating cognitive processes underlying posttraumatic stress disorders (PTSD) and improving symptoms. It is well known that individual neuroanatomy affects the electrical current flow generated by tDCS in the brain. Yet surprisingly tDCS intensity is currently not determined on an individual basis; instead a fixed current intensity, e.g. 1 or 2 mA, is commonly used. Individualizing tDCS intensity based on neuroanatomy ensures consistent dose across individuals and could improve tDCS efficacy as well as assessment in clinical trials. Here we present unpublished data on variability in electrical field values in neural regions associated with PTSD and experience and regulation of emotions and fear response using a fixed tDCS intensity as part of an ongoing clinical trial of tDCS plus virtual reality for PTSD (NCT03372460). Given the aim of this clinical trial to specifically augment ventromedial prefrontal cortex (VMPFC)-based habituation, we also calculated the needed tDCS intensity to achieve a predetermined electrical field in the VMPFC.

Methods: Structural MRI scans from 27 adults with PTSD were included for individual electrical field modeling of a fixed 2 mA tDCS intensity with the anode placed over FP1 and the cathode over P08 using 3x3 cm electrodes. Individual electrical field modeling was performed using the open-source Realistic, vOlumetric Approach to Simulate Transcranial electric stimulation tool. Extracted regions included the VMPFC, amygdala, dorsolateral prefrontal cortex, insula, and hippocampus. Average extracted electrical field values were then reverse-calculated to establish the individualized tDCS intensity for the VMPFC as the target region in this clinical trial.

Results: Repeated measures ANOVA demonstrated that obtained electrical field values differed significantly across neural regions of interest ($F(1.3, 33.3)=18.98$, $p<.001$, $\eta p2=0.42$) with observed individual values ranging between 0.12 - 0.99 V/m. Obtained electrical field values in VMPFC were significantly higher compared to values in amygdala, hippocampus, and insula (all $p<.001$). Obtained electrical field values in VMPFC did not differ significantly from those obtained in the dorsolateral prefrontal cortex ($t(26)=1.46$, $p=.16$). In order to ensure the average obtained value in VMPFC, tDCS intensity should have ranged from 1.55 - 3.01 mA for individual participants.

Conclusions: Obtained electrical field values demonstrated greater current density in regions associated with down-regulation of fear and regulation of emotions more broadly, e.g. ventromedial and dorsolateral prefrontal cortex, over regions associated with fear responding, e.g. amygdala, insula. Nonetheless, the wide range in obtained electrical field values and subsequent reverse-calculated tDCS intensities validates the need for individualized electrical field modeling in clinical trials of tDCS.

Keywords: TDCS, vmPFC, Electrical Field Modeling, Clinical Trial, PTSD

Disclosure: Nothing to disclose.

W11. A Rodent Analogue of the Fearful Face Task via Ultrasonic Vocalization Playback: Evidence From Behavioral, Neural, and Task-Based fMRI Analyses

Jennifer Honeycutt*, Lauren Granata, Camila Demaestri, Praveen Kulkarni, Craig Ferris, Heather Brenhouse

Bowdoin College, Brunswick, Maine, United States

Background: Limbic regions, such as the amygdala and bed nucleus of the stria terminalis (BNST) play a crucial role in processing emotionally salient information, including fear and anxiety. They are involved in the initiation [basolateral amygdala] and maintenance [BNST] of anxiety-like states in response to potential threat. Changes in the underlying neural circuitry in anxiety and hypervigilance can be probed clinically in humans through use of the Fearful Face Task (FFT) in fMRI studies.

Currently, there exists no established analogue of the FFT to systematically study anxiety-related changes in task-based connectivity in preclinical rat models of affective dysfunction. Thus, development and implementation of novel assessments of anxiety-related circuitry in rodents is needed for researchers to gain an ethological understanding of - and assess translational validity of - anxiety models. To this end, we can harness existing communication modalities in rats that convey comparable affective information as would be communicated through human facial expressions. The use of ultrasonic vocalizations (USVs) as a means of communication in rats is well established, with specific USV frequency ranges thought to be representative of different affective states. Aversive USVs (22kHz range) are typically emitted by adult rats as alarm calls in situations where the animal may be experiencing anxiety and/or fear due to a real or perceived threat. Conversely, appetitive USVs (55kHz range) are thought to be indicative of rewarding or pleasurable experiences. Previous work from our group and others indicate that playback of USVs to a listening rat leads to acute changes in affective state, as evidenced by increased anxiety-like or approach behaviors following either 22 or 55kHz playback, respectively. We leveraged this knowledge to determine whether USV playback could elicit acute affective changes, as measured through behavior, immunohistochemistry, and awake task-based fMRI brain activity changes.

Methods: Ultrasonic speakers (for behavioral and IHC studies; male rats) and custom ultrasonic ear buds (task-based fMRI study; male and female rats) were used to introduce aversive (22kHz) and appetitive (55kHz) USVs to awake listening adult rats. In study 1, rats were evaluated in the open field or elevated plus maze during USV playback, with brains collected for cFos characterization in multiple brain regions. In study 2, awake restrained rats (previously habituated to testing conditions) were fitted with ultrasonic earbuds and imaged in a Bruker 7T magnet and scanned at 300MHz in a custom quadrature transmit/receive coil. Anatomical scans were collected using RARE pulse sequence and functional scans were collected using a spin-echo triple-shot EPI sequence. USVs were presented in a box car design (2min on/2min off with a 14min total duration) and voxel-based BOLD activity during playback was compared to baseline. All experiments have group n's ranging from 8-12.

Results: In study 1, playback of aversive, but not appetitive, USVs increased cFos expression in the basolateral amygdala ($p<0.05$) compared to silence. Aversive USV playback also increased cFos expression in the anterodorsal nucleus of the BNST ($p<0.0001$) compared to both silence and appetitive playback. Conversely, appetitive USV playback increased cFos activity within the oval BNST compared to aversive playback ($p<0.05$), suggesting that these nuclei differentially process affective information. These neural data were supported by changes in anxiety-like behavior in both the open field and elevated plus maze when animals listened to sustained aversive playback, but not when listening to appetitive playback. Preliminary analysis of the BOLD data following USV playback to awake rats in the fMRI reveal comparable findings, in that aversive and appetitive USV playback selectively activate distinct brain regions/circuits. Transformed Z statistics of BOLD data during playback compared to baseline show significant (absolute $Z > 2.3$) voxel-based changes in different brain regions depending on type of playback. Specifically, aversive USV playback showed significant changes in activity from baseline in the basolateral amygdala, central amygdala, and BNST, among others; while appetitive playback showed changes from baseline in the nucleus accumbens and BNST, among others. Interestingly, within this preliminary data, there does not seem to be an obvious effect of sex (though additional data is still being collected).

Conclusions: Our data provides exciting preliminary evidence suggesting that both aversive and appetitive USV playback activates affective circuits in a manner similar to that seen in the human fearful face task. Development and implementation of this novel methodology will allow researchers to leverage the advantages of rodent modeling in a task that is highly translational to human populations, thereby providing enhanced understanding of anxiety-related etiology.

Keywords: Anxiety & PTSD, Affective Neuroscience, Ultrasonic Vocalization, Task-Based Functional Connectivity, Rodent Models

Disclosure: Nothing to disclose.

W12. A Prospective Study of the Effects of Prior PTSD on Stress Reactivity to COVID-19

Sabra Inslicht*, Vincent Rozalski, Thomas Neylan

University of California, San Francisco, California, United States

Background: The COVID-19 pandemic is an unprecedented crisis that poses a significant risk to the physical health and well-being of much of the world population to an extent that is yet unknown. The potential threat of death to oneself and loved ones that some may experience with COVID-19 and the disruption to routines, finances, and social networks likely increases chronic stress and exacerbate PTSD. Additionally, increased behavioral avoidance and impulsivity that is commonly found in PTSD could impact adherence to public health safety recommendations meant to mitigate this disease.

Our overall objective is to determine whether individuals with prior PTSD experience heightened stress reactivity to the pandemic, report greater self-reported anxiety and riskier health behavior. We predicted that individuals with prior PTSD will have greater intrusive and avoidant thoughts related to the pandemic compared to trauma-exposed controls and less adherence with public health recommendations.

Methods: We are conducting a prospective study of a pre-existing cohort of PTSD participants and trauma-exposed controls. The cohort is drawn from 2 prior studies that had a 2 x 2 design, with 4 groups: male PTSD, female PTSD, male controls, and female controls ($n = 310$). Participants included Veterans and civilians, ages 18-65. PTSD status was determined by the Clinician Administered PTSD Scale for DSM-IV. Individuals in the control group had been exposed to a traumatic event but never met PTSD criteria. For this study, participants will complete questionnaires at 4-month intervals over the 36-month study to evaluate exposure to COVID-19, health behavior, and intrusions and avoidance related to COVID-19 on the Impact of Event Scale. This data will be linked with pre-collected measures on trauma history and PTSD status that were administered prior to the pandemic. This prospective data will allow us to determine the impact of COVID-19 on individuals with prior histories of PTSD, current stress symptoms, health behavior and risks on trajectories of functioning over the course of the pandemic.

Results: Preliminary analyses of the initial 40 respondents indicated an association between prior PTSD diagnosis and greater intrusion and avoidance symptoms related to COVID-19 ($r = .39$, $p < .05$). Greater intrusions and avoidance to COVID-19 were associated with greater adherence to COVID-19 health precautions, including social distancing and wearing a mask ($r = .36$, $p > .05$). Analyses from the larger sample will be presented at the meeting.

Conclusions: Among trauma-exposed individuals, prior PTSD was strongly associated with stress reactions to the COVID-19 pandemic. However, contrary to expectations, greater COVID-19 stress symptoms were associated with greater adherence to health precautions, suggesting that greater attention to threat may be protective in the context of a current stressor and may affect risk for disease.

Keywords: PTSD, COVID-19, Health Promotion

Disclosure: Nothing to disclose.

W13. The Effects of Pharmacological Inactivation of the Nucleus Reuniens During Predator Odor Exposure on Contextual-Induced Stress Memory

Laura Ornelas*, Kalynn Van Voorhies, Joyce Besheer

Bowles Center for Alcohol Studies, The University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, North Carolina, United States

Background: Two out of the four clusters of diagnostic criteria for Post-Traumatic Stress Disorder (PTSD) within the DSM-5 include 1) intrusive distressing memories of the traumatic event(s) and 2) persistent avoidance of the stimuli associated with the traumatic event(s). Understanding the behavioral and neurobiological mechanisms associated with these persistent and long-lasting symptoms can be invaluable tools for effective treatment and prevention strategies for PTSD. In animal models, re-exposing animals to stress-related stimuli or the environment in which the stressor was presented can induce contextual fear or stress responses that can serve as an index of memory of that context. The nucleus reuniens (RE), a midline thalamic nucleus, is an emerging area of the brain underlying symptom profiles of PTSD, such as stress and depression. Here we examined the functional involvement of the RE in stressor-related memory via inactivation of the RE prior to stressor exposure.

Methods: Female (n=75) Long Evans rats were implanted with a unilateral cannula aimed at the RE. One week after surgery, rats received muscimol (0.5 mM) or aCSF microinjections 10 min prior to stressor exposure. Rats underwent predator odor stressor exposure using trimethylthiazoline (TMT; synthetically derived fox feces component) for 20 min in a distinct context with no bedding material or with bedding. Controls underwent water exposure under the same conditions. To measure reactivity to the TMT-paired context, 2 weeks following the initial TMT exposure, rats were re-exposed to the TMT-paired context (in the absence of TMT).

Results: During TMT exposure (without bedding), rats in the TMT groups showed a significant increase in immobility behavior and avoidance of the TMT side (immobility $F(1,24) = 139.5$, $p < 0.05$; avoidance $F(1,24) = 66.43$, $p < 0.05$), but no significant interaction for either immobility or avoidance ($p > 0.05$). There were no differences in immobility behavior or time spent on the side in which TMT was located in rats administered muscimol compared to vehicle ($p < 0.05$). During TMT exposure (with bedding), rats in the TMT groups engaged in defensive digging, immobility behaviors and avoidance of the TMT side (digging $F(1,30) = 19.77$, $p < 0.05$; immobility $F(1,30) = 46.07$, $p < 0.05$; avoidance $F(1,30) = 16.59$, $p < 0.05$, $p < 0.05$). There were no main effects of drug treatment or interactions ($p>0.05$). There were no differences in defensive digging, immobility behavior or time spent on the side in which TMT was located in rats administered muscimol compared to vehicle.

During context re-exposure (no bedding), rats previous exposed to TMT showed decreased immobility compared to controls (main effect of TMT ($F(1,24) = 4.70$, $p < 0.05$), indicating reactivity to the context and likely related to increased exploration of the environment. There was a significant exposure x treatment interaction ($F(1,22) = 4.94$, $p < 0.05$), with rats in the TMT group that received RE inactivation showing decreased immobility relative to rats that received aCSF ($p<0.05$), suggesting that muscimol pre-treatment blunted context reactivity. During context re-exposure (with bedding), inactivation of the RE had no effect on context re-exposure behavior. That is, rats previously exposed to TMT showed decreased immobility behavior (similar to the previous no bedding cohort) and increased defensive digging (immobility $F(1,30) = 38.39$, $p < 0.05$; digging $F(1,30) = 38.39$, $p < 0.05$;), but no main effect of drug treatment or significant interaction ($p < 0.05$)

Conclusions: The results demonstrate several important findings. First, RE inactivation blunts context reactivity (as measured by immobility behavior) in rats previously exposed to TMT when no bedding is present in the context. This suggests that the RE is important for the expression of this context-induced stress memory. However, when providing rats with bedding during TMT exposure, RE inactivation does not affect context reactivity (as measured by immobility behavior and defensive digging), suggesting that the memory of the stressor-related environment is still intact.

In conclusion, recruitment of the RE in stressor-related contextual memory appears to be dependent on the contextual environment and whether the animal is able to engage in different stress coping strategies.

Keywords: PTSD, Nucleus Reuniens, Stress Coping

Disclosure: Nothing to disclose.

W14. Effect of Acute Stress and Stress-Conditioned Cues on Matrix Metalloproteinases in the Nucleus Accumbens Core

Ritchy Hodebourg*, Constanza Garcia-Keller, Peter Kalivas

Medical University of South Carolina, Charleston, South Carolina, United States

Background: Post-traumatic stress disorder (PTSD) is a psychiatric disease that is seen in a subpopulation of people experiencing a traumatic event. It is well established that there is a strong comorbidity between PSTD and substance use disorders (SUDs). For example, 40-50% of veterans diagnosed with PTSD being comorbid for SUDs. Preclinical studies have shown that acute stress produces long-lasting neuroadaptations at glutamatergic synapses in the nucleus accumbens core (NAcore) that parallel those produced by drug self-administration. Thus, 2h of restraint stress followed by 3 weeks induces an increase in the AMPA/NMDA ratio and dendritic spine density and downregulates the glutamate transporter (GLT-1) in the NAcore. Furthermore, a stress-conditioned stimulus (CS) is able to reinstate drug seeking behavior. Based on these data we hypothesized that, like drug-associated cues, a stress-CS induces transient-synaptic potentiation (t-SP) in the NAcore. Knowing that cue-induced t-SP and drug seeking requires activation of NAcore matrix metalloproteinases (MMPs), we sought to determine whether a stress-CS also activates MMPs.

Methods: To quantify the MMP-2,9 activity, male Sprague Dawley were microinjected with FITC-quenched gelatin into the NAcore immediately before 30 min of acute restraint stress or 30 min in the home cage. The MMP-2,9 activity was analyzed using confocal microscopy. To evaluate the role of a stress-CS on the behavior and MMP-2,9 activity, Long Evans rats were restraint stressed for 2h and simultaneously exposed to an odor that became the stress-CS. Control rats were exposed to the same odor in the home cage. Three weeks after the stress, FITC-quenched gelatin was injected into the NAcore and the effect of the CS or a novel stimulus (NS) was tested in a defensive burying task for 15 min. The burying and immobility behaviors, as well as MMP-2,9 activity, were quantified.

Results: We found that 30 min of acute stress increased the MMP-2,9 activity in the NAcore. The stress-CS induced burying behavior and elevated MMP-2,9 activity compared to the sham control. However, there is no difference between stressed rats exposed to the CS and an unconditioned odor. Then, to show that the stress-CS induced burying was mediated by MMP-9, an MMP known to be involved in cue-induced t-SP and reinstated drug seeking, we pretreated with an MMP-9 inhibitor to reduce stress-CS induced MMP activity.

Conclusions: These findings contribute to a growing literature suggesting that PTSD and SUDs share common neural substrates and offer new targets for treating both disorders.

Keywords: PTSD, Acute Stress, Nucleus Accumbens, Matrix Metalloproteinase-9 (MMP-9)

Disclosure: Nothing to disclose.

W15. The Novel Insomnia Drug Daridorexant Also Reduces Behavioral and Physiological Stress Responses in Rats

Michel Steiner*, Rebekka Locher, Hugues Lecourt, Francois Jenck

Idorsia Pharmaceuticals Ltd, Basel, Switzerland

Background: The orexin neuropeptide system is a natural regulator of wakefulness in the mammalian sleep-wake cycle. Dual orexin receptor antagonists (DORAs) such as daridorexant reduce wakefulness in rats, dogs, and humans. DORAs constitute a new class of insomnia medications that start to compete with the traditionally prescribed positive GABA-A receptor modulators. Patients suffering from anxiety disorders often have difficulties in getting enough and restful sleep. They may experience delayed sleep onset and frequent awakenings during the night due to sudden surges of anxiety and nightmares. A sleep medication, which also reduces feelings of fear and anxiety, could provide additional therapeutic benefits to many insomnia patients. The current study explored the potential anxiolytic-like effects of daridorexant in experimental simulations of fear and anxiety states in the rat.

Methods: Male rats were treated with single oral doses (10, 30, 100 mg/kg vs vehicle control) of daridorexant (n=10-17/treatment group). One hour later their stress-reactivity was tested in experimental models of anxiety states including fear-potentiated startle, social stress-induced hyperthermia and tachycardia, ultrasound-induced defensive behavior, and schedule-induced polydipsia.

Results: Daridorexant dose-dependently reduced fear-potentiated startle (ANOVA; $p<0.001$), social stress-induced-hyperthermia, and tachycardia (ANOVA; $p<0.001$), and schedule-induced polydipsia (ANOVA; $p<0.01$) with effective oral doses (post-hoc tests; $p<0.05$) starting at 10 mg/kg. Daridorexant had no effect on ultrasound-induced defensive behavior.

Conclusions: Daridorexant exerted anxiolytic-like effects in three rat paradigms modeling aspects of human anxiety states reminiscent of post-traumatic stress disorder, social anxiety disorder, and obsessive-compulsive disorder. Daridorexant did not reduce panic-like flight reactions induced by ultrasonic danger signals in the rat. The positive impact of decreased wakefulness on attenuating the aversive emotional responses to non-vital environmental stress is discussed.

Keywords: Behavioural Neuroscience, Behavioral Pharmacology, Animal Research

Disclosure: Idorsia Pharmaceuticals Ltd: Employee (Self), Idorsia Pharmaceuticals Ltd: Stock / Equity (Self)

W16. PTSD Affects Fear Extinction Recall Among Trauma-Exposed Individuals

Vincent Rozalski*, Sabra Inslicht

UCSF and San Francisco VA Medical Center, San Francisco, California, United States

Background: Trauma exposure and PTSD alter physiological reactions to threat and potential threats. Fear conditioning is a well-established model that has been used to examine the acquisition and extinction of threat stimuli. However, several variations on the model exist, and we sought to examine differences in physiological reactivity among diagnosis-group using a three-phase model that combines several different methodologies used in the literature.

Methods: Data were drawn from an experimental study of fear conditioning in trauma-exposed adults. Participants were diagnosed as either having PTSD or not. Participants were exposed to a mild shock (CS+ condition) or no shock (CS- condition) following a visual stimulus across several trials in an acquisition task. Two more tasks were administered a day later (extinction) and then a week later (retention) where the same stimuli were shown but no shocks were administered. Skin conductance (SC) was measured as the primary dependent variable to measure sympathetic nervous system reactivity. Mixed level modeling was used to examine rate of change between groups.

Results: Participants (N=131, 56% women) were trauma-exposed civilians (65%) and military Veterans, and 77% met criteria for PTSD. During the acquisition and extinction tasks, participants' SC did not differ significantly in magnitude or rate of change with regards to PTSD diagnosis. During the retention task, individuals with a PTSD diagnosis had a non-significant reduction in SC during the first two trials of the CS+ presentation, while those without PTSD showed a significant reduction from the first to second trial of the CS+ presentation.

Conclusions: Among trauma-exposed individuals, those with PTSD show deficits in fear extinction recall to previously threatening stimuli a week after extinction learning. Diagnosis may be a key factor in discerning learning deficits to future stimuli. Additionally, a three-task paradigm is appropriate to detect physiological differences among trauma-exposed individuals with and without PTSD.

Keywords: PTSD, Fear Conditioning and Extinction, Skin Conductance Responses

Disclosure: Nothing to disclose.

W17. Psychophysiology-Based Clusters of Trauma-Exposed Individuals Predict Trauma-Related Psychopathology

Lana Grasser*, Cassandra Wanna, Charis Wiltshire, Mariam Reda, William Davie, Antonia Seligowski, Karlye Phillips, Jennifer Stevens, Vishnu Murty, Steven Bruce, Kerry Ressler, Samuel McLean, Tanja Jovanovic

Wayne State University, Detroit, Michigan, United States

Background: Trauma exposure occurs in approximately 70-90% of individuals over the life course, yet only approximately 7-10% develop subsequent posttraumatic stress disorder (PTSD). Biomarkers research has defined objective variables which may indicate individuals who are more likely to experience PTSD, or other trauma-related psychopathology (including anxiety and depression, which are often comorbid with PTSD), in order to direct focused early intervention. Fear conditioning and extinction paradigms quantify responses to threat cues, how well individuals differentiate between threat and non-threat cues, and how well individuals can learn new, competing safety information to inhibit previously learned fear. During the acquisition phase, a neutral cue (conditioned stimulus, CS+) is repeatedly paired with an aversive stimulus (unconditioned stimulus, US) resulting in a conditioned fear response. Another neutral cue that is never paired with the US is also presented (CS-). During the extinction phase, the CS+ is repeatedly presented without simultaneous presentation of the US, so that a new safety memory is formed that inhibits the initial fear response. Throughout the task, psychophysiological data are recorded, including the startle response (eye blink, measured via electromyogram, or EMG), heart rate (HR), and electrodermal activity (EDA). Previous studies have typically looked at EMG, HR, and EDA in isolation to predict

current or future trauma-related psychopathology. Identifying how these variables may be used in combination to provide more representative and descriptive indications that capture individual variability may enhance predictive accuracy and therefore clinical utility. In the present study, we conducted an exploratory cluster analysis to identify clusters based on EMG, HR, and EDA data collected during the acquisition and extinction phases of FPS in a sample of adults exposed to a variety of traumatic events. Post-hoc analyses then investigated psychological symptoms of the individuals in each cluster.

Methods: Data were obtained from the AURORA study, a large cohort study investigating biomarkers and pathogenesis of adverse posttraumatic neuropsychiatric sequelae. Individuals exposed to a traumatic event and presenting in emergency departments within 72 hours of exposure were enrolled in this longitudinal study. Data were collected within the ED and up to 6 months following trauma exposure through both in-person lab sessions and via mobile data collection through smartphone apps and wearable devices. EMG, HR, and EDA data from a fear conditioning and extinction test at two weeks following trauma exposure was available for n=117(78%F, mean age 35). Data were scaled to a normal distribution and outliers more than 3 standard deviations above or below the mean were replaced by the next-highest value. Hierarchical agglomerative clustering (performed in R) was applied following Ward's criterion (agnes). Nonparametric bootstrapping was applied to the cluster solutions, with 500 iterations. Clusters were then plotted across each physiological measure, as well as across self-report measures of posttraumatic stress, anxiety, and depressive symptoms obtained at 2-week, 8-week, and 3-months post-trauma timepoints.

Results: Based on silhouette and distance metrics, a 4-cluster solution was identified. The agglomerative coefficient, which is a measure of the strength of the clustering solution where values closer to one indicate stronger clustering solutions, was equal to .84. Bootstrapping indicated that cluster 1 reliably replicated 77% of iterations, cluster 2 57% of iterations, cluster 3 65%, and cluster 4 44%. Examination of the patterns of physiological reactivity on EMG, HRV, and EDA across acquisition and extinction indicated that individuals in cluster 1 showed blunted physiological responses, individuals in cluster 2 showed high fear load, individuals in cluster 3 showed higher vagal tone, and individuals in cluster 4 showed normative physiology. When mapping the four clusters onto longitudinal symptoms data, cluster 1 showed a late/delayed emergence of more severe psychopathology worsening over time, cluster 2 showed a highly symptomatic non-recovering clinical presentation, cluster 3 showed a recovering phenotype, and cluster 4 showed a resilient phenotype.

Conclusions: These preliminary findings provide an indication of how physiological variables may be used in combination to provide more representative and descriptive indications regarding individual responses to threat and safety cues during both fear learning and fear extinction to identify longitudinal trajectories of trauma-related psychopathology. While at the time of abstract submission the sample is limited to n=117, ongoing data collection for the AURORA study will yield a larger sample size. This initial analysis will be replicated and expanded in the larger cohort, and with the larger sample size will allow for statistical testing of differences in symptoms severities, diagnostic frequencies, and demographic traits between clusters.

Keywords: Anxiety & PTSD, Biomarkers, Fear-Potentiated Startle, Psychophysiology, Trauma

Disclosure: Nothing to disclose.

W18. Prefrontal Somatostatin Interneurons Encode Fear Memory

Kirstie Cummings*, Sabina Bayshtok, Roger Clem

Icahn School of Medicine At Mount Sinai, New York, New York, United States

Background: While the prelimbic subdivision of the rodent medial prefrontal cortex is required for fear expression, it remains unclear whether plasticity in this area contributes to memory encoding and if so, which specific circuits and cell types are involved. In addition to excitatory projection neurons (PNs), which have been considered to be the substrate of memory, prefrontal cortex harbors a network of local inhibitory interneurons, comprised primarily of parvalbumin- (PV-INs) and somatostatin-(SST-INs) expressing neurons. Since interneurons signal primarily through GABAergic synaptic connections, they have generally been considered to function in an inhibitory capacity to constrain memory. In contrast to their hypothesized role, we found that prelimbic somatostatin-expressing interneurons (SST-INs) display cue-related activity and plasticity following fear conditioning and mediate circuit disinhibition to recruit a distributed fear-related brain network. These results suggest that rather constraining memory, SST-IN activation is central to fear memory acquisition/expression. Since we observed plasticity only in certain SST-INs, it is likely that fear memory encoding is directed by a specialized subset of SST-INs, rather than the entire prelimbic population. Using intersectional activity-dependent neural tagging, immunohistochemistry, and in vivo optogenetics, we are investigating which SST-INs are recruited during fear memory encoding and the mechanisms supporting their selective recruitment.

Methods: Male and female mice (C57Bl/6J and SST-FlpO) were used throughout all experiments. Sample sizes range from 4-15 mice per group in each experiment. Mice were randomly assigned to behavior groups and all experiments and analyses were done blind to experimental group. Parametric tests (paired and unpaired two-tailed t-test, repeated measures ANOVA, one-way ANOVA) were used except in cases where data exhibited unequal variance or were not normally distributed, in which case non-parametric tests were used (Mann-Whitney U test, Kruskal-Wallis ANOVA).

Results: We identified a prelimbic neural ensemble recruited following cued fear learning using an activity-dependent neural tagging approach. In addition to principal neurons, we captured a sparse population of fear-activated SST-INs, but not other GABAergic neuron types. To specifically capture this SST-IN ensemble and examine its behavioral contributions, we developed a novel approach that combines activity-dependent tagging and intersectional genetics. Using this approach, we found that fear-tagged SST-INs are reactivated during memory expression and their optogenetic activation was sufficient to drive fear expression.

Conclusions: Our data suggest that a potentially specialized ensemble of SST-INs may orchestrate fear memory encoding in prefrontal cortex of mice.

Keywords: Auditory Fear Conditioning, GABAergic Interneurons, Memory Encoding and Retrieval, Circuit Optogenetics, Electrophysiology

Disclosure: Nothing to disclose.

W19. Ventral Hippocampal Neural Ensemble Activity Modulates (R,S)-Ketamine's Prophylactic Fear Buffering Effects

Josephine McGowan*, Christine Ann Denny

Columbia University/New York State Psychiatric Institute, New York, New York, United States

Background: Stress can lead to a wide variety of psychiatric illnesses such as major depressive disorder (MDD) and post-traumatic stress disorder (PTSD). However, some individuals are more susceptible to developing psychopathology in response to

stress, while others exhibit stress resilience, but the research into the underlying neurobiology of this phenomenon is still in its infancy. We have previously shown that (R,S)-ketamine can act as a prophylactic against stress-induced behavioral despair when administered 1 week prior to various stress models and can buffer fear expression. We have also demonstrated that this prophylaxis is partially mediated by activity in ventral CA3 (vCA3) region of the hippocampus, which has been previously implicated in mood and anxiety disorders. Furthermore, dysregulated neural activity, specifically in regions such as the hippocampus, are hallmarks of major MDD and PTSD pathology. Yet, how activity is changed, particularly in vCA3, during prophylactic ketamine treatment, stress, or expression of behavioral despair or fear is unknown.

Methods: Here, we used Inscopix nVoke's in vivo calcium imaging system in freely moving mice to determine the neural dynamics underlying prophylactic (R,S)-ketamine efficacy in vCA3. A GCaMP6f virus was injected into vCA3 of 129S6/SvEv mice at 7 weeks of age, and subsequently a GRIN lens was implanted over the injection site. Four to 6 weeks later, a baseplate was installed to visualize calcium transients, and 1 week later, calcium activity was recorded during and immediately following saline or (R,S)-ketamine (30 mg/kg) administration (n = 4-6 mice per group). One week following drug administration, mice underwent contextual fear conditioning (CFC) and were tested in assays to measure avoidance behavior and behavioral despair. Calcium activity was recorded during and after drug injection, and during each of the behavior sessions.

Results: As we have shown previously, (R,S)-ketamine buffered fear expression in CFC following this prophylactic timeline in the 129S6/SvEv mice. Calcium transient rates during (R,S)-ketamine administration and during freezing in CFC was decreased in vCA3 as compared to saline-treated mice. (R,S)-ketamine-treated mice also demonstrated lower calcium activity in CFC, even though there was a higher percentage of shock-selective cells in these mice. Graph theory network analysis revealed that ventral hippocampal correlated activity was decreased in (R,S)-ketamine-treated mice during fear expression. These effects were most pronounced in CFC, but persisted during assays that assessed avoidance behaviors and behavioral despair.

Conclusions: These data suggest that ketamine's resilience-enhancing fear buffering effects may depend on differential activity changes in vCA3 throughout treatment and behavioral expression. In particular, prophylactic (R,S)-ketamine may be reversing deleterious hyperactivity and hyper-connectivity in the ventral hippocampus, specifically vCA3, induced by stress. These results also propose a potential node in the brain to target in order to increase stress resilience in susceptible populations.

Keywords: Ketamine, Stress, Hippocampus, Calcium Imaging, Fear

Disclosure: Nothing to disclose.

W20. Neural Correlates of Approach-Avoidance Conflict in the Prelimbic Prefrontal Cortex

Jose Fernandez-Leon, Douglas Engelke, Guillermo Aquino-Miranda, Fabricio Do Monte*

The University of Texas Health Science Center at Houston, Houston, Texas, United States

Background: The ability to identify and discriminate cues associated with reward and aversive stimuli allows an organism to select the most appropriate behavioral response. Neurons in the prelimbic subregion of the prefrontal cortex (PL) respond to both reward- and threat-associated cues. However, whether PL activity regulates the animals' decision to approach or avoid such cues remains unknown.

Methods: Male Long-Evans rats with single-unit recording electrodes in PL were initially trained to press a bar for sucrose during the presentation of audiovisual cues. Next, rats were fear conditioned by pairing a neutral odor with electrical foot shocks. During the test session, rats were placed in a rectangular arena (60cm x 26cm x 40cm) comprising two different zones: a food zone where the bar, the sucrose dish, and the odor port were located and an adjacent hidden zone. Rats were exposed to three phases: only audiovisual cues (reward), only odor cues (fear), or both simultaneously (conflict). To search for food during the conflict phase, animals had to leave the hidden zone and confront the conditioned odor presented in the food zone.

Results: During the reward phase, animals approached the food zone and pressed the bar immediately after the onset of the food cues. During the fear phase, animals showed stronger defensive behaviors characterized by a reduction in time exploring the food zone and an increase in time spent in the hidden zone. Interestingly, two subpopulations of rats emerged during the conflict phase: rats that continued searching for food despite an increase in the latency to press the bar (pressers) vs. rats that remained in the hidden area and showed complete suppression of food-seeking responses (non-pressers). PL recordings (n= 367 neurons, 32 rats) revealed that the magnitude of reward-cue responses observed during the conflict phase was similar to the reward phase for pressers, but was drastically reduced for non-pressers (Z-score >2.58 for excitatory responses and <-1.96 for inhibitory responses). Notably, pressers discriminated the reward cues earlier during the training session, and showed ~50% more reward-cue responses in PL during the reward phase, when compared to non-pressers. To better explore the neural correlates of risky sucrose-seeking behavior in pressers, we used a combination of recordings and optogenetics for photoidentification of distinct subtypes of PL neurons. We found that ~30% of PL glutamatergic (PL-GLUT) neurons reduced their spontaneous firing rate during the reward phase, when compared to baseline. Presentation of the conditioned odor during the fear phase disinhibited ~60% of the PL-GLUT neurons, whereas the introduction of reward cues during the conflict phase restored the inhibition of PL-GLUT neurons to the same levels as the reward phase. In contrast, changes in spontaneous firing rate of PL GABAergic (PL-GABA) neurons were similar across the different phases, most likely reflecting the distinct subtypes of interneurons described in this region. Consistently, photoactivation of PL-GLUT neurons during the onset of the reward-cues reduced sucrose-seeking responses in pressers, whereas photoactivation of PL-GABA neurons had not effect.

Conclusions: Our results establish a role for PL in the regulation of approach-avoidance conflict by demonstrating that reduced activity in PL-GLUT neurons correlates with increased risky sucrose-seeking behavior, and activating PL-GLUT neurons is sufficient to attenuate the animal's drive to search reward.

Keywords: Fear Conditioning, Reward, Recordings, Optogenetics, Rats

Disclosure: Nothing to disclose.

W21. Prefrontal Cortex Projections to the Central Amygdala Regulate Defensive Behavior

Chandrashekhar Borkar, Quan-son Le, Jonathan Fadok*

Tulane University, New Orleans, Louisiana, United States

Background: Exaggerated responses to threat, inappropriate defensive responses to non-threatening stimuli, and disruptions in defensive state transitions are characteristic of several trauma- and stressor-related disorders. The neuronal mechanisms that govern appropriate behavioral state transitions in response to escalating, or de-escalating threats are poorly understood.

Central to our current understanding of behavioral scalability is a prominent psychological theory known as the predatory imminence continuum. Within this framework, complex behaviors such defensive responses are scaled in a hierarchical manner in which defensive action selection is contingent upon the perceived proximity and intensity of threat. Based on this theory we developed a novel behavioral paradigm in which mice rapidly transition between freezing and flight in response to conditioned stimuli (Fadok et al., 2017). We used this behavioral conditioning paradigm to reveal a mutually inhibitory neuronal motif in the central amygdala involved in defensive action selection. However, the upstream cortical brain network providing potential top-down regulation of action selection processes in the central amygdala is not yet understood. Here, we describe a glutamatergic projection from the prefrontal cortex to the central amygdala that provides important top-down control of amygdala function and behavioral output.

Methods: All experiments used similar numbers of male and female mice. To determine which areas of prefrontal cortex project to the central amygdala, we stereotaxically injected retrograde tracers (N = 2-4 mice/group). Neuronal activation of this pathway during our novel pavlovian fear conditioning paradigm was confirmed using a neuroanatomical tracing approach combined with analysis of immediate early gene activation (N = 2-4/group). Optogenetic and chemogenetic excitation and inhibition was used to manipulate the function of the pathway at various timepoints (N = 8-10/group). Following the conclusion of each experiment, inclusion of data into analyses was determined by post-hoc histological confirmation of vector and implant targeting. Data were tested using the Shapiro-Wilk normality test ($\alpha = 0.05$) and the appropriate parametric or non-parametric tests were used to determine statistical significance.

Results: injection of fluorescent retrobeads into the CEA of mice retrogradely labeled a large number of cells in the most ventral aspects of the prefrontal cortex, including the dorsal peduncular nucleus (DP), along with the infralimbic region. Double neuronal tracing experiments showed that these projections were distinct from hypothalamus-projecting neurons known to elicit sympathetic responses. Conditioning in the modified fear conditioning paradigm induced abundant Fos expression in CEA-projecting DP cells. Viral labeling revealed that DP efferents to the CEA are predominantly positive for VGLUT1. Furthermore, these neurons strongly innervate the anterior part of the CEA, which contains a large population of CRH neurons known to regulate flight responses. Using in vivo optogenetics and DREADD techniques, inhibition of DP-CEA projections during conditioned stimuli leads to higher freezing and reduced flight, suggesting an important role in regulating defensive behavior. Similarly, inhibition of this pathway during extinction training significantly increased freezing behavior and decreased flight.

Conclusions: Classical neuroanatomical studies indicate that the prefrontal cortex sends a projection to the central amygdala. Here, we demonstrate that the vast majority of these inputs emanate from the most ventral aspects of the medial prefrontal cortex. Our Fos results suggest that this pathway is activated by fear conditioning and by manipulating the function of this pathway we demonstrate its role in regulating defensive behavior. Collectively, our results suggest that rapid and flexible action selection in the CEA is regulated by top-down cortical afferents.

Keywords: Amygdala, Fear Conditioning, Defensive and Motivated Behaviors, Prefrontal Cortex, Stress and Trauma

Disclosure: Nothing to disclose.

W22. Investigating the Role of the Locus Coeruleus Norepinephrine System in Novelty-Induced Anxiety

Abstract not included.

W23. Double-Blind Placebo-Controlled Pilot Study of Acamprosate in Fragile X Syndrome

Craig Erickson*, Ernest Pedapati, Rebecca Shaffer, Kelli Dominick, Lauren Schmitt, Logan Wink, John Sweeney, Elizabeth Berry-Kravis

Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States

Background: Fragile X syndrome (FXS) is the most common inherited form of developmental disability and most common single gene cause of autism spectrum disorder. As an X linked disorder FXS impacts 1 in 4,000 males and 1 in 6-8,000 females with a more severe phenotype noted in males. FXS results from a CGG triple repeat expansion in the promoter region of the fragile X mental retardation (FMR1) gene on the long arm of the X chromosome. FMR1 codes for fragile X mental retardation protein (FMRP), a key modulator of RNA translation impacting the expressing of hundreds of genes involved in brain activity including many genes implicated in the pathophysiology of autism. Deficient FMRP results in likely functional imbalance of GABAergic and glutamatergic neurotransmission. Acamprosate is FDA approved for the treatment of alcoholism. The drug has pleiotropic effects including putative potentiation of GABA(A) activity and inhibition of NMDA and metabotropic type 5 glutamate receptors. Following multiple small open-label pilot reports supporting potential use of acamprosate in FXS targeting interfering behavior and social and communication deficits, we embarked on the first placebo-controlled trial of acamprosate in FXS.

Methods: We conducted a double-blind, placebo-controlled, parallel groups two site (Cincinnati and Rush University) 10-week study of acamprosate in forty-six 5-22 year olds with full mutation FXS. Acamprosate or placebo was flexibly dosed during the first six weeks of study followed by a minimum of four weeks of steady dosing. Maximum dosing was 1,998 mg divided TID for those with weight greater than 50kg and 1,332 maximum dosing for those with weight under 50 kg. Clinician and parent reported outcome measures included the Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), Vineland Adaptive Behavior Scale 2nd Edition (VABS), Anxiety Depression and Mood Scale (ADAMS), and the Clinical Global Impressions Improvement (CGI-I) subscale. Objective measures included expressive language sampling, eye tracking, and molecular blood assays including evaluation of lymphocytic extracellular signal related kinase (ERK) activation and plasma amyloid precursor protein levels pre- and post-treatment.

Results: 46 persons enrolled in this study. Mean final acamprosate dosing at Week 10 was 1612 mg/day with a range of 333-1,998 mg per day. Mean age of those receiving acamprosate was 15.37 years and 15.03 years for those receiving placebo. Eighteen males and five females received acamprosate and 19 males and four females received placebo. The mean IQ of those receiving acamprosate was 47.8 (range 36-80) and 45.7 for those receiving placebo (range 40-75). Regarding tolerability, overall acamprosate was well tolerated with no serious adverse events reported. Gastrointestinal adverse effects were more common in those receiving acamprosate with 17 cases of diarrhea or abdominal discomfort noted in those receiving acamprosate compared to seven such reports in the placebo group. Risk for dose dependent irritability noted in earlier open-label pilot studies in FXS was not noted in this pilot placebo-controlled study. While within the acamprosate treatment group, significant improvement was noted in many outcome measures including the ADAMS, ABC, SRS, VABS, and ADHD-RS, no differences in improvement were noted between acamprosate and placebo use across all parent-reported outcome measures.

Conclusions: In this report, acamprosate use was generally well tolerated in a broad sample of 5 to 22-year-olds with FXS. Significant placebo response was noted in this small double-blind study. While within the acamprosate treatment group, improvements were noted during treatment, these improvements were not significantly greater than improvements noted with placebo treatment. Future directions include analysis of the quantitative outcomes included in the project. Additional future work may require larger sample sizes to more adequately power an analysis of acamprosate in FXS to potentially differentiate from placebo given the known placebo effects inherent in clinician and parent reported outcomes in this field.

Keywords: Fragile X Syndrome, Acamprosate, Clinical Trial

Disclosure: Confluence Pharmaceuticals: Patent (Self)

W24. Intranasal Oxytocin Eliminates the Placebo Response for Hyperphagia and Repetitive Behaviors in Children With Prader-Willi Syndrome: A Randomized Controlled Trial

Eric Hollander*, Kayla Levine, Casara Ferretti, Katherine Freeman, Ellen Doernberg, Bonnie Taylor

Albert Einstein College of Medicine, Bronx, New York, United States

Background: The effects of intranasal oxytocin and placebo on hyperphagia and repetitive behaviors were compared in children and adolescents with Prader Willi Syndrome (PWS). To date, no effective treatments for managing hyperphagia have been found. One possibility that may be promising is the use of intranasal oxytocin (IN-OXT), a neurohormone that has been proposed to play a role in social recognition, pair bonding, anxiety, and maternal behaviors. OXT receptors are expressed in various parts of the brain including the amygdala, ventromedial hypothalamus, and the brainstem. Recent studies suggest that the clinical phenotype observed in PWS may be linked to improper processing of hormones like OXT [Burnett LC, et al. 2017]. This connection is further established based on postmortem studies in individuals with PWS that demonstrate a lower number of OXT-secreting neurons in the paraventricular nucleus of the hypothalamus compared to normal [Swaab DF, et al. 1995].

Methods: Children and adolescents with PWS were enrolled in an 8-week double-blind placebo-controlled intranasal oxytocin randomized trial. Twenty-three (23) subjects were assigned to oxytocin (N=11) or placebo (N=12). Hyperphagia was measured with the Hyperphagia Questionnaire (HQ), and repetitive behavior was measured with Repetitive Behavior Scale- Revised (RBS-R).

Results: There were modest significant treatment by-time interactions indicating reduction in hyperphagia and repetitive behaviors across time for placebo but no reduction for oxytocin. Total HQ score showed a greater average reduction of 1.81 points/week for the placebo group vs. oxytocin, with maximum reduction at week 4. There were also greater reductions on HQ-Drive and HQ-Behavior subscales on placebo vs. oxytocin. RBS-R subscales followed similar patterns to the HQ, with a significantly greater reduction in sameness subscale behaviors (average 0.825 points/week) in the placebo group compared to the oxytocin group. Oxytocin was well tolerated, and the only adverse event that was both more common and possibly related to oxytocin vs. placebo was nocturia (n= 1 vs 0).

Conclusions: Placebo was associated with modest improvement in hyperphagia and repetitive behaviors in childhood PWS whereas intranasal oxytocin was not associated with improvement in these domains.

While some recent studies have demonstrated greater improvement in hyperphagia behaviors in response to intranasal OXT vs. placebo in the treatment of PWS, other studies found little to no differences between treatments. Our data demonstrate modest significant treatment x time interactions for IN-OXT vs placebo in ratings of hyperphagia and repetitive behaviors, although in the opposite direction than initially expected. Notable findings include a decrease in hyperphagia behaviors on total, drive, and behavior subscales of the HQ in the placebo group, whereas the IN-OXT group remained relatively stable. Similar findings were also apparent in the RBS-R scales with significant findings favoring placebo on the sameness subscale vs IN-OXT. Of interest, the two outcome measures followed different patterns of response; the hyperphagia scales depicted a peak drop on placebo at week 6 with subsequent rise, whereas the repetitive behavior scale findings depicted a continual drop on placebo towards endpoint. Another finding of interest was noted in the salivary sample collection. Although the sample size was small (N=7 subjects), an increased salivary OXT at week 4 was positively correlated with an increase in hyperphagia drive but negatively correlated with RBS-R ritualistic behaviors, RBS-R sameness behaviors, and WHO-QOL Physical Health. Thus, higher overall salivary OXT level was associated with increased hyperphagia and diminished quality of life, but with improved levels of rigidity and rituals.

More work is needed to understand the mechanism of how intranasal oxytocin eliminates the placebo response for hyperphagia and repetitive behaviors in PWS.

Keywords: Prader Willi Syndrome, Oxytocin, Repetitive Behavior, Compulsive Eating

Disclosure: Nothing to disclose.

W25. A Longitudinal Study of Resting-State Connectivity and Response to Psychostimulant Treatment in Attention/Deficit Hyperactivity Disorder

Luke Norman*, Gustavo Sudre, Marine Bouyssi-Kobar, Wendy Sharp, Philip Shaw

National Human Genome Research Institute, Bethesda, Maryland, United States

Background: Psychostimulants, including methylphenidate-based and amphetamine-based agents, are the first line pharmacological treatments for Attention Deficit/Hyperactivity Disorder (ADHD). Meta-analytic evidence suggests that these medications are efficacious relative to placebo in children, adolescents and adults with the disorder. However, 30-40% of patients do not respond well to treatment and the mechanisms underlying these differences in treatment response are still poorly understood. In this work, we aimed to examine whether treatment response to psychostimulant medication was associated with resting-state connectivity within a set of cingulo-opercular, striato-thalamic and default-mode brain networks that have been implicated strongly in pathophysiological models of ADHD and in the therapeutic actions of psychostimulants, as well as whether the nature of potential associations changed with age.

Methods: Patients (N=110, 26% female) and typically developing controls (N=154, 43% female) underwent symptom assessments on up to five occasions during childhood and adolescence (age range 5 years to 18 years old). For patients, symptoms were rated on and off medication. Structural and resting-state functional neuroimaging data were also collected at each visit. Patients were scanned while off medication. Resting-state connectivity within and between cingulo-opercular, striato-thalamic and default-mode networks was quantified at each available time-point for each subject using seed-to-seed methods. Linear mixed-effects models examined whether treatment response was associated with resting-state connectivity as well as whether this association changed with age after controlling for gender, off medication symptoms, dosage and head motion during the functional scan. In order to aid the interpretation of any delineated relationships between treatment response and resting-

state connectivity, comparisons with typically developing controls were also performed.

Results: Resting-state connectivity within the cingulo-opercular network was explained by a significant interaction between age and treatment response (B=-0.05, SE=0.01, t=-3.46, p<0.001). Good treatment responders showed high connectivity within this network during childhood which decreased with increasing age, whereas poor responders showed low connectivity during childhood which increased with increasing age. Follow-up comparisons indicated that typically developing controls showed highly similar age-related changes in cingulo-opercular connectivity to the good responders (p>0.4), while both good responders and typically developing controls showed significant differences in age related connectivity changes compared with poor psychostimulant responders (p<0.001).

Conclusions: This work provides the first examination of how the development of resting-state connectivity within a key network implicated in the pathophysiology of the disorder may be associated with treatment response to psychostimulants in patients with ADHD. Specifically, we report that patients with patterns of resting-state connectivity within the cingulo-opercular network that were more in line with age-expected normative development had better responses to psychostimulant treatment. We therefore suggest that functioning and development within this brain network could be an important factor that plays a role in reported individual differences in treatment response. Future clinical trials, using larger samples and machine learning methods, could examine whether baseline connectivity within this network can differentiate good and poor responders to psychostimulants. The present findings also suggest that treatment response to psychostimulants could be improved in poor responders using cognitive training, neurofeedback training and brain stimulation methods shown in previous work to modulate cingulo-opercular functioning in the disorder.

Keywords: ADHD, Psychostimulants, Functional MRI (fMRI), Resting State Functional Connectivity

Disclosure: Nothing to disclose.

W26. Oxytocin Dose-Dependent Effects on ACC and Amygdala Activity During a Socially Dynamic Game in Autism Spectrum Disorders

Elissar Andari*, Andrew Cotton, Joseph Cubells, James Rilling, Larry Young

University of Toledo, Toledo, Ohio, United States

Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is characterized by deficits in social interaction and communication. Oxytocin is a key modulator of social behavior. Acute administration of intranasal oxytocin (IN-OT) enhances social functioning in autism. However, long-term trials with IN-OT have yielded some inconsistent findings. Here, we conducted a dose-dependent study with IN-OT to examine target engagement for this treatment on the brain activity of adults with ASD.

Methods: Subjects and Scanning: A total of 31 ASD subjects were recruited from the Emory Autism Center in Atlanta. ASD subjects underwent four fMRI scanning sessions in which they completed an interactive social ball game. The social ball game consisted of playing with groups of individuals who gave either positive or negative feedback during the game. Participant also played the same game with computers. All patients received 4 doses of IN-OT (8IU, 24IU and 48IU) and IN-placebo in a randomized, double-blind, within-subject, placebo-controlled design. Visits were separated by a one-week interval. Participants played the ball game inside the MRI scanner (3T, Siemens) 60 minutes after receiving IN spray.

Whole Brain Analysis: General linear models were designed in FSL 6.0.3 for two human feedback runs and two computer feedback runs. Intermediate models for each subject session were constructed for human, computer, and human vs. computer runs. 30 subjects passed quality control for excess motion, though some individual runs were also excluded. To analyze the effects of IN-OT dose on brain activation for each of the three intermediate analyses, higher level linear mixed effects models were constructed which modeled IN-OT dose with one explanatory variable and controlled for the subjects' repeated sessions with an additional 30 explanatory variables. All results were reported for $Z > 2.3$ and cluster-$p < 0.05$.

ROI Analyses: A prior regions of interest were selected from the Harvard-Oxford Cortical and Sub-Cortical Atlases as well as the Juelich Atlas for analysis with repeated measures ANOVAs for IN-OT dose. Differences in activation were investigated for the human, computer, and human vs. Computer feedback analyses. For significant ANOVAs, differences in activation for the different doses were assessed using the LSD.

Results: The linear mixed-effects regression showed that there was a significant IN-OT dose-dependent effect on the anterior cingulate when adults with ASD played with other human players who expressed negative feedback compared to when they played with computers (n=30, voxels=448; Z-max= 4.08; MNI peak coor. x=4, y=30, z=10). Also, there was a negative relationship between IN-OT doses and perceptual areas, such as Heschl's Gyrus (voxels=564; Z-max= 3.42; MNI peak coor. x=-46, y=-8, z=-8) and the lateral occipital cortices (voxels=415; Z-max= 3.82; MNI peak coor. x=30, y=-70, z=12), when the subjects played with computers. ROI analysis on the subregions of amygdala, specified with the Juelich atlas, revealed a significant dose dependent effect of IN-OT on the right laterobasal amygdala (LBamy) (ANOVAs repeated measures, n=22, df=2.063, F=3.396) when adults with ASD played with human partners expressing negative feedback. More specifically, small dose of IN-OT (8IU) reduced LBamy compared to placebo and higher doses (p<0.05).

Conclusions: Here, we found that IN-OT enhances, in a dose-dependent fashion, the activity in the ACC, an area involved in emotional empathy, during negative social interactions. We also found that IN-OT reduces the salience to non-social cues (computer games) by reducing the activity of visual and auditory areas in a dose dependent manner. Smaller doses of IN-OT reduce amygdala activity during negative social interactions, and, possibly reducing anxiety and negative emotions associated to these interactions. These findings are promising and inform us about oxytocin mechanisms of action.

Keywords: Oxytocin, Autism Spectrum Disorders, Target Engagement, Amygdala, Anterior Cingulate Cortex (ACC)

Disclosure: Nothing to disclose.

W27. Effects of Maternal Experience of Discrimination During Pregnancy on Neonatal Brain Connectivity

Arline Pierre Louis, Kristiana Barbato, Kiarra Alleyne, Antonette Davids, Bradley Peterson, Catherine Monk, Dustin Scheinost, Marisa Spann*

Columbia University Irving Medical Center, New Haven, Connecticut, United States

Background: There is emerging evidence that chronic exposure to racial discrimination during pregnancy is associated with various negative psychological and physical maternal outcomes including increased risk of depression, anxiety, and systemic inflammation (Chaney et al, 2019; Giurgescu et al, 2016). However, there is a lack of research examining the impact of maternal racial discrimination on offspring brain development.

Methods: The current study aims to determine whether maternal exposure to racial discrimination affects neonatal functional connectivity of the hippocampus and amygdala, areas that have been linked to stress and depression. Forty-five pregnant women, aged 14-19, were recruited from Columbia University Irving Medical Center. They received routine prenatal care and had no major health problems. A majority of the women were Hispanic (88%). At 34-37 weeks gestation, the women completed self-report psychological assessments including the Experiences of Discrimination Scale. Resting-state functional MRI data was acquired in the neonatal period. Standard seed connectivity from the right and left hippocampi and amygdalae were performed.

Results: Women reporting discrimination during pregnancy compared to women who did not had neonates with weaker connectivity between the right hippocampus and dorsolateral prefrontal cortex. In kind, women experiencing discrimination relative to those who did not had neonates with weaker connectivity between the left amygdala and anterior prefrontal cortex. Our findings suggest that maternal exposure to racial discrimination is associated with neonatal connectivity of the hippocampal and amygdala regions to the prefrontal cortex, which are all brain regions implicated in stress and emotion processing. Previous studies with adults have also shown that the prefrontal cortex is involved in racial processing (Katsumi et al, 2018; Knutson et al, 2007), and further, that social evaluative stress is associated with altered prefrontal connectivity with brain regions involved in emotion regulation and attention (Berger et al, 2014). The amygdala has also been implicated in the processing of 'other race' faces in adult minorities (Sankar et al, 2018; Liu et al, 2015).

Conclusions: As our findings with infant brain connectivity are consistent with these brain regions associated with racial processing and social evaluative stress in adults, there could be a intergenerational effect of discrimination that will require further study. Future studies are necessary to relate these connectivity patterns to racial information processing in offspring, and to determine whether racial discrimination during pregnancy is associated with negative psychological and physical outcomes in offspring.

Keywords: Discrimination, Pregnancy, Depression, Stress, Infant

Disclosure: Nothing to disclose.

W28. A Novel CRH-specific Projection from Basolateral Amygdala to Nucleus Accumbens Depresses Reward-Seeking Behaviors

Matthew Birnie*, Annabel K. Short, Christy A. Itoga, Xiangmin Xu, Yuncai Chen, Tallie Z. Baram

University of California-Irvine, Irvine, California, United States

Background: The reward circuit executes the encoding and seeking of rewarding experiences, and disrupted reward circuit function is thought to underlie mental health problems including depression and drug and alcohol use disorders. The nucleus accumbens (NAc) is a major component of the reward circuit and key structure mediating pleasure, motivation, and emotional processes. Multiple inputs converge onto the NAc to modulate reward-seeking behaviors, including the basolateral amygdala (BLA). The BLA mediates associate learning for both aversive and appetitive stimuli, and stimulation of glutamatergic projections from the BLA to NAc selectively promotes appetitive, but not aversive behaviors. We have identified a BLA-NAc projection expressing corticotropin-releasing hormone (CRH). CRH is an evolutionarily conserved stress reactive neuropeptide that plays context dependent roles in the reward circuit. In the NAc, CRH+ axon terminals modulate reward and motivational behaviors, yet their origin is unclear. CRH+ BLA-NAc projections are uniquely poised to influence reward behaviors during current or prior stress, Here, we identify the role of this CRH+ BLA-NAc projection in reward seeking behavior in naïve and stress-experiencing mice.

Methods: To identify CRH+ projections onto the NAc, we utilized viral-genetic approaches to map these pathways using Cre-dependent viruses injected into CRH-IRES-Cre mice. To determine the function of the novel CRH+ BLA-NAc projection we employ chemogenetic and optogenetic strategies in both control and early-life adversity experiencing mice. In CRH-IRES-Cre mice, we injected excitatory or inhibitory Cre-dependent DREADD (hM3Dq and hM4Di) and optogenetic (ChR2 and eNpHR3.0) carrying viruses into BLA, followed by medial NAc shell targeted microinjections of CNO or placement of optic fibers. We tested the function of this pathway using the Sucrose Preference, sex-reward seeking behavior (Scent of a Mouse) and Palatable Food tasks.

Results: Viral genetic tracing identified a novel CRH+ projection from the BLA to the medial NAc shell. Activation of the projection using chemo- and optogenetic excitation reduced preference for sucrose, palatable food, and the scent of a female mouse. Compared with control mice, male mice that experienced ELA had reduced preference for sucrose, palatable food and for the scent of a female mouse. In adult ELA mice, chemo- and optogenetic inhibition of the CRH-specific BLA-NAc projection rescued all three reward-seeking behaviors.

Conclusions: We identify a novel CRH+ BLA-NAc projection and establish its role in mediating the effects of ELA on reward-seeking behaviors. These discoveries provide new, important information about the mechanisms of reward-seeking behaviors and provide potential selective targets for prevention and intervention in the disruption of such behavior that accompanies several psychopathologies.

Funding: This work was supported by National Institutes of Health Grant Nos. MH73136 and MH096889 (to TZB) and the George E. Hewitt Foundation for Medical Research Postdoctoral Fellowship (to MTB).

Keywords: CRH, Early Life Adversity, Reward, Nucleus Accumbens, Basolateral Amygdala

Disclosure: Nothing to disclose.

W29. Early Life Stress Causes Neurobiological and Physiological Impairments That Precede Behavioral Despair in Adulthood

Rushell Dixon*, Serena Wu, Arielle Emile, Christoph Anacker

Columbia University & New York State Psychiatric Institute, New York, New York, United States

Background: Early life stress (ELS) increases vulnerability for neuropsychiatric disorders, such as depression, but little is known about neurodevelopmental abnormalities resulting from ELS. Our lab has previously shown that hyperactivity of the ventral dentate gyrus (vDG) region of the hippocampus increases stress responses and depressive-like behavior. Here, we investigated whether vDG activity may be changed by ELS.

Methods: We used the limited bedding model of ELS to induce maternal stress and fragmented parental care. Offspring were tested as juveniles (PND 35) or as adults (PND 56) in the forced swim test (FST) to study behavioral despair. Following the FST, mice were perfused and brains were stained for the immediate early gene, c-fos, as a proxy marker for neural activity. Blood samples were taken for analysis of corticosterone (CORT) levels.

Results: Juvenile ELS-exposed mice showed comparable levels of mobility in the FST to juvenile controls. Adult ELS-exposed mice had significantly decreased mobility in the FST compared to adult control mice (ELS: 120±13.9 sec ; CTRL: 164±11.8 sec, *p=0.02, n=14-18) indicating increased behavioral despair in ELS-exposed

adults, but not in juveniles. In the vDG of juvenile ELS-exposed mice, the number of c-fos+ cells was significantly increased compared to juvenile controls (*p=0.01; n=5-6 mice), indicating that ELS leads to higher levels of vDG activity that precede behavioral abnormalities in the FST. In addition, CORT levels were increased in ELS-exposed mice compared to controls (**p=0.005; n=8-11 mice).

Conclusions: Our results suggest that ELS induces neurobiological and physiological changes in juvenile mice that precede the development of despair-like behavior.

Keywords: Early Life Stress, Forced Swim Test, Animal Models

Disclosure: Nothing to disclose.

W30. Developmental Emergence of Persistent Memory for Contextual and Auditory Fear in Mice

Maithe Arruda-Carvalho*, Rojina Samifanni, Mudi Zhao, Arely Cruz-Sanchez, Agarsh Satheesh

University of Toronto, Scarborough, Toronto, Canada

Background: The ability to generate memories that persist throughout a lifetime, i.e. memory persistence, emerges in early development across species. Although it has been shown that persistent fear memories emerge between late infancy and adolescence in mice, it is unclear exactly when this transition takes place, and whether two major fear conditioning tasks, contextual and auditory fear, share the same timeline of developmental onset.

Methods: Using behavior and immunohistochemistry, we compared the ontogeny of remote contextual and auditory fear in C57BL/6J mice across early life. Mice at postnatal day (P)15, 21, 25, 28 and 30 underwent either contextual or auditory fear training and were tested for fear retrieval 1 or 30 days later. We also examined the recruitment of prelimbic cortex in 1- and 30-day memory retrieval.

Results: We found that the onset for persistent memory for context- and tone-fear in mice takes place between P21 and P25. Furthermore, 30-day memory retrieval led to an increase in the number of c-Fos positive cells in the prelimbic region of the prefrontal cortex only at an age in which contextual fear memory was successfully retrieved.

Conclusions: These data delineate a precise timeline for the emergence of persistent contextual and auditory fear memories in mice and suggest that the prelimbic cortex is only recruited for remote memory recall upon the onset of memory persistence.

Keywords: Contextual Fear, Auditory Fear Conditioning, Remote Memory, Prefrontal Cortex, Development

Disclosure: Nothing to disclose.

W31. Polygenic Risk for Neurodevelopmental Disorders Predicts Diffuse Measures of Dimensional Psychopathology at Age 9-10

Joshua Roffman*, Dylan Hughes, Casey Hopkinson, Pieter Vuijk, Erin Dunn, Alysa Doyle, Phil Lee

Massachusetts General Hospital, Charlestown, Massachusetts, United States

Background: With few exceptions, genome wide association studies (GWAS) in psychiatry, and related polygenic risk score (PRS) analyses, have focused primarily on disorders that emerge in adulthood and have enrolled adult participants. Effects of polygenic loading for these disorders on psychopathology in children remain largely unexplored. Dimensional measures of psychopathology in children may index risk for emergence of full-blown disorders later in life. As such, an understanding of how genetic loading for these disorders maps onto dimensional symptoms in children may have value for prognosis and early intervention. Leveraging baseline data from the Adolescent Brain Cognitive Development (ABCD) Study, we examined how broad and narrow indices of polygenic risk track with dimensional psychopathology at age 9-10.

Methods: GWAS data from 4,413 non-related ABCD participants of European ancestry across 21 U.S.-based sites were used to calculate PRS using summary statistics from the most recent Psychiatric Genomics Consortium studies of 8 disorders (anorexia nervosa, AN; obsessive compulsive disorder, OCD; Tourette's syndrome, TS; attention deficit hyperactivity disorder, ADHD; autism spectrum disorder, ASD; major depressive disorder, MDD; bipolar disorder, BD; schizophrenia, SCZ). We also computed polygenic loading for cross-disorder (CD) as well as for three factors (compulsive/perfectionistic, mood and psychotic, and neurodevelopmental) that accounted for 51% of the genetic variation across the 8 disorders in the most recent PGC cross-disorder analysis (N=727,126). ABCD participants were characterized for psychopathology across 8 specific dimensions, as well as for broad-band total, externalizing, and internalizing symptoms, through parent-reported ratings via the Child Behavior Checklist (CBCL). Psychosis scores were obtained through the Prodromal Psychosis Scale, again by parent rating. Regressions of PRS scores on phenotypes were conducted in parallel, controlling for age, sex, site, and the top 5 principal components to control for stratification artifact, and p-values were adjusted for 144 comparisons (12 PRS scores x 12 phenotypes) using the False Discovery Rate (q=.05).

Results: Among 8 disorder-specific PRS scores, ADHD and MDD scores both predicted diffuse psychopathology (9 phenotypes each, p's .003 to 2.8E-09), respectively centered on externalizing and internalizing symptoms. Among the three cross-disorder factors, the neurodevelopmental factor predicted the most diffuse categories of psychopathology (11 total phenotypes), also with the strongest effect (p's .004 to 2.6E-09). Neurodevelopmental factor scores outperformed compulsive/perfectionistic and mood and psychotic factor scores in predicting CBCL total (p=5.4E-08), externalizing (p=6.2E-07), and internalizing (p=9.2E-04) symptoms and prodromal psychosis symptoms (p=1.4E-05). Similarly, neurodevelopmental factor scores outperformed CD and ADHD PRS scores in predicting CBCL total (p=.010) and internalizing (p=.010) symptoms and psychosis symptoms (p=.009).

Conclusions: The genetic underpinnings of dimensional psychopathology symptoms at age 9-10 predominantly reflect those of neurodevelopmental disorders. Notably, effects of such genetic loading are clinically diffuse, and include a range of internalizing and externalizing symptoms as well as psychosis scores. As predicted by the NIMH RDoC framework, these data suggest that the alignment between clinical categories of polygenic risk and psychopathology symptoms does not adhere strictly to traditional diagnostic boundaries. Longitudinal data will further inform a revised conceptualization, which may become more parsimonious over the course of adolescent brain development, as behavioral phenotypes become differentiated. In turn, such data may help to refine predictive algorithms that may be used for prognosis and early intervention.

Keywords: ABCD Study, GWAS, Polygenic Risk Scores, Dimensional Child Psychopathology

Disclosure: Nothing to disclose.

W32. Polygenic Risk for Autism Spectrum Disorder and Regional Cerebral Blood Flow

Madeline Hamborg*, Daniel Eisenberg, Michael Gregory, Philip Kohn, Karen Berman

National Institute of Mental Health, Bethesda, Maryland, United States

Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by deficits in language abilities, impaired social functioning, and restricted interests, with strong evidence for genetic etiologies. Prior work has demonstrated that ASD is highly heritable (with estimates up to 80%), though large-scale genome-wide studies have found the genetic risk to be polygenic in nature, with much of the associated genetic variation individually accounting for only a small proportion of the variance. In searching for the neurobiological underpinnings of ASD, previous research has shown a host of abnormalities in MRI- and PET-based resting cerebral blood flow measures in frontal, temporo-parietal, and subcortical regions, although results have been inconsistent. Hypotheses that these abnormalities may be related to underlying etiological mechanisms have been attractive; however, it has been unclear to what degree some of these findings may be affected by epiphenomena and functional sequelae of this illness, which necessarily complicate patient studies. Here, to circumvent these epiphenomenological effects and to attempt to link the genetic and neurobiological correlates of ASD, we used the oxygen-15 water positron emission tomography (PET) method to test whether the polygenic risk for ASD predicts variability in resting regional cerebral blood flow (rCBF) in a large sample of healthy individuals.

Methods: Healthy volunteers (N=152, mean age 34.62 + 10.72, 83 females) provided blood samples for genotyping and were studied with oxygen-15 water PET. Prior to the scan, participants refrained from caffeine or nicotine use for four hours. Scanning was performed on a GE Advance positron emission tomograph. After transmission scanning for attenuation correction, two 12 mCi injections of oxygen-15 water were given six minutes apart to participants in a resting state. Each individual underwent a separate T1 weighted structural MRI scan for co-registration and spatial warping purposes. Attenuation-corrected images were then processed with ANTS and AFNI software, which included spatial warping, normalization, smoothing, and averaging of the two acquisitions for each participant.

Separately, after genotype quality control, phasing, and imputation procedures (using 1000 Genomes Phase 3 as a reference), polygenic risk scores were calculated with Plink software for each healthy volunteer in our sample using the summary statistics from a genome-wide association study of ASD data collected by the iPsych Project (Grove et al. 2019) at fourteen different p-value thresholds. To concentrate the polygenic signal, the fourteen scores were reduced to a single component using the principal component analysis function in SPSS software. With SPM software, these polygenic risk for ASD values were tested for association with voxel-wise cerebral blood flow measures in the PET sample using a general linear model. Covariates of age, sex, and ancestry-related genetic components, and a threshold of $p<0.005$, uncorrected were used.

Results: There was a positive relationship between polygenic risk for ASD and resting cerebral blood flow in bilateral frontal cortical regions, including the anterior cingulate cortex, inferior frontal gyrus, and dorsolateral prefrontal regions, with an inverse relationship identified in the fusiform gyrus.

Conclusions: In this work, we show that even in healthy individuals, the genetic risk for ASD is associated with cortical physiology in brain regions known to be important in the disease. These findings are consistent with prior studies showing resting cerebral blood flow abnormalities in patients with ASD, and similar regions have also been shown to have altered brain structure. Together, the cumulative work suggests that these neural phenotypes are not merely epiphenomenological and may be linked to genetic mechanisms underlying the illness, shedding light on the role genetics play in shaping brain function in ASD.

Keywords: Autism, PET Imaging, Polygenic Risk Score, Neurodevelopmental Disorders, Cerebral Blood Flow

Disclosure: Nothing to disclose.

W33. The Use of a Novel Diffusion Imaging Fiber Cluster Analysis to Assess Frontostriatal Brain Wiring in Male and Female Healthy Subjects

James Levitt*, Fan Zhang, Mark Vangel, Marek Kubicki, Martha Shenton, Lauren O'Donnell

VA Boston Healthcare System, Harvard Medical School, Brockton, Massachusetts, United States

Background: Alterations in brain connectivity may underlie neuropsychiatric conditions such as schizophrenia. We here assess the pattern of structural connectivity between the prefrontal cortex (PFC) and striatum in 100 healthy subjects (HSs) from the Human Connectome Project (HCP); age: 22 to 35; sex: 46 females and 54 males. We propose a novel method, using fiber clustering of whole brain diffusion Magnetic Resonance Imaging (dMRI) tractography, to assess the organization of frontostriatal brain wiring, which allows us to quantify the degree of deviation from a topographic, parallel, arrangement.

Methods: To enable the identification of fiber tract parcels from the prefrontal cortex (C) and the striatum (S), we used a data-driven fiber clustering atlas (Zhang et al, 2018) that allows for a whole brain tractography parcellation into 2000 fiber clusters according to the white matter (WM) anatomy (i.e., fiber geometric trajectory). Then, fiber clusters of interest (i.e., from C to S) from the whole brain WM were identified according to their connected anatomical brain regions. We studied multiple Freesurfer PFC regions including orbital, lateral and medial PFC regions and the striatum. We identified 17 WM fiber clusters that connect C and S in both left and right hemispheres. To quantify the topographical relationship of these fiber clusters, we measured the mean distances between the endpoints of the fiber clusters within the prefrontal cortex (i.e., cortical distance) and the mean distances between the endpoints of the corresponding fiber clusters terminating in the striatum (i.e., striatal distance).

Results: We analyzed the data in several ways. First, in both hemispheres, we generated a plot (not shown) based on the 17 fiber clusters (with 136 pairs of fiber clusters, yielding 136 data points), showing the relationship between the cortical distances and the corresponding striatal distances of the obtained fiber cluster pairs that connect the prefrontal cortex and the striatum. An exponential model was fit to the data points which was superior to a linear model. We showed that the PFC-striatal WM streamline projection pattern was non-linear, which was driven by the results from 10 cluster pairs. Of note, certain clusters, e.g., cluster 6, originating in pars orbitalis PFC, were significantly over-represented in these 10 cluster pairs. We performed 2 additional analyses. First, we generated plots (not shown) for each of the 17 cluster pairs. For each cluster, we fit a least squares line for predicting striatal distance from cortical distance, for the distance from that cluster to each of the other 16. Then we performed two-tailed binomial tests for the smaller of the number of 16 cluster pair distances with striatal < cortical distance and the number with cortical < striatal distance. Adjusting for the 17 comparisons, in both hemispheres we showed clusters 6 and 8 (originating in pars orbitalis PFC), and cluster 10 (originating from both medial and lateral orbitofrontal PFC) significantly deviated from chance with striatal < cortical distance, i.e., in a convergent pattern (adjusted p-value 0.0009). Second, we compared sex differences in male and female subjects across all pairs of clusters (a total of 136 pairs). We did this in the following manner. For each pair of clusters, we measured the ratio of the fiber cluster endpoint distances: (cortical distance - striatal distance) / (cortical distance + striatal distance).

Then, for each subject, we put all ratio numbers (a total of 136values) together and performed a PCA for dimension reduction. For each dimension value (1-46), we computed a p-value using a Hoteling T-Squared test between males and females. When the dimension value was higher or equal to 23, we found $p<0.05$ in the left hemisphere, and when the dimension value was higher or equal to 34, we found $p<0.05$ in the right hemisphere.

Conclusions: Using dMRI fiber cluster topography analysis in HSs, we show that the PFC wiring projection pattern between the PFC and the striatum deviates from a topographic, parallel, organization, due to a pattern of convergence in regionally specific anatomic clusters connecting the PFC and striatum. Second, we find that these clusters originate in a contiguous region covering the medial and lateral orbitofrontal cortex PFC and extending into ventrolateral PFC. Third, we show a bilateral similar organization with the same clusters identified as convergent in both hemispheres. Lastly, we found a sex difference in the ratio of the fiber cluster endpoint distances when the dimension reduction after PCA is higher or equal to 23 and 34 in the left and right hemispheres, respectively, there was a significant group difference. In the future, we plan to test for variation in the pattern of frontostriatal brain wiring in both males and females, separately, in other neuropsychiatric conditions such as schizophrenia.

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

Disclosure: Nothing to disclose.

W34. Neuroimaging Features Predict Subsequent Binge Drinking in Healthy Adolescents

Alejandro Meruelo*, Ty Brumback, Fiona Baker, Sandra Brown, Duncan Clark, Kate Nooner, Kilian Pohl, Michael De Bellis, Susan Tapert

University of California, San Diego, California, United States

Background: Adolescents tend to drink more alcohol in a sitting, yet less frequently than adults. Fourteen percent of 12th graders reported past 2-week binge alcohol use in 2019 (Monitoring the Future), defined as five or more drinks in the same occasion on at least 1 day in the past 30 days in men (four or more drinks in women). Binge drinking has serious consequences including alcohol poisoning, fatal injuries and accidents, and long-term influences on families, other students, and the general community. Neural predictors of initiating binge drinking during adolescence on a large-scale longitudinal sample such as National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA) have not yet been identified.

Methods: Non-binge drinking NCANDA subjects (age 12-21) were assessed with structural neuroimaging at baseline. Participants were categorized based on Year 1 and later (e.g., Year 2, Year 3) follow-up alcohol use data as binge drinkers or non-binge drinkers using Qdec ANCOVA group analysis, controlling for covariates of age, socioeconomic status, and biological sex. FreeSurfer's autosegmentation process was used to estimate vertex-wide cortical thicknesses. FreeSurfer's Qdec was then used to investigate the relationship between development of binge drinking at year 1 and cortical thickness at baseline (Monte Carlo corrected for multiple comparisons, vertex-wise cluster threshold of 1.3, $p < 0.01$).

Results: Results demonstrated thinner cortices for future binge drinkers compared to future non-binge drinkers in the rostral middle frontal, paracentral, superior parietal, and precuneus regions above and beyond effects attributable to age, sex, and socioeconomic status. Sex differences were found in cortical thickness between those who transitioned into binge drinking and those who remained non-binge drinkers specifically in the superior parietal region.

Conclusions: Our results suggest that cortical thinning over regions involved in frontal and parietal regions may predict increased vulnerability towards the future development of adolescent binge drinking. Results may point to neural systems that could be explored as targets of early prevention programs.

Keywords: Binge Drinking, Neuroimaging, Adolescence, Longitudinal, Biomarker

Disclosure: Nothing to disclose.

W35. Toward Development of an Abbreviated PANSS for Pediatric Trials: New Analyses of PANSS TEOSS Data

Joan Busner*, Eric A. Youngstrom, David G. Daniel, Robert L. Findling

Signant Health, Avondale, Pennsylvania, United States

Background: What are the psychometric properties of the PANSS in a pediatric sample, and do they support abbreviation of this instrument for pediatric trials? Challenges in ensuring valid data in pediatric psychopharmacology trials include developmental limitations in symptom description, the need to integrate information from parents and patients, a shortage of child-trained investigators, and the lack of pediatric-validated efficacy measures. Pediatric schizophrenia trials, with few exceptions, have used for primary efficacy assessment the PANSS, a complex and lengthy 30-item (adult) measure that has been extensively studied and shown to pose ratings challenges even when used with the adult patients for whom it was designed. For adult populations there have been a variety of efforts to shorten the PANSS while retaining its clinical and research value. To our knowledge there have been no similar efforts for the pediatric population. The NIMH Treatment of Early Onset Schizophrenia (TEOSS) study affords a unique opportunity to examine the psychometric properties of the PANSS in a pediatric population.

Methods: As part of a NIMH multisite study (completed and previously described), male and female youths with schizophrenia/schizoaffective disorder were administered the PANSS at baseline and weekly throughout an 8-week randomized double-blind study of three antipsychotic agents. In this study we examined the psychometrics of the baseline PANSS to determine if a shortened form could be supported.

Results: N=118 youths (mean age=14.26, SD=2.41 years) had baseline PANSS data. Cronbach's alpha =.85, superficially indicating acceptable internal consistency, but the average inter-item correlation was .15 (where .30 is considered minimal)1, and Guttman's lambda-6 was .92, suggesting considerable "lumpiness" among the items. Similarly, multiple decision rules confirmed a 5-factor structure that was highly consistent with the solutions described in adults, with two notable differences: (a) several items did not load substantively on any factor, (b) "positive symptoms" split across 3 factors (or did not load anywhere). The 5 factors had a median correlation of .13 (range -.13 to +.48), indicating that they measured distinct symptom dimensions. IRT graded response models confirmed that half of the items did not cohere with a global factor. Dropping 10 items that had no loading, or cross-loading or unexpected primary loadings, created a 20 item set keeping 5 factors with total alpha = .79 and r = .968 with the full length score. A 10 item short form, keeping the two strongest loadings from each factor, had r=.89 with the full length, and the 5 2-item subscales each with alpha from .66 to .84. Criterion correlations showed excellent preservation of validity.

Conclusions: Findings confirm a 5-factor structure of symptoms in youths aligning closely with adult models 2,3, but with several items not tapping any factor, and with low factor correlations and item correlations challenging the idea that there is a single

underlying construct. Pruning the psychometric "dead wood," omitting items that do not provide information about any of the 5 symptom dimensions, reduced scale length by 33%, with excellent content coverage. An ultra-brief form, with the 2 best items for the 5 factors, still showed good coverage (r=.89) with a 67% reduction in length. Examining criterion correlations showed minimal loss of precision (or even improvement), reducing interview length and burden on patients and raters. Future directions include comparing the pruned and precision-focused forms to a third version optimized to maximize sensitivity to acute treatment effects, as well as examining psychometrics of the shortened forms in an "extracted" instead of "embedded" administration.

Keywords: Children and Adolescents, Assessment, Child Psychopharmacology, Psychosis, Pediatrics

Disclosure: Signant Health: Employee (Self)

W36. Digital Phenotyping of Pediatric Irritability: Clinical Findings

Amanda Chue*, Reut Naim, Ashley Smith, Hannah Grassie, Julia Brooks, Daniel S. Pine, Melissa Brotman, Ellen Leibenluft, Katharina Kircanski

National Institute of Mental Health, Bethesda, Maryland, United States

Background: The ability to assess psychiatric symptoms remotely in real-time holds promise for advancing clinical understanding and interventions. Irritability symptoms are common and impairing in youth, yet little is known about their naturalistic, real-time expression; clinical reports of youth and parents are typically retrospective. In this study, we used ecological momentary assessment (EMA) to digitally phenotype irritability and related constructs across diagnoses including disruptive mood dysregulation disorder (DMDD) and attention-deficit/hyperactivity disorder (ADHD). Here, we focus on clinical questions concerning core irritability symptoms as reported by youth and parents, as well as functional impairment and co-occurring anxiety and mood symptoms.

Methods: A transdiagnostic sample of 59 youth ages 8-18 (M age=12.1 years, SD=2.8; 71% male) and their parents completed the EMA protocol. To obtain variability in irritability symptoms, participants were recruited on the basis of primary DMDD (n=25), ADHD (n=20), or clinically significant irritability not meeting the threshold for DMDD (n=13). Trait irritability levels were assessed using the self- and parent-report Affective Reactivity Index (ARI; Stringaris et al., 2012; M child-report=3.9, M parent-report=5.8). During the one-week EMA protocol, youth and parents were prompted concurrently on smartphones three times per day to complete items that assessed core irritability symptoms in the child, including temper outbursts and irritable mood, as well as functional impairment and co-occurring anxiety and mood symptoms. Data were analyzed using multilevel modeling to account for the nested structure (prompts within persons).

Results: Compliance rates with the EMA protocol were high: on average, youth completed 83% of prompts, and parents completed 85% of prompts. We found evidence for modest agreement between youth and parents regarding reports of irritability symptoms. With respect to temper outbursts, when the child reported having had an outburst since the previous prompt, the parent agreed 48% of the time. In contrast, when the parent reported that the child had outburst, the child agreed 32% of the time. However, the degree of youth-parent agreement was moderated by ARI score, such that for youth with higher ARI scores there was greater agreement (b=.06, p<.01). In addition, youth and parent reports of irritable mood were significantly correlated (b=.22, p<.001). Again, there was a trend for a stronger youth-parent concordance in those with higher ARI scores (b=.02, p=.05).

Trait levels of irritability as measured using ARI scores also significantly moderated other clinical phenomena. Specifically, youth with higher ARI scores had a stronger positive association between momentary irritability and depressive symptoms (b=.09, p<.001). Moreover, youth with higher ARI scores showed a stronger autocorrelation of irritability symptoms from one prompt to the next (b=.01, p<.05), and a stronger prospective relationship between irritability symptoms at one prompt and anxiety (b=.02, p=.01) and depressive (b=.01, p=.01) symptoms at the next prompt.

Conclusions: This study represents one of the first attempts to digitally phenotype pediatric irritability symptoms in real-time using smartphone technology and EMA. The findings indicate modest agreement between youth and parents in their concurrent reports of temper outbursts and irritable mood. Importantly, informant agreement appears stronger when the child has higher trait levels of irritability. Moreover, trait irritability was associated with a more autocorrelated or persistent daily course of symptoms, and a greater co-occurrence of anxiety and other mood symptoms. Further analyses will examine contextual effects on the naturalistic expression of irritability, including the role of parent responses to the child's irritability symptoms. These findings, which contribute to our clinical understanding of pediatric irritability symptoms and their correlates, may also help facilitate the development of novel interventions that target problematic behaviors or mood patterns related to pediatric irritability in real-time.

Keywords: Pediatric Irritability, Digital Phenotyping, Ecological Momentary Assessment

Disclosure: Nothing to disclose.

W37. Effects of Methylphenidate on Verbal Creativity, Verbal Fluency, and Convergent Problem Solving Performance in Adults With Attention-Deficit Hyperactivity Disorder

David Beversdorf*, Molly McBride, Alyssia Gonzalez, Andrea Schaeffer, Amanda Zand, David Wang, Alinna Sam, Eric Hart, Aneesh Tosh, Bradley Ferguson

University of Missouri, Columbia, Missouri, United States

Background: A common pharmacological treatment for attention-deficit/hyperactivity disorder (ADHD) is methylphenidate (MPH), which is a psychostimulant that acts as a norepinephrine-dopamine reuptake inhibitor. Both the noradrenergic and dopaminergic systems have been shown to have an impact on aspects of creativity. Anecdotal reports from patients with ADHD suggest that their creativity is impaired by MPH. MPH does not generally impair creativity among those without ADHD, but effects in those with ADHD is not known. Therefore, this study examined effects of MPH on convergent and divergent tasks associated with creativity in adults with ADHD.

Methods: Seventeen adults aged 18-40 with a diagnosis of ADHD and prescribed MPH participated in the study. Participants attended two sessions in a counterbalanced order, once after taking the prescribed amount of MPH, and once after withholding their MPH. Participants completed problem-solving tasks (anagrams and compound remote associates) and generative tasks (letter fluency, semantic fluency, and Verbal Torrance Test for Creative Thinking, or VTTCT) in a counterbalanced fashion.

Results: There was a significant increase in the number of words generated on the semantic fluency task, t(16)=3.27, p=0.005 for the MPH session. Furthermore, the solution latency for correct anagrams was significantly greater for the MPH session, t(15)=2.63, p=0.02. In addition, there was a significant increase in the MPH session for the originality scores generated on the VTTCT,

t(8) = 1.867, p=0.049, and a trend toward significance was found for the overall VTTCT battery average score, (p=0.056).

Conclusions: In adults with ADHD, MPH enhanced verbal fluency and divergent thinking abilities, but impaired convergent thinking abilities on problem solving tasks. Furthermore, MPH appeared to enhance the originality aspect of verbal creativity. Therefore, MPH appears to have opposing effects on divergent and convergent task performance in adults with ADHD, possibly due to contrasting effects due to impact on the noradrenergic and dopaminergic systems. Future studies are needed to understand the roles of norepinephrine and dopamine in this effect.

Keywords: Creativity, ADHD, Stimulants

Disclosure: Nothing to disclose.

W38. The Inflammasome in Early Life Stress: How the Progressive Loss of CB2 Receptors Leads to Delayed Depressive Behavior in an Animal Model

Susan Andersen*

McLean Hospital, Belmont, Massachusetts, United States

Background: Clinically, individuals with an abuse history use more 'medicinal' marijuana than non-abused individuals. Cannabidiol (CBD; the agent in medicinal marijuana) principally targets the type 2 CBD receptor (CB2). Activity at the CB2 receptor confers anti-inflammatory effects. Inflammation is elevated in both human abuse survivors and in an animal model of early life stress. We and others show that this early life stress-induced inflammation is further associated with reduced parvalbumin (PV) and depressive-like behavior that emerges in adolescence. Individuals with an abuse history are often resistant to traditional anti-depressants, indicating a need for a new treatment approach. Here, we investigated the inflammasome, a protein complex involved in the innate immune response in an animal model of early life stress. Research in the periphery show that CB2 agonists reduce inflammasome activity, but little is known about the effects on the brain. The current study determined the inter-relationship between CB2 receptors, PV neurons, and found that CB2 agonist treatment altered depressive behavior in animals with a history of early life stress.

Methods: Sprague-Dawley rats were separated from their mother between postnatal day 2-20 for four hours/day. These maternally separated rats (MS) were compared to rats raised in the colony under standard conditions (Con). One cohort of subjects was used for Western immunoblotting at P40 (n=6/6 males) to examine prelimbic prefrontal cortex levels of the nucleotide-binding and oligomerization domain (Nod)-like receptor family pyrin domain-containing 3 (NLRP3) and the E3 ubiquitin enzyme MARCH7, which modulates NLRP3. The second cohort of subjects (n=3/group) was perfused at P25 (juvenile), 40 (adolescence), and 100 (adulthood). The brains were sliced (40μm) and double-labeled with PV (1:10,000; Sigma anti-mouse) and CB2 (1:50; Cayman, anti-rabbit), enhanced with secondary, and visualized with DAB and DAB+Ni, respectively. Cells were counted with StereoInvestigator software (MBF Bioscience) at 40x following our established methods. For qualitative analysis, a separate set of tissue was processed with fluorescence, and images were taken with a Leica confocal microscope at 100x with oil submersion. Behavioral effects of the CB2 agonist, HU-308 (5 mg/kg), on sucrose preferences were ascertained in a two-bottle choice task (water vs.1% sucrose; n=4/group male and female).

Results: MS animals have a 2-fold increase in NLRP3 expression ($p<0.05$) and a 20% reduction in MARCH7 ($F1, 8 = 0.5$, $p<0.05$). A second cohort replicated this finding. Images from confocal microscopy show a CB2-immunoreactive cell wrapped around a PV cell, consistent with the localization of CB2 cells in microglia; microglia co-localization was confirmed by double labeling of CB2 with iba-1 (a microglia marker). Data across age and condition were expressed as a CB2/PV for each subject to account for lower PV neurons in MS male rats. The number of CB2/PV neurons sharply increased at P25 relative to controls and then progressively decreased in MS rats across P25, 40, and 100. CB2/PV increased modestly in Con across age ($F2, 12 = 7.07$, $p=0.009$). To demonstrate an anti-depressive role for CB2 activity, the CB2 agonist, HU-308 (5 mg/kg), increased sucrose consumption in a two-bottle choice task (water vs.1% sucrose).

Conclusions: Our results offer new support for the use of treatment targeting the CB2 receptors in subjects exposed to early life stress. We have established dysregulation of the inflammasome in our model at P40. Our new observations of a progressive decrease in CB2/PV expression across age in MS animals parallels the delayed onset of depressive effects in the animal model at P40 and in humans exposed to early trauma. The initial increase in CB2 expression may represent a compensatory mechanism that fails over time. Intervention with a CB2 agonist is expected to decrease inflammasome activity and redirect the trajectory back onto a healthier path. These studies are preliminary, requiring dose-response assessments and a narrowing of the ideal age to treat the abuse subjects for maximal effect.

Keywords: CB2, Early Life Stress, Inflammasome

Disclosure: Nothing to disclose.

W39. Sex Differences in Resting-State Functional Connectivity in High-Functioning Adults With Autism

Kevin Sun, Rafi Ayub, Vicky Lam, Manish Saggar, Lawrence Fung*

Stanford University School of Medicine, Palo Alto, California, United States

Background: Are females with autism spectrum disorder (ASD) biologically and phenotypically different from their male counterparts? Males are four times more likely than females to be identified with ASD. This male-bias in ASD has raised the question of how the sex ratio might be relevant to the physiological mechanisms underlying ASD. Behaviorally, high-functioning adult females with ASD displayed more lifetime sensory symptoms, fewer current socio-communication difficulties, and more self-reported ASD traits than HFA males with ASD. Neuroanatomically, resting-state functional magnetic resonance imaging (rsfMRI) studies revealed consistent patterns of hypo-connectivity in ASD males (compared to TD males), and hyper-connectivity in ASD females (compared to TD females). These data have provided functional evidence for the sex differences in the neurobiology of ASD. However, investigations on the sex differences in neuroanatomic region-specific functional connectivity are lacking in the field of ASD. The goal of this study was to examine the sex differences in thalamocortical functional connectivity (FC) in high functioning adults with ASD compared to an age- and sex-matched control group and analyze its relationship with clinical symptoms.

Methods: Participants

Twenty-three individuals with ASD (mean[SD] 27.1[8.9] years; 39% female; full-scale IQ 103.7[16.1]) and 20 age- and sex-matched typically developing (25.1[7.2] years; 30% female; full-scale IQ 111.1[14.6]) individuals were included in the present study.

Imaging data acquisition

High-resolution 3D T1-weighted structural images were acquired using a 3T GE Signa PET/MR scanner. Gradient echo

pulse sequence was used for resting-state functional imaging with the following parameters: TR=2000ms, TE=30ms, flip angle=80°, acquisition matrix=64×64, field of view=24cm, slice thickness=4mm, and interleaved slices. A fixation cross was displayed throughout the duration of the scan. Subjects were instructed to let their mind wander and encouraged to keep their eyes-open and fixate on the cross while lying in the scanner, promoting a state of wakeful rest. Total scan time was eight minutes for each subject.

Seed-based correlation analysis

The left and right thalami were individually seeded by extracting the average time-series of all voxels within the region. The resulting time-series from the five ROIs were placed in a first-level GLM to determine the relationship of each seed with every other voxel in the brain. The resulting parameter estimates for each participant were incorporated in a higher-level GLM, which included any variables with significant group differences as covariates. This higher-level GLM analysis was run using FSL randomise using threshold-free cluster enhancement to correct for family-wise error rate in order to determine brain areas with group differences in parameter estimates between TD and ASD. Finally, for each of these FSL-identified "clusters," its mean parameter estimate was extracted for each individual using Featquery. The significance threshold for identified clusters was set to p=0.01 after Bonferroni correction for five seeds (alpha=0.05, m=5).

Behavioral assessments

The diagnosis of ASD in participants was confirmed by a combination of Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule, Second Edition-2 (ADOS-2), as well as clinical interview. Verbal and nonverbal IQ were measured using the Stanford-Binet Intelligence Scales, Fifth Edition. All participants completed a series of self-report questionnaires to assess ASD symptoms, including the AQ.

Post-hoc analyses of age and sex

Post-hoc GLM analyses were used to explore the potential effects of age and sex on group differences in resting-state FC (RSFC). ASD/TD group and age were included as independent variables in one GLM setup; group, age, and a group x age interaction term, in another GLM. Then, we performed the same analyses replacing age with sex. Significance level was set at alpha=0.05 (two-tailed).

Results: Sex differences in RSFC-behavior relationships. Relationships between right thalamus—left pre/post-central gyrus RSFC and ASD symptom severity were found to differ by sex in the ASD cohort. In males, across ASD and TD groups, a positive linear relationship was noted between AQ total score and right thalamus—left precentral and postcentral gyrus RSFC ($r = 0.57$, $p = 0.00144$). In females, a similar relationship across groups was not evident. Further GLM analyses of the HFASD cohort were performed to explore sex differences in the relationship between ASD behavioral measures and right thalamus–left pre/post-central gyrus RSFC.

Moderation analysis in ASD

In ASD participants alone, we performed a moderation analysis to investigate whether sex moderates the effect of RSFC on AQ. We included sex, RSFC, and a sex x RSFC interaction term as independent variables of a GLM predicting behavioral score. Significance was set at $p < 0.05$. Sex was found to be an effect modifier of the relationship between AQ and RSFC (model $F(3,19) = 5.059$, $p = 0.00962$, adjusted $R2 = 0.3563$; sex x RSFC interaction $p = 0.0238$).

Conclusions: We present novel evidence for resting-state thalamocortical hyperconnectivity in high-functioning adults with ASD that is associated with symptom severity in a sex-specific manner.

Keywords: Autism, Resting-State fMRI, Sex Difference

Disclosure: Nothing to disclose.

W40. In Vivo Examination of mGluR5 Availability in Chronic Pain: A [18F]FPEB PET Study

Margaret Davis*, Sarah Debonee, Ryan Cool, Irina Esterlis

Yale University School of Medicine, Waterbury, Connecticut, United States

Background: Chronic pain (CP) is a significant source of both personal and public health burden, accounting for an estimated $635 billion in care-related costs each year. Of further concern, in many cases (up to 66%) CP is treated with opioid-based analgesic medications, leading to high rates of drug misuse and frequently to opioid addiction. Critically, research suggests that the consequences of opioid prescription in CP impact women more negatively than men; women are more likely to be prescribed opiates for pain and the prevalence of opiate addiction and rate of opioid overdose is growing at a faster rate in women compared to men. Identification of alternative pharmacological targets capable of rapidly reducing pain in CP without leading to substance misuse must be prioritized. Recent preclinical, genetic, and postmortem evidence implicates mGluR5 - a mostly postsynaptic metabotropic glutamate receptor believed to induce pronociceptive behavior – as a potential treatment target for CP with low abuse-potential. Using positron emission tomography (PET) and the radioligand 18F-FPEB, we quantified mGluR5 availability in vivo for the first time in individuals reporting CP, and matched controls with no current pain experience (NCP).

Methods: Participants (n= 25 CP [M Age=36.76, 13 female, 52% Caucasian, 16 with comorbid MDD or PTSD, M MADRS Score=16.83]); n=25 NCP [M Age=38.08, 13 female, 52% Caucasian, 16 with comorbid MDD or PTSD, M MADRS Score=15.61]) were recruited and matched for age, gender, comorbid psychiatric diagnostic status, and smoking status. All participants completed 1 MRI scan and 1 PET scan with 18F-FPEB, as well as psychiatric and cognitive assessments. The radiotracer was injected as bolus plus constant infusion and subjects were scanned during steady state (90-120mins post-injection). Volume of distribution (VT: the ratio of activity in tissue relative to that in blood) was computed using a venous input function. Primary analyses focused on 6 frontal and limbic brain regions relevant to the pathophysiology of chronic pain: dorsolateral prefrontal cortex (dlPFC); orbitofrontal cortex (OFC); ventromedial prefrontal cortex (vmPFC); anterior cingulate cortex (ACC); amygdala; hippocampus.

Results: A multivariate analysis of variance (MANOVA) with mGluR5 availability (VT) in the specified regions of interest entered as dependent variables, and group (CP/NCP) as the independent variable, was significant ($F6,42=4.91$, p=.001). Post hoc analyses indicated CP individuals had significantly higher mGluR5 availability in frontal (dlPFC p=.003, 16% difference; OFC p=.001, 21%; vmPFC p=.004, 16%; ACC p=.001, 17%), but not limbic (amygdala p=.10, 10%; hippocampus p=.08, 8%) regions of interest. Of note, results remained significant when comorbid psychiatric diagnosis was controlled for ($F6,41=4.84$, p=.001). Interestingly, in the CP group ACC mGluR5 availability was significantly higher in females relative to males (p=.04, 13% difference). No gender differences in mGluR5 availability were observed in NCP individuals, or across groups. Exploratory analyses revealed that mGluR5 availability was inversely correlated with performance on measures of attention (r's=.-45-.50; p's= .02-.03), and working memory (r's=-.48-.57; p's= .007-.04) in CP.

Conclusions: Individuals with CP had higher mGluR5 in frontal brain regions relative to matched NCP with comorbid psychiatric diagnostic status controlled for. Females with CP had higher mGluR5 availability than males in the ACC only; no gender differences were observed in NPC or across groups. Further, mGluR5 availability was negatively associated with performance in select domains of cognitive functioning (attention and working

memory). Of note, deficits in both attention and working memory are common in individuals with CP; present findings raise the possibility that mGluR5 plays a role in CP related cognitive dysfunction. Observed differences in mGluR5 availability suggest a possible role for glutamate neurotransmission, and mGluR5 specifically, in the pathophysiology of CP. Further evaluation of glutamatergic targets for the treatment of chronic pain, especially in women, is highly warranted.

Keywords: Chronic Pain, Opioid Abuse, Chronic Pain Treatment, PET Imaging Study, mGluR5 Receptors

Disclosure: Nothing to disclose.

W41. Ethanol Withdrawal Differentially Induces the Spatiotemporal Neuron and Astrocyte Activities in the Dorsal Striatum Associated With Social and Anxiety-Like Behaviors

Seungwoo Kang*, Doo-Sup Choi

Mayo Clinic College of Medicine, Rochester, Minnesota, United States

Background: Anxiety is one of most common co-occurring disorders with impairments in social and cognitive-related disorders. However, little is known about the common or distinctive profiles of brain activities to shape those co-occurred neuropathological behaviors. The dorsal striatum (DS) has been considered a hub brain area controlling context-dependent decision-making process and coordinating movements because the cellular activities in the DS are linked to go and no-go pathway and lead to biased behavior in exploratory tasks according to the existence of avoidance conflict. Interestingly, recent studies reported that aberrant sociability and anxiety-like behavior are accompanied by the structural or functional abnormalities of the DS. We demonstrated that repeated ethanol exposure induces the striatopallidal neuronal adaptation in the dorsomedial striatum (DMS) accompanied with increased anxiety-like behavior and social impairment. Thus, we examined the cell-type specific spatiotemporal signaling in the DS on the different behavioral outputs and their contributions to neuropathological conditions induced by repeated ethanol exposure.

Methods: Using multi-regional fiber-photometry calcium imaging, we examined the spatiotemporal activities of the astrocytes and neurons in the medial and lateral parts of the DS (DMS and DLS) that represent context-dependent signatures in sociability and anxiety-like behavior. We selectively expressed the GCaMP6s, a genetically encoded calcium-dependent fluorescent indicator, in direct-pathway striatonigral medium spiny neuron (dMSN), indirect-pathway striatopallidal medium spiny neuron (iMSN), and astrocyte of transgenic mice expressing both Cre-dependent GCaMP6s and D1R-Cre, A2AR-Cre, or ALDH1L1-Cre. The brain calcium imaging was acquired during self-paced movements of mice in three-chamber social approach and elevated plus maze tasks. In the three-chamber task, "social preference" was measured as the comparison between the time spent with a stranger mouse and a novel object. In the elevated plus maze task, "anxiety level" was measured as the amount of the time spent in open- and closed-arms. We investigated whether the chronic intermittent ethanol (CIE) exposures alter the synchronization of the cellular activities with the specific behaviors in the tasks in the DMS and DLS.

Results: We observed that, at 3 to 4 days withdrawal from repeated ethanol exposure, mice showed the decreased social preference and increased anxiety-like behavior compared to those of ethanol-naïve counterparts. The CIE-induced spatiotemporal activity changes in the DMS are largely iMSN selective in both tasks, suggesting the iMSN's involvement in common progressive adaptation following the withdrawal from repeated ethanol exposure. While in the social approach task, the astrocyte activity in the DMS was increased during the mice explored for social behavior, the activity was decreased when the mice explored in the elevated plus maze, which could be upregulated parallelly with the ethanol withdrawal. This suggests that the dorsomedial striatal astrocytes represent the distinctive profiles in shaping the sociability and anxiety behavior in the context-dependent exploratory tasks. The cellular activities in the DLS largely did not show behavioral-context specific differences.

Conclusions: Our results indicate that the DMS iMSN's activities are common signatures that could be adapted by the withdrawal from repeated ethanol exposure and the DMS astrocytic activities are distinctive in the ethanol withdrawal-induced impairment of sociability and increased anxiety-like behavior. These findings provide new insights into striatal cellular activities how they temporally coordinate the context-dependent behavioral outputs.

Keywords: Anxiety, Sociability, Alcohol Withdrawal, Dorsal Striatum

Disclosure: Nothing to disclose.

W42. Association of Psychiatric Disorders and Psychotropic Medications With Bone Quality as Measured With Trabecular Bone Score

Sina Hafizi*, Lisa Lix, Didier Hans, James Bolton, William Leslie

University of Manitoba, Brandon, Canada

Background: Osteoporosis, a major cause of disability and mortality among ageing adults, is characterized by loss of bone strength. Current research suggests mental disorders and psychotropic medications as possible contributors to osteoporosis. Some mental disorders are shown to be associated with higher risk of fractures. Also, psychotropic medications such as anti-psychotics and antidepressants are suggested to increase the risk of fracture and alter bone density. Supporting this, bone turnover biomarkers are altered by psychotropic medications. Moreover, comorbidities of mental health disorders such as smoking and substance use, increase the risk of osteoporotic fractures. The majority of current evidence on the link between mental disorders and osteoporosis are based on bone mineral density (BMD) - as quantified by dual x-ray absorptiometry (DXA). Although DXA, as the gold standard tool for osteoporosis diagnosis, quantifies bone mineral density (BMD), it does not provide information about the quality of bone tissue. Furthermore, BMD measurement using DXA can falsely underestimate the risk of fracture. To address the limitations of the previous studies we studied the association between lumbar spine trabecular bone score (TBS) and mental disorders as well as psychotropic medications in a large population-based DXA registry from Manitoba, Canada. TBS is a novel FDA-approved technique for assessing bone quality from lumbar spine DXA that predicts fracture risk independently from BMD. Using TBS as adjunct to BMD and the Fracture Risk Assessment Tool (FRAX) significantly improves fracture prediction. We aimed to investigate – for the first time - whether mental disorders and related psychotropic medications are related to bone quality as measured with TBS.

Methods: A total number of 47,006 individuals (95.1% female) 40 years of age or older at the time of TBS assessment were included in this study. The psychiatric disorders studied included anxiety disorders, depression, psychotic disorders, and alcohol use disorder. The psychotropic medications examined in this study were classified as follow: selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants (TCA), other antidepressants, lithium, non-lithium mood stabilizers, antipsychotics, and benzo-diazepines. A general linear model was used to evaluate the association of TBS with psychiatric disorders and psychotropic medications while adjusting for relevant covariates (such as age and BMI) and controlling for confounders (such as history of glucocorticoid exposure prior to TBS assessment).

Results: Overall 20.6% of our sample suffered from psychiatric disorders with anxiety disorders being the most common diagnoses. About 17.6% of our sample used psychotropic medications and SSRIs were the most common prescribed medications. We observed significant negative effects of alcohol use disorder ($F(1, 46183) = 20.53$, $p < 0.001$, 95% CI = -0.043, -0.017), SSRI ($F(1, 46183) = 10.75$, $p = 0.001$, 95% CI = -0.011, -0.003), TCA ($F(1, 46183) = 5.14$, $p = 0.023$, 95% CI = -0.011, -0.001), other antidepressants ($F(1, 46183) = 9.57$, $p = 0.002$, 95% CI = -0.014, -0.003), and lithium ($F(1, 46183) = 8.91$, $p = 0.003$, 95% CI = -0.06, -0.012) exposure on TBS.

Conclusions: The findings of the current study, while preliminary, suggest that mental disorders and psychotropic medications can negatively affect bone quality.

Keywords: Psychotropic Medications, Mental Disorders, Side Effects

Disclosure: Nothing to disclose.

W43. Use of Actigraphy and Ecological Momentary Assessment to Monitor the Impact of COVID-19 on Mood and Behavior in Psychiatric Outpatients: Social Media and Smartphone App Use Predicts Maintenance of Physical Activity

Agnes Norbury, Shelley Liu, Maria L. Barrigon, Juan José Campaña-Montes, Lorena Romero-Medrano, Emma Smith, Elizabeth Ramjas, Antonio Artes, Enrique Baca-Garcia, M. Mercedes Perez-Rodriguez*

Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: There is growing concern that the social and physical distancing measures implemented in response to the Covid-19 pandemic may negatively impact health in other areas, via both decreased physical activity and increased social isolation. We used actigraphy and ecological momentary assessment to characterize the longitudinal impact of COVID-19 lockdown measures on physical activity, social behavior and psychological wellbeing in a prospective cohort of psychiatric patients.

Although social media and smartphone use have been proposed to have negative effects on mood and mental health, the strength and reliability of evidence for this assertion is disputed, and the reality is likely to be more nuanced. In particular, it has been suggested that digital social media tools may help individuals to foster and maintain social support networks during periods of stress and isolation. We explored whether increased engagement with digital social tools may help mitigate effects of enforced isolation on physical activity and mood.

Methods: 323 psychiatric patients were prospectively monitored using smartphone-based ecological momentary assessment and actigraphy before and during the COVID-19 pandemic. Physical activity and smartphone use were passively monitored, and emotional state was actively self-reported. Generalized linear mixed effect models were used to assess the effects of COVID-19 lockdown measures on physical activity, social media use and emotions, across four time periods: 1) Pre-COVID-19; 2) Post-COVID-19 first case, with schools still open; 3) Post-COVID-19 with schools closed; 4) Lockdown. In a subsample of 163 subjects, we used Gaussian graphical models (a form of network analysis which gives insight into the predictive relationships between measures across timepoints) to investigate whether increased time spent using social media apps would predict maintenance of higher physical activity levels, pre- vs post- imposition of lockdown conditions

Results: Daily steps decreased by 349 (95%CI:147-550) after the first COVID-19 case, when schools were still open; by 1040 (95% CI:670–1411) after schools closed, and by 3000 (95% CI:2784–3217) during lockdown. Daily social media use increased by 21 minutes (95%CI:16–27) after school closures, and by 30 minutes (95%CI:27–33) during lockdown. Rates of negative emotional states doubled during the lockdown (OR:2.23 [1.41-3.52]).

The Gaussian graphical models showed that within-individuals, there was evidence of a positive predictive path between digital social engagement, general smartphone use, and physical activity – selectively under lockdown conditions (N=127 individual users, M=6201 daily observations). Further, we observed a positive relationship between social media use and total daily steps across individuals during (but not prior to) lockdown.

Conclusions: Physical activity decreased during lockdown, increasing disease and mortality risk. Negative emotions doubled during lockdown, an early sign of psychological distress. Social media use increased, which may be a potentially adaptive mechanism to maintain social connections during social distancing. In fact, we found preliminary evidence that increased engagement with digital social media tools helps preserve physical activity -and likely psychological wellbeing- during periods of enforced social distancing. Exercise and virtual social contact are modifiable factors that are ideal targets for public health interventions to prevent the negative consequences of enforced social distancing.

Keywords: Actigraphy, Ecological Momentary Assessment, COVID-19, Social Isolation, Physical Activity

Disclosure: Neurocrine Biosciences: Grant (Self), AI Cure: Grant (Self), Takeda/Millennium Pharmaceuticals: Grant (Self), Neurocrine Biosciences: Advisory Board (Self), Merck: Grant (Self), American Foundation of Suicide Prevention: Consultant (Self)

W44. New Edition: A Model Psychopharmacology Curriculum for Teachers of Psychiatric Residents

Ira Glick*

Stanford University School of Medicine, Stanford, California, United States

Background: Started by the ACNP training committee in 1984, the ASCP Psychopharmacology Committee has developed unique and widely disseminated curricula for teaching clinical psychopharmacology to psychiatric residents, medical students and primary care physicians. It has increasingly had global penetration. We present here the 10th edition of the resident curriculum, and the joint 5th edition for medical students and for primary care.

Methods: The ASCP Curriculum Committee composed of directors of both resident education as well as medical student education educators have developed materials related to the "what, why, and how" to teach and evaluate. In addition, for each curriculum, we included both a core series of lectures as well as optional lectures developed by experts in their fields. We have done follow-ups on all three curriculums within the last 2 years. We describe here the process of revising, updating, and moving to a web-based curriculum. We present the content for the three curriculums.

Results: Based on the follow up of all three curricula, we have revised every lecture and updated the pedagogy. Depending on the size/resources of the program, teachers use the curriculum in its entirety or in parts. It works even in non-English speaking countries as committee members work with users to adapt/translate to local conditions and teaching strategies. It has been difficult to connect with primary care training programs.

Conclusions: For residents, the curriculum is now in its 10th edition and has 88 lectures and over 4,000 slides. For teaching medical students and primary care physicians, there has never been a generally accepted curriculum or set of teaching materials specifically designed for them. There is a great deal to teach in the four-year curriculum and medical students have widely divergent

career paths. This curriculum has 22 lectures. Having the curriculum web-based has improved availability although some programs globally still need a hard copy version.

Keywords: Education and Training, Psychiatric Education, Curriculum

Disclosure: Nothing to disclose.

W45. Insulin Resistance and Striatal Glutamate in Obese Humans: Preliminary Findings

Fernando Caravaggio*, Edgardo Torres, Julia Kim, Wanna Mar, Sri Mahavir Agarwal, Margaret Hahn, Gary Remington, Philip Gerretsen, Ariel Graff-Guerrero

Centre for Addiction and Mental Health, University of Toronto, Toronto, Canada

Background: Lower insulin sensitivity (%S) has been associated with lower dopamine levels in the right striatum of healthy non-obese persons. Concurrently, lower %S has also been associated with lower glutamate+glutamine (Glx) levels in the right striatum of healthy non-obese persons. However, it is unknown whether this positive relationship between %S and Glx in the right striatum is also present in obese persons (body mass index > 30).

Methods: Using proton magnetic resonance spectroscopy (1H-MRS), we quantified Glx levels from the right striatum of twenty obese participants (5 male, mean age: 47.95 ±12.93; body mass index: 38.68±4.97) and estimated %S from fasting plasma levels (12 hours) of insulin and glucose using the Homeostasis Model Assessment II.

Results: Consistent with previous findings, we observed that % S was positively correlated with levels of Glx in the right striatum ($r(18)=.41$, $p=.04$). Other potential moderating variables, such as age, body mass index, cholesterol levels, and triglycerides were not associated with %S . Despite confirming our a priori hypothesis, a post-hoc analysis revealed our sample only achieved 62% power to detect our effect. Our goal is to double our sample to achieve at least 85% power.

Conclusions: We preliminary report that %S is positively correlated with Glx levels in the right striatum of obese persons ($r(18)=.41$, $p=.04$). This is a preliminary replication and extension of our previous findings in non-obese persons in an explicitly obese sample. Data collection is on-going to achieve appropriate statistical power. These findings will have important implications for understanding how insulin may modify dopamine and glutamate concentrations in the striatum of humans.

Keywords: Dopamine, Glutamate, 1H-MRS, Dorsal Striatum, Insulin

Disclosure: Nothing to disclose.

W46. Implicit Priming Reduces High-Calorie Food Appeal and Neuronal Response to High-Calorie Food Cues

Kristina Legget*, Marc-Andre Cornier, Christina Erpelding, Brianne Sutton, Jason Tregellas

University of Colorado School of Medicine, Aurora, Colorado, United States

Background: Obesity rates are rapidly rising in the United States, giving rise to myriad health and quality-of-life concerns. Weight loss is associated with a reduction in comorbid conditions, such as cardiovascular disease and hypertension. Weight loss can be difficult, however, and preventing weight regain is even more challenging. As such, the development of effective strategies to promote weight loss and maintenance is critical. Toward this goal, we developed an evaluative conditioning paradigm (implicit priming [IP]), in which positively or negatively valenced images are presented immediately prior to food images, but not consciously perceived. We hypothesize that this bottom-up sensory-level conditioning approach alters affective associations with food items. For example, pairing ice cream with a negative prime (e.g., maggots on food) could reduce positive response to the ice cream itself. This approach has the potential to reduce the appeal of hedonic (high-calorie) foods, which could promote reduced intake and support weight loss and maintenance efforts. Preliminary behavioral investigation of this intervention found that active IP vs. a control IP condition (with scrambled images as primes) was associated with reduced high-calorie food preference ratings. The present study used fMRI to study the neurobiology of this approach. We hypothesized that active IP, compared to control IP, would result in reduced reward-related neuronal responses to high-calorie food cues.

Methods: Forty-one adults 18-65 years old with BMI > 25 kg/m2 completed the study. Participants were randomized to active or control IP (active IP: $N = 23$ [4M, 19 F]; control IP: $N = 18$ [9M, 9F]). The day before the study visit, participants completed a 1-day, macronutrient-controlled, eucaloric run-in diet. On the study day, participants arrived in the fasted state and completed a food image ratings task, during which they rated 96 food images (48 high-calorie, 48 low-calorie) for "desire to eat" using a visual analog scale (0-100). Participants then consumed a standardized breakfast meal. Functional magnetic resonance imaging (fMRI) was completed 1.5 hours after breakfast, such that participants were in a neutral state of hunger. During the scan, participants completed a visual food cues task, during which they viewed 96 food images (48 high-calorie, 48 low-calorie). Images were different from those used during the image ratings task to reduce habituation potential. The 10-minute IP intervention (active or control) was then completed during scanning to examine neuronal mechanisms involved. In active IP, images of high- and low-calorie foods were paired with implicitly presented (below perceptual threshold, for 20ms) images of either negative or positive valence (high-calorie images with negative; low-calorie images with positive). Control IP matched active IP, but with scrambled images instead of negative/positive priming images. Following IP, participants repeated the visual food cues task to determine effects of IP on neuronal response to food cues. Four hours after breakfast, participants repeated the food image ratings task ("desire to eat" high- and low-calorie food images).

Results: Behavioral results from our first study were replicated, with a significant time by group interaction ($F\ [1,33] = 4.81$, $p = 0.035$), such that the active group reduced "desire to eat" high calorie foods, but the control group did not. Interestingly, desire to eat low-calorie foods was increased in the control group, but not the active group (time by group interaction: $F\ [1,33] = 8.28$, $p = .007$). During the priming paradigm, a greater response to high-calorie foods was observed in the insula and anterior cingulate cortex in the active compared to control group ($p < 0.05$, corrected). In the visual food cues task, a reduced response to high-calorie food images was observed in the insula, striatum, and dorsolateral prefrontal cortex (dlPFC) in the active compared to control group ($p < 0.05$, corrected), after vs. before the intervention.

Conclusions: Replicating our preliminary behavioral investigation of the IP intervention, active IP reduced ratings of "desire to eat" high-calorie foods, an effect not observed in control IP. This effect was specific to high-calorie foods. That low-calorie ratings were not increased following implicit priming with positive images may be due to the likely greater salience of the negative images that were paired with high-calorie images. The negative images chosen were selected to elicit disgust, which would be expected to elicit an insula response and is commonly associated with strong evaluative conditioning effects. The fMRI results reported here support this hypothesis, with greater response to high-calorie food images (paired with disgust images in active IP) observed in

the insula during active compared to control IP. This suggests that the intervention is affecting the hypothesized biological target. Furthermore, neuronal response to high-calorie food cues was reduced following active IP, compared to control IP, in brain regions associated with food-based motivation, reward processing, and stimulus salience. The next step will be to conduct a larger investigation to determine the impact of IP on longer-term food preferences, how results translate to eating behaviors, and to further delineate neuronal mechanisms underlying the intervention.

Keywords: Obesity, fMRI, Implicit Priming, Food Cues

Disclosure: Nothing to disclose.

W47. Laparoscopic Sleeve Gastrectomy Enhances Functional Connectivity of Brain Regions Associated With Self-Referential and Sensory Processing in Obese Patients: A Longitudinal fMRI Study

Jia Wang, Guanya Li, Yang Hu, Wenchao Zhang, Dardo Tomasi, Nora D. Volkow, Gang Ji, Yi Zhang, Gene-Jack Wang*

National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland, United States

Background: Obesity is a prevalent health problem defined as abnormal or excessive fat accumulation. Previous studies have identified various brain regions and neurocircuit abnormalities associated with obesity. To date, bariatric surgery is the most effective intervention for weight reduction in severe obesity. Following surgeries, such as laparoscopic sleeve gastrectomy (LSG), sustained weight-loss is driven by changes in eating behaviors and physiological signals of energy availability. Neuroimaging studies have revealed that bariatric surgery can promote functional and structural changes in regions implicated in executive control (dorsolateral prefrontal cortex), reward (caudate), memory (hippocampal gyrus), emotion (amygdala), and self-referential processing (posterior cingulate cortex-PCC). However, since the stomach fundus is removed with LSG, we questioned whether it affected the activity of sensory processing regions in the brain and their functional connectivity with other regions involved with homeostasis and eating behaviors. To investigate this, we employed resting-state fMRI (RS-fMRI) and functional connectivity density (FCD) mapping to identify regions affected by LSG, which we use as seeds to map their resting-state functional connectivity (RSFC) and their association with behavior.

Methods: Twenty-five obese patients who underwent LSG and 25 age- and sex-matched normal weight participants (NW) underwent eyes open resting-state whole-brain 3T fMRI (TE=30 ms, TR=2000 ms, scan time = 360 s). LSG group was tested before (PreLSG), and one- (PostLSG_1) and twelve-month (PostLSG_12) after LSG. Image time series were denoised, motion corrected and normalized to the standard MNI space. Framewise displacement (FD), which was used to assess subject motion during scanning, did not differ for obese (FD=0.089±0.009) and NW (FD=0.067 ±0.022). Local (lFCD) and global FCD (gFCD) were calculated from fMRI time series using Matlab. Repeated measures ANOVA was used to assess time effects in lFCD and gFCD. Regions of interest (ROIs) were identified after family wise error (FWE) correction for multiple comparisons at a cluster-level correction approach (PFWE< 0.05) with a minimum cluster size of k=50 and a cluster-forming threshold of P< 0.001. ROIs were used as seed regions for subsequent seed-voxel correlation RSFC analysis. FCD and RSFC values were extracted from NW based on ROIs coordinates, and two samples t-tests were performed between NW and PreLSG, PostLSG_1, PostLSG_12, respectively.

Results: There were significant time effects on lFCD in thalamus (THA) and PCC/precuneus (PreCun) in LSG group. Post-hoc tests showed LSG significantly increased lFCD in THA at PostLSG_12 compared with PreLSG and PostLSG_1, and lFCD was equivalent to that in NW at PostLSG_1 and PostLSG_12; decreased lFCD in PCC/PreCun at both PostLSG_1 and PostLSG_12 compared to PreLSG and NW. Changes in lFCD in THA correlated negatively with changes in BMI and positively with changes in cognitive-control at PostLSG_12 compared to PreLSG whereas changes in lFCD in PCC/PreCun at PostLSG_1, compared to PreLSG correlated positively with changes in BMI. THA had stronger RSFC with PreCun at PostLSG_12 compared to PreLSG and PostSLG_1, and changes in RSFC of THA-PreCun were negatively correlated with changes in hunger at PostLSG_12, compared to PreLSG. PCC/PreCun had weaker positive RSFC with insula (INS), both at PostLSG_1 and PostLSG_12, compared to PreLSG. RSFC of PCC/PreCun-INS were negatively correlated with hunger and anxiety at PostLSG_1 and PostLSG_12 respectively, and changes in reward for high calorie food-cues (HC_Reward) were negatively correlated with changes in RSFC of PCC/PreCun-INS at PostLSG_1, compared to PreLSG.

Conclusions: These findings suggest that LSG-induced sustained weight loss after the surgery is associated with functional changes in brain regions involved with self-referential (PreCun) and gustatory sensory processing (THA, INS) as well as eating behaviors.

Keywords: Bariatric Surgery, Brain Imaging, Functional Connectivity

Disclosure: Nothing to disclose.

W48. Accessible Wireless Technologies to Automate Behavioral and Circuit Neuroscience

Kyle Parker, Raza Qazi, Choong Yeon Kim, Rudriger Rill, Makenzie Norris, Jaeyoon Chung, Jenny Kim, John Bilbily, Marie Walicki, Graydon Gereau, Hyoyoung Lim, Yanyu Xiong, Jenna Lee, Melissa Tapia, Matthew Will, Sangtae Ha, Jae-Woong Jeong, Jordan McCall*

Washington University in St. Louis, St. Louis, Missouri, United States

Background: Behavioral and circuit neuroscience is labor-intensive and often requires researchers to be physically present to conduct experiments in limited numbers of animals at once. Recent advances in materials science and electrical engineering have enabled miniaturized, flexible and wireless neural devices to help mitigate issues associated with traditionally tethered schemes that restrict animals' natural movements. However, these wireless platforms have significant disadvantages which limit their widespread adoption in neuroscience laboratories. Notably, the wireless control of these devices is not selective for a particular animal or device function. While there are instances when one may want to use the same neural manipulations across animals, this is not ideal for more complex behaviors or large experiments testing multiple conditions. Furthermore, these systems often rely on expensive and highly specialized equipment that may not be portable to other uses and the available platforms are not easily modifiable or scalable for automated or high-throughput experimentation. To overcome these limitations, we introduce a remotely-programmable, globally-accessible hardware and software infrastructure of miniaturized wireless networks, which we refer to as the "Internet of Things in Neuroscience" (IoTN).

Methods: The IoTN system integrates wireless neural devices and other laboratory equipment with Bluetooth Low Energy (BLE) and Wireless Fidelity (Wi-Fi) technologies to enable semi-automated and fully-automated behavioral studies, respectively. The wireless IoTN network can either be controlled locally through BLE piconets using a smartphone, or can be accessed globally over the Internet using Wi-Fi or cellular network. We used multiple established animal models of behavior with both male and female mice for proof-of-principle integration of IoTN infrastructure into

in vivo neuroscience experiments. To demonstrate different control schemes, we stimulated agouti-related protein (AgRP) expressing neurons to drive food consumption in ad libitum fed condition. To demonstrate the principles of IoTN control systems for multiple simultaneous experiments, we simultaneously targeted the secondary motor cortex (M2) of six mice expressing ChR2 under the Thy1 promoter. To demonstrate independent selectivity, we used multicolor optogenetics to bidirectionally control the midbrain dopaminergic system (VTA-DA) to modulate social interaction. For automated experimentation we tested whether the known arousal modulating properties of the locus coeruleus noradrenergic system extend to its projections into the prefrontal cortex (LC-PFC).

Results: Blue-light photostimulation wirelessly activated via Local Piconet or Internet IoTN control significantly increased food consumption above baseline levels (Repeated measures one-way ANOVA, $p < 0.01$, $p < 0.001$), validating the reliability of both IoTN control modes for wireless neuromodulation. Following a baseline exposure to an open arena, smartphone-based Local Piconet control simultaneous photostimulation of M2 increased total locomotor activity as well as induced rotation behavior in a time-locked fashion (Paired t-test (0 vs 20 Hz), $p<0.05$, $p<0.01$). VTA-DA neuron stimulation increased social interaction with non-cagemate "stranger" mice (Paired t-test, $p<0.01$). In contrast, inhibition of VTA-DA activity reduced social interaction, but did not reach significance ($p=0.08$). Early light phase scheduled LC-PFC stimulation increased locomotor activity (Repeated measures one-way ANOVA, *$p<0.05$), but late light phase stimulation had no clear effect.

Conclusions: The IoTN system employed here gives the user the means to effortlessly scale and rapidly adapt to their needs or preference, allowing for remote, scalable, modular, and chronic high-throughput neuroscience studies. While we demonstrate use of the IoTN in home-cage and socially-interacting behavioral experiments, overcoming the line-of-sight handicap seen in other technologies makes experiments in truly unconstrained environments possible. This system can be used immediately in any laboratory with equipment that uses transistor-transistor logic. In other words, the IoTN can readily trigger DPSS laser stimulation, timestamp electrophysiology experiments, activate behavioral apparatuses, and initiate drug or fluid delivery. It is reasonable to imagine using the IoTN infrastructure in large or complex environments that more closely mimic the natural world or in rapidly moving, unstructured settings that would be relevant to many neuropsychiatric disorders.

Keywords: Optogenetics, Internet of Things, Smartphone

Disclosure: Nothing to disclose.

W49. Expand Your Mind: Evidence for Psychedelic Enhanced Brain Stimulation

Lucas Dwiel, Wilder Doucette*

Dartmouth Hitchcock Medical Center, Lebanon, New Hampshire, United States

Background: Serotonergic psychedelics have recently enjoyed a resurgence in neuroscience and psychiatry with promising success in psychedelic-assisted therapy for the treatment of anxiety, depression, and addiction. The mechanism of action behind this therapeutic effect relies, at least in part, on 5-HT2 agonism which can be negated with the 5-HT2A antagonist, ketanserin. At the cellular level, psychedelics have been shown to increase neuritogenesis, spinogenesis, and synaptogenesis (observed 24 hours after acute administration), effectively priming neural circuits for neuroplasticity, which may help explain the sustained efficacy of psychedelic-assisted therapy. At a neural systems level, the acute effects psychedelics have been described with functional imaging and neural oscillations. Briefly, these studies have shown that the drugs increase the entropy of spontaneous cortical activity, thus relaxing the weight of high-level priors (e.g., beliefs) and allowing for increased bottom-up information flow. However, it appears that the strongest and most reliable therapeutic effects of psychedelics manifest when the drugs are paired with a targeted intervention (e.g., psychotherapy). In order to better understand this synergistic interaction without human bias, we paired local field potential (LFP) recordings from rats following lysergic acid diethylamide (LSD) or saline administration and determined if exposure to these treatments altered the effect of a targeted intervention (deep brain stimulation – DBS).

Methods: Sprague-Dawley rats of each sex were implanted with electrode arrays that targeted the bilateral infralimbic cortex, orbitofrontal cortex and the nucleus accumbens core and shell to record local field potentials and deliver electrical stimulation. In the first cohort of animals, the acute effects of 0.02 µg/kg LSD ($n=6$) vs. saline ($n=5$) was assessed using neural oscillations (LFPs). Then we determined if a difference between the LSD and saline exposed group was detectable 24 hours after acute administration. Brain states were quantified using 216 LFP features (power and imaginary coherence within 6 frequency ranges). To estimate the distance between the brain states of pairs of experimental conditions (e.g., LSD vs. Saline), the machine learning algorithm Lasso was used to classifying brain activity (LFPs) from the two data sets being classified and the accuracy of those models provided an estimate of brain state distances. The second cohort of animals ($n=6$) had LFPs recorded during DBS of the infralimbic cortex (3 sessions) and then 24 hours after acute administration of LSD. In the third cohort of animals ($n=5$), the effect of infralimbic DBS on the delay discounting task was evaluated with and without pre-treatment with LSD.

Results: The recorded data demonstrate that acute LSD administration leads to a predictable change in brain activity across individuals compared to saline injected animals (AUROC $=$ 0.79 ± 0.02) which disappear after 24 hours (AUROC $= 0.45 \pm 0.04$). Interestingly, the effect of DBS on these similar brain states (24 hours after a saline or LSD injection) resulted in brain state shifts that were distinguishable from each other (AUROC $= 0.84 \pm$ 0.006). When rats were pre-treated with LSD, DBS induced changes in brain state were larger (AUROC $= 0.9 \pm 0.005$) than when the same animals received DBS only (AUROC $= 0.84 \pm$ 0.006). Specifically, LSD pre-treatment caused DBS to produce more significant changes in imaginary coherence, meaning DBS was able to more robustly modulate functional connectivity between brain regions. The specific LFP features we have previously shown to correlate with the level of impulsivity in the delay discounting task were not differentially modulated with LSD +DBS compared to DBS alone. This likely explains why only subtle differences were observed in the effect of DBS vs. LSD+DBS on delay discounting behavior.

Conclusions: These results further characterize the acute changes induced by serotonergic psychedelics (e.g., LSD) on cortical-striatal brain states and demonstrate that these altered states return to baseline after 24 hours. Interestingly, a focal intervention (DBS) that is applied in a critical period (24 hours after LSD) produces a distinct shift in brain state compared to DBS alone. These results suggest that a single dose of a psychedelic opens a critical window that expands the repertoire of brain state changes that can be induced with a focal intervention – possibly expanding their therapeutic potential for neuropsychiatric illnesses.

Keywords: Psychedelics, LSD, Deep Brain Stimulation, Local Field Potentials, Machine Learning

Disclosure: Nothing to disclose.

W50. Developing an Empirical Definition of Impulsive Aggression and Mood Latent Profiles in Children and

Adolescents With External Validation Across Three Outpatient Samples

Eric Youngstrom*, Andrea Young, Joshua Langfus, Ekaterina Stepanova, Kathryn Van Eck, Robert Findling

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

Background: Impulsive aggression (IA) may distinguish a subset of youth from those with attention problems, oppositional behavior or a mood disorder, and may indicate differential treatment response. Yet, DSM-5 and ICD-10 do not include a diagnosis that adequately captures IA. Our objective was to empirically group youths into profiles based on their IA, manic, depressive, rule-breaking, and self-harm behaviors; examine which profiles replicated across three samples; and characterize the case sets on demographic and clinical features.

Methods: After harmonizing data from three samples (NIH R01MH066647, n=679; Stanley Medical Research Institute, n=392; NIH R01MH073967, n=634), Latent Profile Analysis (LPA) assigned youth to classes based on caregiver-reported measures of AIR, manic, depressive, rule-breaking, and self-harm behaviors. Classes from each sample were grouped into sets based on profile similarity. Chi-squared and logistic regressions tested set differences in diagnoses, sex, and race, and ANOVA and deviation-coded regressions tested differences in age, functioning, and mood severity.

Results: An eight-class solution fit best in each sample and seven profiles replicated across samples: (1) high AIR and self-harm, lower depressive and manic scores; (2) high AIR, manic symptoms, and self-harm; (3) high internalizing symptoms with unipolar depression; (4, 5, 6) smaller sets with elevations on both manic and depressive symptoms and moderate AIR; and (6, 7) high rates of bipolar diagnoses and family bipolar history. Two sets (5, 6) were high on both AIR and mood symptoms, were the most impaired, and had the highest comorbidity. Set differences were all significant omnibus p<.0005 for diagnostic and functioning criteria, as well as age, with large effect sizes on diagnostic rates, mood symptom severity, and overall functioning.

Conclusions: These analyses support an empirical definition of IA in youth separate from mood disorders. LPA profile sets distinguished by level of AIR and mood symptoms differed in demographic and diagnostic characteristics as well as functioning. Importantly, there was a set with high AIR but low mood indicators and with high rates of ADHD and ODD, but not mood disorder.

Keywords: Irritability/Aggression, Depression, Mania, Classification

Disclosure: Supernus: Consultant (Self), Guilford Press: Royalties (Self), American Psychological Association: Royalties (Self)

W51. Evaluation of the Discriminative and Reinforcing Potential of Centanafadine and Reference Comparator ADHD Drugs by Drug-Discrimination and Intravenous Self-Administration Testing in Rats

David Heal*, Helen Rowley, Sharon Smith, Reem Elbekai

DevelRx Ltd., Nottingham, United Kingdom

Background: Centanafadine is a triple inhibitor of monoamine reuptake with greater potency at the human transporter for norepinephrine (NET) compared with dopamine (DAT) or serotonin (SERT) (Bymaster et al, 2012, Synapse 66: 522-32). Centanafadine is undergoing clinical evaluation as a treatment for attention deficit hyperactivity disorder (ADHD). Part of the safety assessment of all new CNS-active drugs is a non-clinical evaluation of the risks they pose for human abuse. To fulfill this regulatory requirement, we have investigated the psychoactive properties of centanafadine in a rat d-amphetamine-cued drug-discrimination model and its reinforcing potential by intravenous self-administration (IVSA) in cocaine-trained rats. The reference comparators were methylphenidate and bupropion (IVSA and drug-discrimination) and d-amphetamine and phentermine (drug-discrimination only).

Methods: Drug-discrimination experiments were performed using freely-fed, adult, female, Lister hooded rats. Using sweetened milk rewards rats were trained to discriminate between d-amphetamine (0.3mg/kg i.p.) and saline (i.p.) in a 2-choice lever-pressing test on a FR5 schedule of reinforcement. Test sessions were 10 min and consisted of 2.5min (non-rewarded) and 7.5min (rewarded on either lever). To avoid any food reward-related lever bias, only the results from the non-rewarded part of the test were used. Generalization to d-amphetamine was set at >75% responding on the drug assigned lever. Results are reported as mean±SD for 7-8 rats/group. IVSA experiments were conducted in mildly food-restricted, adult, male, Sprague-Dawley rats trained to self-administer cocaine (0.36mg/kg/inj [injection]) on a FR5 schedule in 2hr sessions. After robust, stable self-administration was established (≥12inj/session), the responding of the rats was extinguished with saline on FR5 (≤6inj/session) before they were divided into 3 groups to evaluate the potential reinforcing effects of centanafadine (0.003, 0.01, 0.03 and 0.1mg/kg/inj), methylphenidate (0.01, 0.03 and 0.01mg/kg/inj) or bupropion (0.03, 0.1 and 0.3mg/kg/inj).

Self-administration was capped at 20inj/session. Results are reported as mean±SEM for 8-9 rats/group. Both studies were conducted to GLP and the methods are described in detail by Heal et al (2013, Neuropharmacology 73: 348-58).

Results: Orally administered d-amphetamine (0.125-1.0mg/kg) dose-dependently generalized to the intraperitoneally injected d-amphetamine cue (80.9±20.9% at the highest dose). Centanafadine did not generalize to d-amphetamine at doses of 0.3, 3.0, 5.0, or 6.0mg/kg p.o.; only the highest dose (10mg/kg, p.o.) generalized to d-amphetamine (76.3±18.9%). Dose-dependent generalization to d-amphetamine with >75% generalization at the highest dose of each drug was observed with methylphenidate (1.0-5.0mg/kg, p.o.; 82.9±13.2%), phentermine (1.0-3.0mg/kg, p.o.; 93.4 ±6.4%) and bupropion (1.0-30mg/kg, p.o.; 83.0±20.8%). The rank order of potency based on the lowest oral dose required for generalization to d-amphetamine was d-amphetamine > phentermine > methylphenidate > centanafadine > bupropion. In the IVSA study, cocaine (0.36mg/kg/inj) maintained self-administration at levels significantly greater than saline (19.9±0.1inj/session vs 4.8±0.2inj/session, p<0.001). The 2 lowest centanafadine doses (0.003 and 0.01mg/kg/inj) did not maintain levels of self-administration significantly greater than saline (7.2±1.2 and 5.8±1.3inj/session, respectively). The 2 highest centanafadine doses (0.03 and 0.1mg/kg/inj) maintained greater self-administration than saline (12.8±2.4 and 15.2±2.9inj/session, respectively; both p<0.001 vs saline). All doses of methylphenidate (0.01, 0.03 and 0.1mg/kg/inj) maintained self-administration above saline values (8.8±2.2, p<0.05; 18.8±1.5, p<0.001; 20.0±0.0inj/session, p<0.001). Similarly, all doses of bupropion (0.03, 0.1 and 0.3mg/kg/inj) maintained self-administration above saline values (10.4±2.2; 20.0±0.1; 20.0±0.0inj/session; all p<0.001). The rank order of potency based on the lowest dose maintaining self-administration was methylphenidate > bupropion = centanafadine.

Conclusions: Drug-discrimination testing revealed that moderate doses of centanafadine were not recognized as d-amphetamine-like by the rats. However, centanafadine did generalize to d-amphetamine at very high dose. In IVSA, centanafadine served as a positive reinforcer in cocaine-trained rats, but again this effect was seen at high doses. When the results

for centanafadine are compared with those of other drugs that are either approved medications for ADHD or have been reported to show efficacy in this indication in clinical trials, the results consistently predict that centanafadine will pose a lower risk for abuse than methylphenidate (C-II) or d-amphetamine (C-II) and a level of risk similar to that of bupropion (not scheduled as a controlled drug).

Keywords: Centanafadine, Self-Administration, Drug Discrimination, Attention Deficit Hyperactivity Disorder

Disclosure: DevelRx Ltd: Employee (Self), DevelRx Ltd: Stock / Equity (Self)

W52. A Role for Reward Sensitivity in the Serotonergic Modulation of Impulsivity

Stephanie Desrochers, Emma Lesko, Valerie Magalong, Peter Balsam, Kate Nautiyal*

Dartmouth College, Hanover, New Hampshire, United States

Background: While the neural substrates of impulsive behavior are commonly studied in humans and preclinical models, the behavioral and cognitive substrates which contribute to pathological impulsivity are unclear. Understanding what behavioral and cognitive processes can drive pathological levels of impulsivity will allow us to better model and treat disorders with dysregulated impulsive behavior.

Methods: Our approach uses a mouse model with elevated impulsive action, and probes related behavioral and cognitive substrates to determine contributors to the impulsive behavior. Mice lacking the serotonin 1B receptor (5-HT1BR) show increased impulsive action, but not impulsive choice, making this an ideal model to dissect out substrates related specifically to impulsive action. Additionally, the 5-HT1BR is implicated in a number of psychiatric disorders which include dysregulation of impulsive behavior, such as ADHD and substance use, gambling, and conduct disorders. Specifically, in our studies, we modulated 5-HT1BR expression and then measured phenotypes theoretically related to impulsive action including motivation, activity, habitual-like behavior, effort-based decision making, and reward sensitivity.

Results: Consistent with past work, we first report that mice lacking 5-HT1BR expression in adulthood have elevated levels of impulsive action measured in a Go/No-Go task, but do not show elevated levels of impulsive choice as measured in a delayed discounting paradigm. Interestingly, these mice also show increased goal-directed responding and motivation as measured in appetitive operant tasks. However, they show no differences in learning or extinction rate and do not have alterations in habitual-like responding as measured in satiety-induced devaluation and contingency degradation tasks. Interestingly, mice lacking 5-HT1BR did show increased hedonic responses to sweet rewards as measured by lick rates to sucrose (>50% increase from control mice). This led to the hypothesis that the increased impulsive action may be a direct result of increases in reward valuation or the hedonic response to reward. In order to test this, we developed a novel paradigm - a variable value go/no-go (VV-GNG) task to test the direct effect of increasing reward value (by 3X) on impulsivity in a trial-by-trial manner. First, in normal controls, we show that increasing reward value increases impulsive action (increasing both hit rate and false alarm rate). Furthermore, we used the VV-GNG paradigm to show that the elevated impulsive action seen in 5-HT1BR KO mice could be ameliorated by decreasing the reward value (by 1/3).

Conclusions: Overall we find that a lack of 5-HT1B R expression results in increased reward sensitivity, which is a likely a behavioral substrate for the increased impulsive action. This is supported by our findings that increasing reward value results in increases in impulsive action, and that decreasing reward value can return impulsive action to normal control levels in mice lacking 5-HT1B R expression. Taken together these data provide a broad analysis of behavioral and cognitive processes that may contribute to

Keywords: Impulsivity, Reward Sensitivity, Serotonin 1b Receptor, Serotonin

Disclosure: Nothing to disclose.

W53. Chrna5 Speeds the Response to Endogenous Acetylcholine: Rescue by NS9283 in Prefrontal Cortex

Abstract not included.

W54. Contributions of the Circadian Clock to Apoptosis and Survival in Fibroblast and Neuronal Models of Lithium-Responsive Bipolar Disorder

Himanshu Mishra, Heather Wei, Melissa LeRoux, Insu Ko, Angelica Luis, Noelle Ying, John Kelsoe, Michael McCarthy*

University of California, San Diego, California, United States

Background: Lithium is an effective treatment for bipolar disorder (BD), but only about 1/3 of BD patients fully respond to the treatment. Previous work has determined that individual differences in circadian rhythms can help predict which BD patients will be lithium responders (Li-R) and lithium non-responders (Li-NR). Other studies have determined that lithium has neurotrophic properties that may contribute to its therapeutic effects. However, it is not clear if Li-R and Li-NR differ in regard to the neuroprotective benefits of lithium, or to what extent do the differences in circadian rhythms that predict lithium-responsiveness overlap with neuroprotection.

Methods: In this study we developed cellular models of apoptosis in BD patient-derived fibroblasts and induced pluripotent stem cell (iPSC)-derived neuronal precursor cells (NPC). We used bioluminescent caspase and survival assays to measure cell death and circadian rhythms. Apoptosis assays were conducted in conjunction with genotyping, gene expression analyses, drug treatments and gene knockdown using siRNA to identify underlying mechanism of apoptosis that differ in BD and control cells. Data were analyzed by analysis of variance (ANOVA).

Results: We found in fibroblasts that lithium protects against apoptosis preferentially in BD cells compared to controls, but that the effects do not differ between Li-R and Li-NR. Genetic variation in PER3 predicts cell survival in fibroblasts, but individual differences in circadian rhythms did not. The cellular mechanisms of apoptosis and protection were examined in detail in NPCs. In these neuronal cells, staurosporine induced distinct patterns of expression of genes involved in survival pathways including BAX, BCL2, and CACNA1C and the clock gene PER1. PER1 knockdown reduced the viability of NPCs, with the greatest effect observed in Li-NR samples. Knockdown of BMAL1 and REV-ERBα had effects on NPC viability that did not differ by group. Finally, we found that small molecule agonists of REV-ERBα have neuroprotective effects in NPCs that are comparable to lithium. In mouse neurons, the same drugs had neuroprotective effects against glutamate excitotoxicity and modulated circadian rhythms.

Conclusions: Our data demonstrate overlap between the circadian clock and the cell survival pathways engaged by lithium, but not in a simple way in which an individual's rhythm is directly predictive of neuronal vulnerability. Regulation of PER1 may distinguish Li-R and Li-NR, and drugs targeting REV-ERB may have neuroprotective potential in treating BD.

Keywords: Bipolar Disorder, Circadian Rhythm, iPSC, Lithium, Apoptosis

Disclosure: Nothing to disclose.

W55. Sex Differences in ARC Regulation and Behavioral Relevance in the Nucleus Accumbens

Rachel Penrod*, Dalia Martinez, Brandon Hughes, Christopher Cowan

Medical University of South Carolina, Charleston, South Carolina, United States

Background: The Nucleus Accumbens (NAc) is a key brain region mediating reward, motivation, and mood-related behaviors. The NAc consists of a number of neuronal sub-types, though the vast majority of the NAc is comprised of Medium Spiny Neurons (MSNs). MSNs are the primary output neuron of the NAc, integrating dopamine and glutamatergic inputs, and consisting of two sub-types defined by dopamine receptor expression (D1- or D2 MSNs). Sex differences are observed in numerous behaviors relevant to NAc function including anxiety-like and drug-related behaviors. Despite the importance of NAc function, and the observed sex differences in relevant behaviors, we have little understanding of the molecular mediators of these differences. Recent work from our laboratory has identified activity-regulated cytoskeleton associated protein (Arc, also known as Arg3.1) as a potential mediator of sex differences in NAc-relevant behavior. Arc is an immediate early gene and a known regulator of AMPAR surface expression, both through the promotion of endocytosis and the regulation of GluA1 expression. Arc mRNA is upregulated by stress-experience and acute cocaine exposure in the NAc, and our studies of Arc-deficient mice (Arc KO) demonstrated changes in mood- and drug-related behavior. Recent studies using viral-mediated Arc knockdown in the NAc identified a number of impacts on NAc-relevant behavior, many with effects restricted to males. The focus of current studies is to understand how Arc expression and function may differ between sexes and cell types in the NAc.

Methods: To examine Arc induction following behavioral experience, NAc samples from adult wild-type male and female mice were collected following gentle-handling, elevated plus maze exposure (EPM), or cued fear conditioning and mRNA was isolated for qRT-PCR analysis. To assess cell type-specific regulation of Arc induction, male and female transgenic mice expressing cell type-specific fluorophores (D1R-tdTomato; D2R-GFP) were exposed to gentle-handling, EPM exposure, or acute cocaine injection. Immunohistochemistry was used to localize Arc protein to specific MSN sub-types. To examine the behavioral relevance of Arc in the NAc, male and female wild-type mice received intra-NAc infusions of Arc shRNA or control shRNA. To assess cell type-specific behavioral relevance of Arc, male and female transgenic mice expressing Cre recombinase in either D1- or D2-expressing MSNs received intra-NAc infusions of a Cre-dependent Arc shRNA or control. Following recovery and Arc knockdown, mice were assessed for mood- and drug-related behaviors.

Results: We find that males induce Arc in the NAc following EPM exposure, the same context where loss of Arc decreases anxiety-like behavior. In contrast, females fail to induce Arc following EPM exposure, a context where Arc knockdown did not influence behavior. Females can induce Arc in the NAc, to a similar extent as males, following cued fear conditioning exposure. Despite induction, loss of Arc did not influence cued fear conditioning learning in either sex. Consistent with our results from constitutive Arc knockdown, cell type-specific manipulations do not appear to alter female anxiety or drug-related behaviors. However, our preliminary findings suggest that reduction of NAc Arc in D2-MSNs of male mice recapitulates behavioral phenotypes observed following cell type-independent Arc knockdown in males. Ongoing studies in the D1-tdTomato/D2-EGFP mice will help determine if Arc expression and/or induction differences in male mice help to explain our cell type-specific behavioral effects.

Conclusions: These findings demonstrate sex differences in the induction and requirement for Arc in NAc-relevant behaviors. They also elucidate a role for Arc in D2-MSNs in anxiety-like behavior, specifically in males. Future work will examine the relationship between sex hormones and Arc expression, circuit-level sex differences following anxiety-like behavior, and further examinations of cell type-specific localization and action of Arc. Together, this work will extend our understanding of a potential molecular mechanism of sex differences in NAc-related behavior and further our understanding of how Arc action influences NAc function in behaviorally relevant contexts.

Keywords: Sex Difference, Nucleus Accumbens, Activity-Regulated Cytoskeleton-Associated Protein

Disclosure: Nothing to disclose.

W56. Molecular Neurobiology of Enrichment Loss: Multi-Omics Analysis Reveals a Role of Microglia

Marissa Smail*, Brittany Smith, Rammohan Shukla, James Reigle, Khaled Alganem, James Herman, Robert McCullumsmith

College of Medicine, University of Cincinnati, Cincinnati, Ohio, United States

Background: Psychological loss impacts nearly all of our lives, yet little is known about what happens in the brain during this experience. Loss, which is a negative valence construct in the Research Domain Criteria, occurs when one is deprived of something perceived as important, such as a valued relationship, home, or job, and its symptomology resembles atypical depression. Given the common occurrence of loss-precipitating events and the fact that that loss can precipitate depression, there is an urgent need to uncover the mechanisms underlying loss and identify potential therapeutic targets. Our laboratory can simulate loss in rats by removing environmental enrichment, producing unique behavioral and physiological phenotypes that resemble loss in humans. Here we probe the molecular neurobiology of enrichment loss with a multidimensional approach that spans big data techniques, brain regions, and sex.

Methods: Male and female rats were divided into 3 groups: environmentally enriched (EE), enrichment removal (ER), and control (CON). EE and ER animals were housed in groups of 10 in large, multi-level cages with toys. CON animals were pair-housed. ER animals were removed from enriched housing after 4 weeks and moved to single-housing. Two weeks after removal, brains were collected. Bilateral micropunches were taken of regions implicated in loss by c-fos expression, including the infralimbic prefrontal cortex, basolateral amygdala (BLA), and medial amygdala. These regions were run on parallel RNAseq, shotgun proteomics, and kinomics platforms. A series of bioinformatics analyses were then used to derive molecular signatures of loss that span RNA, protein, and kinase activity levels. Tools used include GSEA, iLINCS, OmiClust, and KRSA. The resulting multi-level signatures yield in-depth information about the proteins and pathways that are altered in loss.

Results: ER rats exhibited differential expression in several pathways that may contribute to loss phenotypes. Immune signaling, MAPK activity, and cell-matrix communication were consistently dysregulated in the BLA of ER males. Many of the genes driving these pathways were predominantly expressed in microglia, and these results were supported across RNA, protein, and kinase levels. Given that the BLA is a positive mediator of stress, impaired microglial regulation of neurons in this region could permit increased anxiogenic signaling, contributing to ER behavioral and physiological phenotypes. We have also identified signaling hubs in these processes that represent intriguing candidates for ameliorating ER phenotypes. Further analyses will

continue to expand these signatures of enrichment loss across brain regions and sex.

Conclusions: Taken together, these results allow us to start to understand what is happening in a rat's brain during enrichment loss, offering insight into the mechanisms that could underlie psychological loss in humans. They also point to potential therapeutic targets that may offer relief to people experiencing loss and have broader applications in depression.

Keywords: Bioinformatics, Environmental Enrichment, Loss, RNAseq, Multi-omic

Disclosure: Nothing to disclose.

W57. Dysregulation of Mitochondrial Dynamics, Mitophagy and Apoptosis in Major Depressive Disorder: Does Inflammation Play a Role?

Giselli Scaini*, Alexandre Paim Diaz, Brittany Mason, Manish Jha, Jair Soares, Madhukar Trivedi, Joao de Quevedo

University of Texas Health Science Center at Houston, Houston, Texas, United States

Background: Recent studies have suggested that mitochondrial dysfunction and dysregulated neuroinflammatory pathways are involved in the pathophysiology of major depressive disorder (MDD). Thus, in this study, we tested the hypotheses that the changes in mitochondrial morphology and number are associated with an imbalance in the mitochondrial dynamics and mitophagy in MDD patients. We assessed differences in molecular markers of mitochondrial dynamics, mitophagy, general autophagy, and apoptosis in peripheral blood mononuclear cells (PBMCs) of MDD patients and healthy controls (HCs). Moreover, we studied inflammation engagement as a moderator of mitochondria dysfunctions on the severity of depressive symptoms.

Methods: Our sample included 24 healthy controls (HC; 39.45 ± 2.60 years; 16 females), and 84 MDD (44.91 ± 1.48 years; 59 females). PBMCs were separated using LeucoPREP brand cell separation tubes, and Immunoblotting and Multi-Plex interrogated the MQC, autophagy, and intrinsic apoptotic-related proteins.

Results: One-Way ANCOVA after controlling for age, gender, ethnicity, BMI, smoking status, and antidepressant treatment showed that the protein levels of the mitochondrial fusion-related protein Mfn-2 and fission-related protein Fis-1 were increased in MDD patients when compared to HCs. Moreover, we observed a lower ratio of long to a short isoform of Opa-1 in PBMCs from patients with MDD, suggesting that mitochondrial morphology was shifted toward a fragmented network. We also found that MDD patients had higher levels of Pink-1, p62/SQSTM1, LC3B, and caspase-3 active compared to HCs. On the other hand, our study demonstrated lower levels of Parkin in MDD patients. Another notable finding was that CRP levels were a significant predictor of higher levels of Mfn-2, Pink-1, and LC3B levels, and this relationship persisted when depressive symptoms were controlled for. Moreover, MDD patients with low CRP levels, Opa-1 levels contribute to the depression severity. However, in MDD patients with higher CRP levels, Mfn-2 levels were a significant predictor of depression severity.

Conclusions: Overall, our study demonstrated that mitochondrial fragmentation caused by a disruption in mitochondrial fusion could initiate a cascade of abnormal changes relevant to the critical pathological changes during the course of MDD and lead to progression of the disease, and poor outcomes. Moreover, the subtle regulation of mitochondrial quality control network during disease progression may be a possible therapeutic strategy to improve treatment response and disease progression.

Keywords: Major Depression Disorder, Mitochondria, Mitochondrial Dynamics, Mitophagy, Inflammation

Disclosure: Nothing to disclose.

W58. Association Between Epigenetic Aging Acceleration and Oxidative Stress in Bipolar Disorder

Camila Nayane de Carvalho Lima, Jair Soares, Joao de Quevedo, Gabriel R. Fries*

University of Texas Health Science Center at Houston, Houston, Texas, United States

Background: Bipolar disorder (BD) has been associated with many signs of accelerated aging, early-onset dementia, and a higher incidence of age-related conditions. As a marker of biological aging predicted based on genome-wide DNA methylation levels, we have recently reported on alterations in epigenetic aging in BD patients compared to controls, although the mechanisms underlying this acceleration are unknown. Of note, aging has been repeatedly linked to an accumulation of molecular oxidative damage (free radical theory of aging), and the methylation of numerous blood CpGs sites has been correlated with markers of oxidative stress. Moreover, BD has been associated with oxidative stress. Thus, we aimed to investigate the cross-talk between oxidative stress markers and epigenetic aging in a sample of patients with BD and controls.

Methods: Euthymic BD patients ($n = 93$) and healthy controls ($n = 40$) matched for chronological age, sex, ethnicity, and body mass index were enrolled for this analysis. Peripheral blood samples were analyzed for genome-wide DNA methylation levels using the EPIC BeadChip (Illumina) and assessed for epigenetic age and intrinsic epigenetic age acceleration (IEAA) using the Horvath calculator. Oxidative stress was measured with commercial kits and included the following markers: 8-oxo-2′-deoxyguanosine (8-oxo-dG), thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCC), and total antioxidant capacity. We compared groups showing slower (IEAA < 0) or accelerated aging (IEAA > 0) using univariate analyses. Linear regressions and binary logistic regressions were also performed to check for the combined effects of the markers and covariates on the likelihood that participants would show a slower or accelerated epigenetic aging.

Results: Oxidative stress markers were not significantly different between aging acceleration groups within patients or controls ($p>0.05$ for all comparisons). In a multiple regression, TBARS, PCC, antioxidant capacity, and 8-oxo-dG significantly predicted epigenetic aging acceleration in the whole sample ($F(4,89) = 3.227$, $p = 0.016$, $R2 = 0.127$), but not when the analysis was performed within each group (controls – $F(4,26) = 1.766$, $p = 0.166$, $R2 = 0.214$; patients – $F(4,58) = 1.344$, $p = 0.265$, $R2 = 0.085$). Similarly, binary logistic regression models were not statistically significant in controls ($X2(6)=8.956$, $p=0.176$) or in patients ($X2(7)=3.502$, $p=0.835$).

Conclusions: We did not find major influences of oxidative stress on epigenetic aging acceleration in patients or controls, although the combination of markers was able to predict epigenetic aging acceleration when taking the whole sample (patients and controls combined) into account. These results are supported by studies showing that epigenetic aging may be accelerated by chronic exposure to stress, inflammation, and many other aging-inducing stimuli, which may not have been fully captured by the acute assessment of oxidative stress performed in this study. Replication analyses with a longitudinal design and with the incorporation of other biological aging markers are warranted.

Keywords: Bipolar Disorder, Oxidative Stress, Aging, DNA Methylation

Disclosure: Nothing to disclose.

W59. Evaluate the Effects of Pimavanserin on Anxious Depression in Patients With Major Depressive Disorder: Secondary Analysis of the Clarity Study

Abstract not included.

W60. Mechanism of Action and Safety of Seltorexant as a Monotherapy for Patients With Major Depressive Disorder: A Multicenter, Placebo-Controlled, Randomized, Double-Blind, Phase 1 Study

Adam Savitz*, Sofie Mesens, Iva Kezic, Peter Van Der Ark, Mila Etropolski, Wayne Drevets, Peter De Boer

Janssen Research & Development, LLC, Titusville, New Jersey, United States

Background: Major depressive disorder (MDD) is a common, serious, recurrent disorder, with worldwide lifetime prevalence estimates ranging from 10-15% in most countries. Seltorexant is a selective orexin-2 receptor antagonist that is being developed as an adjunctive treatment for MDD. This phase 1b study was designed as a monotherapy study to characterize the antidepressant mechanism of action of seltorexant in patients with MDD not currently treated with an antidepressant drug.

Methods: A multicenter, placebo-controlled, randomized, double-blind (DB) study assessed the mechanism of action and safety of seltorexant in MDD patients currently not receiving antidepressant therapy. The study consisted of a screening phase (of up to 3 weeks), a DB treatment phase (lasting 8 weeks), and a follow-up phase (1 week). The DB treatment phase consisted of 3 periods, a DB placebo lead-in period, a DB treatment period (5 weeks), and a DB withdrawal period. Patients were randomized 1:1:1 to 20- or 40-mg seltorexant or placebo, and randomization was stratified by placebo response status and absence/presence of subjective sleep disorder. Investigators and patients were blinded to the duration of the lead-in and withdrawal period and to the lead-in response criteria

All efficacy analyses were based on the intent-to-treat analysis set from the enriched population (eITT) which consists of randomized lead-in placebo non-responders (< 30% reduction from baseline score in HDRS17 at the end of the lead-in period and who received ≥ 1 dose of study medication and had ≥ 1 post-baseline HDRS17 assessment during the treatment period).

The primary objective was to determine the magnitude of treatment effect (seltorexant, placebo) on symptoms of depression (measured by Hamilton Rating Scale for Depression-17 [HDRS17] in the intent-to-treat analysis set from the eITT (placebo non-responders

Key efficacy variables included changes in HDRS17 total score from treatment baseline to Treatment Week 5. The changes in HDRS17 total score at treatment Week 5 were analyzed using a mixed-effects model for repeated measures (MMRM) model with subject as random effect, subjective sleep disorder (presence, absence), treatment, time and time-by-treatment interaction as factors and baseline HDRS17 total score as a continuous covariate. In addition, the changes in HDRS17 total score at treatment Week 5 were analyzed by subjective sleep disorder (presence, absence), by baseline insomnia severity index (ISI) score (≤ 15, > 15) and by baseline ruminative response scale [RRS] total score (< 50, ≥ 50) using an analysis of covariance model; HDRS17 remission rates over the treatment period (HDRS17 total score ≤ 7); HDRS17 response rates over the treatment period (≥ 30% and ≥ 50% improvement in HDRS17 total score from treatment baseline).

Results: A total of 128 patients with MDD were enrolled; 86 were placebo non-responders, 79 completed the study. The mean (SD) age was 39.4 (9.85) years, ranging from 19 to 55 years, and 74% of patients were male.

The primary endpoint analysis showed a significant positive treatment effect at Week 5 for seltorexant versus placebo at the one-sided 0.10 significance level (p=0.0456). The estimated least square (LS) mean differences with 80% CI at Treatment Week 5 between seltorexant and placebo were: -2.9 [-4.37, -1.48] and -0.9 [-2.32, 0.61], for 20- and 40-mg, respectively (p=0.0049 and 0.2271). Seltorexant 40 mg dose did not show a statistically significant effect at Week 5.

Seltorexant's efficacy increased in patients with an ISI > 15 at both the 20- and 40-mg dose level (-2.9 [-4.69, -1.02] and -1.9 [-3.66, -0.09], respectively), while showing less efficacy in patients with an ISI ≤ 15 (-1.4 [-4.39, 0.86] and +2.9 [-0.73, 5.49], respectively). In patients with more ruminations (RRS ≥ 50), both 20- and 40-mg dose levels showed increased efficacy (-5.1 [-7.04, -3.07] and -2.1 [-4.04, -0.15], respectively), but not in patients with low levels of rumination (RRS < 50) (+0.9 [-1.36, 3.12] and +0.5 [-1.95, 2.90], respectively).

The percentage of patients with ≥ 50% improvement in HDRS17 total score at Treatment Week 5 in the eITT population was 30.8% for 20 mg seltorexant, 29.2% for 40 mg seltorexant, and 10.3% for placebo.

Week 5 remission rates (HDRS17 ≤ 7) were comparable for 20- and 40-mg seltorexant (eITT: 26.9% and 29.2%) and higher than placebo (eITT: 13.8%).

Seltorexant showed a good safety and tolerability profile in MDD patients with no deaths or serious adverse events. The most common treatment-emergent adverse events in the seltorexant combined group during the treatment phase was headache (7.1% 20 mg, 14.6% 40mg 22.7% placebo) and nasopharyngitis (9.5% 20 mg, 12.2% 40mg, 6.8% placebo).

Conclusions: Statistically significant and clinically meaningful improvements in depressive symptoms were observed for seltorexant 20 mg versus placebo in MDD patients not treated with antidepressant therapy. These effects of monotherapy seltorexant treatment in MDD were more pronounced in patients with insomnia symptoms and/or higher rumination. No new safety signals were observed. Overall study results support the development of seltorexant in MDD.

Keywords: Major Depressive Disorder (MDD), Orexin Receptor Antagonist, Seltorexant, Clinical Trial

Disclosure: Stock Shareholder: Employee (Self)

W61. Low Doses of LSD Acutely Increase BDNF Blood Plasma Levels in Healthy Volunteers: Preliminary Findings

Nadia Hutten, Natasha Mason, Patrick Dolder, Eef Theunissen, Friederike Holze, Matthias Liechti, Nimmy Varghese, Anne Eckert, Amanda Fielding, Johannes Ramaekers, Kim Kuypers*

Maastricht University, Maastricht, Netherlands

Background: Preclinical research has demonstrated that psychedelic substances from different structural classes such as 2,5-dimethoxy-4-iodoamphetamine (DOI), lysergic acid diethylamide (LSD), N,N-dimethyltryptamine (DMT), and psilocybin affect neuroplasticity after acute and chronic administration. While, these effects were similar across psychedelic classes and the dissociative ketamine, LSD was most potent. Additional experiments with DMT also showed these morphological changes, even after the administration of low DMT doses that are considered to be sub-hallucinogenic. The latter is important in light of the increased scientific interest in using low psychedelic doses, also known as 'microdosing'. Despite preclinical evidence for psychedelic-induced neuroplasticity, confirmation in humans is grossly lacking. Given the interest in brain-derived neurotrophic factor (BDNF) as a key player in several neurodegenerative and

neuropsychiatric disorders, and the preclinical data showing psychedelics-induced neuroplasticity, even in low doses of psychedelics, the present, double-blind placebo-controlled, within-subjects study aimed to investigate whether LSD in low doses (0, 5, 10, and 20 mcg) affects BDNF plasma levels in healthy volunteers.

Methods: Participants were recreational psychedelic users (N=24, both sexes) who provided informed consent, fell within the inclusion criteria, and passed medical screening including standard blood chemistry, hematology, and urinalysis, prior to inclusion. Test days were scheduled with minimally five days in between. LSD (5, 10, and 20 mcg LSD base, dissolved in 96% Vol ethanol) or placebo (1 mL of ethanol) was administered orally in the morning (10:00 AM). Blood samples were collected every two hours over a six-hour period, and BDNF levels were determined afterwards in blood plasma using ELISA. Difficulties with the peripheral venous catheter during blood sample collection resulted in missing data. Four trapezoidal areas under the curve (AUC) were calculated for the three LSD doses and placebo.

The study adhered to the code of ethics on human experimentation, it was approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University, and registered in the Dutch Clinical Trial register (number: NTR7102 https://www.trialregister.nl/). A permit for obtaining, storing, and administering LSD was obtained from the Dutch Drug Enforcement Administration.

Complete within-subjects cases (N(5 mcg)=10; N(10 mcg)= 9; N (20 mcg)=8) entered statistical analyses. Non-parametric Wilcoxon signed-rank (S-R) tests for related samples (placebo versus LSD dose) were conducted on BDNF AUC's, and on BDNF plasma levels at -0.5h, +2h, +4h and +6h after dose administration of 5, 10 and 20 mcg LSD. Separate Friedman tests per treatment condition were performed to test at which timepoint LSD differed statistically from placebo. In case of a main effect Dunn's tests for pairwise comparisons including baseline versus 2h, 4h, and 6h, 2h-4h, and 4h-6h were conducted. Effect sizes and 95% confidence intervals (95% CI) are given in case of statistically significant effects at alpha= 0.05. The alpha level was corrected for multiple comparisons with sequential Bonferroni in case of Dunn's tests.

Results: Wilcoxon Signed-Rank (S-R) tests revealed a statistically significant difference between AUC BDNF levels following 5 (Z= -2.60, p= 0.009, r= 0.58, 95% CI [0.11-1.06]) and 20 mcg LSD (Z= -2.52, p= 0.01, r= 0.63, 95% CI [0.09-1.17]) compared to placebo; the difference between AUC BDNF levels after 10 mcg LSD and placebo was not significant (Z= -1.01, p=0.31). Wilcoxon S-R tests revealed higher BDNF levels at +4h after administration of 5 (Z= -2.80, p< 0.01, r= 0.63, 95% CI [0.15-1.11]) and 10 mcg LSD (Z= -1.95, p= 0.05, r= 0.46, 95% [-0.04-0.97]) compared to placebo. The latter CI included zero, indicating non-significance. Tests at +6h after LSD administration revealed significant effects of 5 (Z= -2.29, p= 0.02, r= 0.51, 95% CI [0.03-0.99]) and 20 mcg (Z= -2.52, p= 0.01, r= 0.63, 95% CI [0.09-1.17]) LSD compared to placebo.

Friedman tests investigating BDNF changes in function of time demonstrated that BDNF plasma levels remained stable in the placebo conditions throughout the test frame. BDNF plasma levels in the LSD conditions showed their highest levels at four hours after administration of 5 mcg LSD (8.95 ng/mL), and at 6 hours after administration of 10 mcg (8.28 ng/mL) and 20 mcg of LSD (11 49 ng/mL).

Conclusions: This study provides preliminary evidence that low doses of LSD increase BDNF plasma levels in healthy volunteers up to 6 hours after administration, suggesting a window of opportunity for therapeutic response and/or and cognitive enhancement that might be of use in patient populations. This line of thinking is supported by recent findings with ketamine and ayahuasca (containing the psychedelic DMT) demonstrating increased serum BDNF levels respectively 24 and 48 hours after a single dose, compared to placebo, which was related to fast antidepressant actions. Besides emphasizing the need to sample BDNF beyond the elimination stage, in addition to including behavioral and/or imaging measures, future studies could focus on similarities between underlying biological pathways of the well-studied ketamine, and LSD, as it will contribute to understanding the scope of effects LSD, might have, based on ketamine findings.

Keywords: LSD Microdosing, Neuroplasticity, BDNF

Disclosure: Nothing to disclose.

W62. Effect of Esketamine Nasal Spray on Depressive Symptoms in Adults With Severe Major Depressive Disorder: A Post Hoc Analysis of the ASPIRE I and ASPIRE II Studies

Abigail I. Nash*, Ibrahim Turkoz, Dong-Jing Fu, John Verbanac, Lew Lanera

Janssen Scientific Affairs, LLC, Titusville, New Jersey, United States

Background: ASPIRE I and ASPIRE II were 2 identically designed, global, double-blind, placebo-controlled phase 3 studies (NCT03039192, NCT03097133) designed to evaluate the efficacy and safety of esketamine (ESK) nasal spray vs placebo (PBO) nasal spray, given in the context of comprehensive standard of care (SoC; ie, hospitalization, initiation, or optimization of antidepressant therapy), in adults with major depressive disorder (MDD) who had active suicidal ideation with intent. These studies were the basis for the recent approval of ESK for the treatment of depressive symptoms in adults with MDD with acute suicidal ideation or behavior. To further understand the rapid effect of ESK +SoC vs PBO+SoC on depressive symptoms in patients with severe depression at baseline, we conducted a post hoc analysis of pooled data from ASPIRE I and ASPIRE II to examine changes in Montgomery-Åsberg Depression Rating Scale (MADRS) total scores and Clinical Global Impression-Severity of Suicidality–Revised (CGI-SS-r) scores after ESK treatment.

Methods: The ASPIRE I and II trials enrolled adults aged 18-64 years with MDD confirmed by the Mini International Neuropsychiatric Interview and a minimum total MADRS score of >28 at baseline. Patients were randomized 1:1 to ESK 84 mg or PBO nasal spray twice weekly plus comprehensive SoC antidepressant treatment for 4 weeks. For this post hoc analysis, a subset of trial patients was identified as having severe depression per baseline total MADRS score >34 (Snaith Br J Psychiatry 1986). Change in MADRS total score was calculated from baseline to 4 hours post–first dose, 24 hours post–first dose, and predose on day 25 (ie, last day of double-blind treatment phase). Differences between least squares mean (LSM) changes from baseline between groups in MADRS total score were examined using a mixed model for repeated measures. Percentages of subjects achieving response (ie, change in MADRS total score ≥50%) and remission (ie, MADRS total score ≤12) were also assessed using Cochran-Mantel-Haenszel tests. Generalized estimation equations were used to determine the relative odds of achieving a clinically meaningful improvement on the CGI-SS-r (≥1-point decrease) scale with ESK+SoC vs PBO+SoC. A multiplicity adjustment procedure was not carried out.

Results: Of 450 patients enrolled in the trials, 370 (82.2%) had severe MDD at baseline, as defined by MADRS score, and were included in this post hoc analysis (n=189, ESK+SoC; n=181, PBO +SoC). Demographic and psychiatric characteristics were similar between ESK+SoC and PBO+SoC groups at baseline (mean age: 40.3; 61.4% female). Mean (SD) MADRS total scores were 41.9 (4.62) and 42.5 (4.64), respectively. The ESK+SoC group had significantly greater decreases from baseline in MADRS total score vs the PBO+SoC group at 4 hours post–first dose (−12.6 vs −8.6; LSM difference [95% CI] −4.0 [−5.9, −2.1]; P<0.001), at 24 hours

post–first dose (–16.4 vs –11.7; LSM difference [95% CI] –4.7 [–6.9, –2.5]; P<0.001), and on day 25 (–26.0 vs –22.8; LSM difference [95% CI] –3.2 [–5.8, –0.7]; P=0.013). Significantly more patients in the ESK+SoC group vs PBO+SoC group attained response at 4 hours post–first dose (24.5% vs 11.1%; P<0.001), at 24 hours post–first dose (34.8% vs 22.4%; P=0.009), and on day 25 (76.7% vs 58.3%; P<0.001). The percentages of patients who attained remission were also greater in the ESK+SoC group vs PBO+SoC group at 4 hours post–first dose (8.5% vs 2.8%; P=0.017), at 24 hours post–first dose (18.2% vs 6.7%; P<0.001), and on day 25 (51.3% vs 36.1%; P=0.008). Patients in the ESK+SoC group were 78% more likely to experience a ≥1-point decrease in CGI-SS-r score at 4 hours post–first dose (odds ratio [95% CI] 1.78 [1.15, 2.74]; P=0.009) and 99% more likely at 24 hours post–first dose (odds ratio [95% CI] 199 [1.24, 3.20]; P=0.005] than patients in the PBO+SoC group.

Conclusions: Similar to findings from the ASPIRE global phase 3 clinical trial program to assess efficacy of ESK+SoC vs PBO+SoC in reducing depressive symptoms in adults with moderate to severe MDD and suicidal ideation with intent, patients with severe MDD, as defined by MADRS total score >34, demonstrated significant improvement in depressive symptoms when treated with ESK +SoC compared with PBO+SoC as early as 4 hours after the first ESK dose. Improvements in depressive symptoms continued in favor of ESK+SoC treatment through study end of the double-blind treatment phase. Additional analyses will be conducted to assess efficacy outcomes in patients with severe MDD defined through other validated instruments. ESK+SoC may provide an effective treatment of depressive symptoms with continued significant benefit for this population with challenging-to-treat depression.

Reference: Snaith RP et al. Br J Psychiatry 1986;148:599-601.

Keywords: Esketamine Nasal Spray, Severe Major Depressive Disorder, Patient Outcomes

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

W63. Transcranial Magnetic Stimulation in US Military Veterans – A Naturalistic Study in the Veterans Health Administration

Noah Philip*, Michelle Madore, Frank Kozel

Brown University & Providence VA Medical Center, Providence, Rhode Island, United States

Background: Repetitive transcranial magnetic stimulation (rTMS, hereafter TMS) has emerged as evidence-based treatment for pharmacoresistant major depressive disorder (MDD) and has been cleared by the US Food and Drug Administration for over a decade. In that time, data has emerged supporting the use of TMS utilizing different devices, settings, and patient populations. One particular patient population of interest in the US are military Veterans; this patient group is thought to have generally higher levels of medical and psychiatric comorbidity, as well as elevated risk for suicide. A recent negative study of TMS indicated that active stimulation did not separate from sham, and poor response was particularly prevalent in Veterans who had comorbid posttraumatic stress disorder. Yet, other studies have consistently indicated efficacy of TMS for Veterans including with PTSD in other studies. To this end, the VA instituted a nationwide rollout of TMS, with the purpose to evaluate clinical outcomes in naturalistic settings and thus inform real-world practice. This abstract reports clinical outcomes of the first cohort of patients to receive TMS at the VA as part of this program, where we hypothesized that TMS would be safe, well tolerated, and provide clinically meaningful reductions in depressive symptoms as well as comorbid PTSD symptoms.

Methods: VA Palo Alto Healthcare System Mental Illness Research Education and Clinical Center served as the central coordinating site for this naturalistic study; the VA Palo Alto/ Stanford Institutional Review Board approved procedures related to this report. Individual sites received training through the coordinating site and data gathered through a centralized VA REDCap database. Veterans were eligible for TMS if they met standard inclusion/clearance criteria for TMS (e.g., failure of at least one antidepressant in the current major depressive episode, etc.); in general other treatments were unchanged for approximately six weeks before stimulation, and remained unchanged during stimulation, although this could be modified depending on a Veteran's clinical situation. TMS parameters were at local clinical discretion, and generally were high frequency stimulation (10Hz or intermittent theta burst) delivered to the left dorsolateral prefrontal cortex at 120% of motor threshold over 6-8 weeks. Depression and PTSD symptoms were measured using the 9-item patient health questionnaire (PHQ9) and PTSD symptom checklist for DSM5 (PCL-5); outcomes of interest included mean changes in both rating scales, as well as dichotomous outcomes describing response (>50% reduction) and remission (score<5) on the PHQ9, and clinically meaningful reduction (>10 points) and those no longer meeting threshold criteria for PTSD (score <33) on the PCL-5. Safety outcomes were measured through database queries and separate inquiries across the sites. Outcomes were analyzed using paired-sample t-tests and missing data were addressed using multiple imputations. This report includes all Veterans that received TMS up until March 2020 (i.e., pre-COVID).

Results: The cohort included N=413 Veterans with major depression. 380 (92%) reported some degree of PTSD symptoms, and 275 (66.6%) had threshold-level PTSD symptoms. Average age was 51 years (SD = 14.14, range 22 – 89), and 74.2 % identified as male. The majority of Veterans were treated with high frequency stimulation to the left dorsolateral prefrontal cortex. There was a significant decrease in self-reported depression symptoms on the PHQ9 ($t(412) = 24.15$, $p < 0.01$; pre-TMS: mean = 18.2, standard deviation = 5.06, post-TMS: mean = 10.66, standard deviation = 7.12). Depression response and remission rates were 46.2% (n=191) and 23.7% (n=98), respectively. There was also a significant decrease on self-reported PTSD symptoms on the PCL-5 ($t(379) = 18.85$, $p < 0.01$; pre-TMS : mean = 43.80 SD = 18.36, post-TMS: M = 28.15, SD = 20.42). Of the Veterans with threshold-level PTSD symptoms at baseline, n=159 (57.5%) demonstrated clinically meaningful reduction and n=130 (47.2%) no longer met PTSD threshold criteria post-treatment. TMS was generally safe as delivered; four Veterans experienced unexpected side effects requiring significant medical intervention. One Veteran reportedly had a seizure (H-coil), and five were hospitalized for worsened psychiatric symptoms.

Conclusions: This data represents the first multisite naturalistic study of TMS for Veterans with pharmacoresistant major depression. Consistent with this patient population, the majority reported comorbid MDD & PTSD. TMS resulted in statistically significant and clinically meaningful improvements in depressive and PTSD symptoms. Stimulation was safe as provided, with side effects consistent with the known profile of TMS. Limitations include those inherent to naturalistic cohort studies. Furthermore, all Veterans were engaged in concurrent clinical care, and we did not differentiate between different stimulation parameters. Future reports, using the associated network of clinical sites, will focus on key related questions including evaluating the impact of different TMS parameters or approaches, as well as the study of biological outcomes such as functional magnetic resonance imaging and electroencephalography to better identify biomarkers of treatment response in this population. Caveats notwithstanding, this is the largest report of TMS in Veterans and provides a clear indication of efficacy and safety in real-world environments.

Keywords: PTSD, Depression, rTMS, Prefrontal Cortex, Veterans, Repetitive Transcranial Magnetic Stimulation (rTMS)

Disclosure: Nothing to disclose.

W64. Real Time Cloud-Based fMRI Neurofeedback Reduces the Negative Attentional Bias in Depression: A Proof-Of-Concept Study

Anne Mennen, Nicholas Turk-Brownes, Grant Wallace, Darsol Seok, Adna Jaganjac, Janet Stoct, Megan deBettencourt, Johnathan Cohen, Kenneth Norman, Yvette Sheline*

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Depressed individuals show an attentional bias toward negatively valenced stimuli and thoughts and have difficulty disengaging attention away from negative stimuli. They tend to have larger, more prolonged neural responses to negative stimuli. Clinically they have ruminative thoughts, automatic replay of negative thoughts, catastrophizing and more generally, an inability to exercise cognitive control over negative stimuli. Paradigms that have been used to train participants specifically to reduce negative bias include Attention Bias Modification training and Cognitive Bias Modification for Interpretation. Clinically, cognitive behavior therapy incorporates exercises to train patients using reappraisal to modify cognitive biases. In this proof-of-concept study, we present a novel closed-loop neurofeedback procedure that seeks to remediate this negative bias. Internal attentional states were detected by applying machine learning techniques to fMRI data in real-time, and externalized using a visually presented stimulus that the participant could learn to control.

Methods: We trained 15 depressed (MDD; n female = 8, mean age = 27.3 years) and 12 healthy control (HC; n female = 6, mean age = 25.4 years). Participants were unmedicated, R handed, English speaking consenting adults recruited from the University of Pennsylvania Center for Neuromodulation in Depression and Stress (CNDS) laboratory. MDD participants underwent SCID screening and scored >16 on the Montgomery-Åsberg Depression Rating Scale (MADRS) clinical interview during Visit 1; HC had no lifetime MDD and scored <8 on MADRS. Participants were trained over three fMRI sessions on a 3T Siemens Prisma scanner, preceded and followed by behavioral and clinical assessments. Participants completed 7–9 neurofeedback runs per visit. Each neurofeedback run contained 8 blocks: the first 4 blocks ("stable blocks") showed only neutral stimuli with constant opacity and served as training data for the face versus scene classifier; in the last 4 blocks, the attended category was neutral scenes and the distractor category was negative faces. These blocks served as neurofeedback blocks: opacity changed depending on the relative degree of neural representation of scenes versus faces as indicated by a pattern classifier applied to fMRI. Participants were told that change in opacity was determined by their brain activity rather than button-pressing accuracy. During neurofeedback, a realtime cloud-based multi-voxel pattern classifier was used to decode the extent to which attention was directed at the task-relevant scene or the task-irrelevant face for every time point of image acquisition (TR). The classifier model was re-trained for each neurofeedback run based on the most recent eight stable blocks from before the current run. During neurofeedback, the difference between the amount of classifier evidence for scenes (ranging from 0 to 1) and faces (ranging from 0 to 1) was used as the output neurofeedback score. This score was saved as a text file and sent back from the cloud to the local computer to influence the display during the following time point. The neurofeedback score was converted to an opacity value for the neutral scene using a sigmoidal transfer function (the more participants attended to the negative faces vs. neutral scenes, the more visible the negative faces became). We quantified the extent to which attentional states persisted over time by first dividing the continuous range of neurofeedback scores into discrete bins, or attentional states. Then, we operationalized persistence in a state as the conditional probability that the scene minus face difference remained in the same bin across time points.

Results: MADRS scores decreased significantly from pre-training to post-training (one-tailed $t(14) = 3.61$, $p = 0.0014$), to the one-month follow-up visit (one-tailed $t(13) = 2.85$, $p = 0.0069$), and to the three-month follow-up (one-tailed $t(12) = 3.43$, $p = 0.0025$). At the start of neurofeedback training, the largest difference between groups in the persistence of attentional states occurred for the most negative state (i.e., the state with the highest attention to the negative face and the greatest face visibility), with MDD participants showing a greater tendency to get stuck in this state (one-tailed $t(24) = 2.80$, $p = 0.0049$). At the end of neurofeedback training, MDD participants were marginally less likely to get stuck in the most negative state, compared to the start of neurofeedback training (paired one-tailed $t(13) = 1.67$, $p = 0.059$). Additionally, there was a significant interaction between group and visit, such that the MDD group decreased in their probability of getting stuck in the most negative state from early to late in training relative to the HC group (unpaired one-tailed t-test comparing change in MDD group to change in HC group, $t(24) = 2.04$, $p = 0.026$).

Conclusions: This initial proof-of-concept study highlights the practicality and potential clinical benefits of real-time fMRI neurofeedback procedures that target specific cognitive states. By tracking sustained attention over time, our technique provides a face-valid way of detecting the difficulties that MDD patients experience in getting "stuck" in negative states. These results demonstrate that our method is sensitive to the negative attentional bias in depressed individuals and showcase the potential of this novel technique as a treatment that can be evaluated in future clinical trials.

Keywords: Real-Time fMRI, Depression, MRI

Disclosure: Nothing to disclose.

W65. BI 1358894 Leads to Significant Attenuation of Brain Activity in the Cortico-Limbic System as Shown by Task-Based BOLD-fMRI Using Emotional Paradigms in Patients with MDD

Simone Grimm, Christian Keicher, Christian Paret, Inga Niedtfeld, Christian Beckmann, Maarten Mennes, Stefan Just, Vikas Sharma, René Fuertig, Lena Herich, Salome Mack, Claus Thamer, Christian Schultheis, Anne Weigand*, Christian Schmahl, Andreas Wunder

MSB Medical School Berlin, Berlin, Germany

Background: BI1358894 is a small-molecule inhibitor of transient receptor potential cation channel subfamily C (TRPC) being developed for the treatment of major depressive disorder (MDD) and borderline personality disorder (BoPD). Abnormal emotional processing is a key feature in both diseases.

Methods: To investigate the effects of BI1358894 and the antidepressant Citalopram as a control on acute neural responses elicited by emotional faces and scenes, functional magnetic resonance imaging measurements (BOLD-fMRI) were performed in a single dose, randomized, placebo-controlled phase I study in 73 MDD patients (male and female). Primary endpoints were BOLD signal changes during the faces task in key brain regions involved in emotional processing, i.e. bilateral amygdala (AMY), dorsolateral prefrontal cortex (DLPFC), insula (INS), and anterior cingulate cortex (ACC). Secondary endpoints were BOLD signal changes in these regions based on the scenes task. For each region, ANOVA models were computed for the comparison of treatments (BI 1358894 and Citalopram) vs. placebo.

Results: In the faces task, BI1358894 induced signal reduction in the left and right AMY (effect=-0.08 each, p-value=0.011 and 0.013) as well as in the left INS (effect=-0.08, p-value=0.042). Citalopram showed a trend in signal reduction only in the right AMY (effect= -0.07, p-value=0.078). Both compounds did not significantly alter BOLD signals during this task in other brain areas. In the scenes task, BI1358894 was associated with broad and robust circuit engagement in bilateral AMY, as well as in additional brain areas (left DLPFC, bilateral INS, and bilateral ACC). Citalopram did not significantly attenuate the BOLD signal in this task.

Conclusions: In conclusion, results demonstrate that BI1358894 reduced brain activation in several brain regions involved in emotional processing, regardless of the task. The broadened circuit modulation through BI1358894 supports the further clinical development in patients with MDD and BoPD in future studies. This study is listed in clinicaltrials.gov. under NCT03854578.

Keywords: Antidepressant, Emotion Processing, Emotion Modulation

Disclosure: Nothing to disclose.

W66. Ventral-Hippocampal Afferents to Nucleus Accumbens Encode Both Latent Vulnerability and Stress-Induced Susceptibility

Abstract not included.

W67. Symptoms and Quality of Life in Depression: Characterizing Differential Profiles of Intrinsic Reward Circuit Connectivity Underlying Antidepressant Treatment Response

Adina Fischer*, Bailey Holt-Gosselin, Scott Fleming, Laura Hack, Tali Ball, Alan Schatzberg, Leanne Williams

Stanford University, Stanford, California, United States

Background: Up to 50% of patients with major depressive disorder (MDD) fail to respond to antidepressant medication (ADM) based upon traditionally defined and clinically-assessed symptoms. Moreover, the majority of individuals with MDD continue to experience impairment in function and quality of life that significantly contribute to morbidity and mortality in MDD regardless of ADM treatment response. Thus, the first premise of the current study was that it is important to consider both clinical response as assessed by change in symptoms as well as functional response as assessed by change in quality of life. A second premise was that in order to advance our understanding of the neural mechanisms by which some patients do not respond to ADMs, in regard to symptoms and/or quality of life, it is important to also undertake trials that incorporate direct measures of neural function such as with functional neuroimaging. While reward neurocircuitry profiles have been critically implicated in the underlying pathophysiology of MDD and in ADM treatment response, prior studies have focused on neurobiological markers of change in symptoms; however, the severity of those symptoms may not necessarily directly correlate with functional impairment or translate directly to public health costs. To address the current gap in knowledge, we examined change in intrinsic functional connectivity (FC) of reward circuitry with repeated neuroimaging scans to characterize how change in reward circuitry FC relates to both symptom and quality of life responses following ADM. We also investigated whether this relationship differs according to type of ADM. The International Study to Predict Optimized Treatment for Depression (iSPOT-D) is among the largest prospective ADM treatment trials to include repeated functional neuroimaging to examine underlying change in neural circuitry and the opportunity to assess change in reward circuit FC in relation to change in both symptoms (primary outcome measure) and in quality of life (secondary outcome measure).

Methods: Data from 128 patients with MDD were included in this analysis, which included functional neuroimaging acquired pre-treatment and following an 8-week course of ADM (n=42 sertraline, n=46 escitalopram, n=40 venlafaxine-XR). Given that impairment in reward function is implicated in both clinical symptoms of MDD and diminished quality of life, we examined intrinsic FC of reward circuitry using bilateral nucleus accumbens (NAc) seed regions of interest and conducted whole-brain seed-to-voxel analyses (FDR-corrected cluster-level $p<0.05$ threshold). We examined pre-to-post treatment change in intrinsic FC of reward circuitry that distinguished (1) symptom responders from non-responders (defined as a 50% of greater improvement on the Hamilton Depression Rating Scale), and (2) functional responders versus non-responders in environmental, physical, social, and psychological domains of function (defined as a 25% or greater improvement on the World Health Organization Quality of Life scale). Lastly, we explored differential reward circuitry FC treatment response profiles with sertraline, venlafaxine-XR, and escitalopram. Regression modeling was used to test whether discovered associations remained significant after correcting for the effect of possible confounding covariates of age, gender, race, duration of illness, presence of co-occurring anxiety disorder, and equivalent medication dose.

Results: Symptom responders were characterized by a pre-to-post ADM increase in NAc-dorsal anterior cingulate cortex (ACC) FC relative to non-responders (voxel-level $p<0.001$), that was significantly associated with improvement in physical function (beta = 0.322, $p<0.0003$). By contrast, functional (i.e. quality of life) responders were characterized by a pre-to-post ADM increase in (1) NAc-ventral ACC FC for the environmental domain, (2) NAc-thalamus FC for the physical domain, and (3) NAc-paracingulate gyrus FC for the social domain (voxel-level $p<0.001$). Symptom responders to sertraline were also distinguished by decreased pre-to-post treatment NAc-insula FC relative to non-responders (voxel-level $p<0.001$), while symptom responders to venlafaxine-XR were distinguished by increased NAc-inferior temporal gyrus FC relative to non-responders (voxel-level $p<0.005$).

Conclusions: The present study is the first to examine whether change in reward circuit FC from pre-treatment to post-treatment with 8-weeks of antidepressant medication is associated with response in both symptoms and quality of life domains of function in depression. We identified unique profiles of reward circuit FC change pre-to-post treatment with ADM that distinguish symptom and quality of life response, particularly in environmental, physical and social domains of function. The findings highlight the promise of functional connectivity measures in delineating mechanisms of treatment-related change in neural circuitry that map onto quality of life outcomes that are directly relevant to burden of illness as well as traditionally defined clinical symptoms of depression. The inclusion of quality of life outcomes in neuroimaging trials also has the potential to derive more precise determinants of 'response' and thereby to develop more targeted and individualized treatments to alleviate debilitating symptoms and functional impairments that extend beyond clinical symptoms in depression.

Keywords: Major Depressive Disorder (MDD), Antidepressant Response, Intrinsic Functional Connectivity, Brain Imaging, fMRI, Reward Neural Circuitry

Disclosure: Nothing to disclose.

W68. Anterior Cingulate Cortex and Depressive Symptoms: A Structural MRI Study

Abstract not included.

W69. Current-Based Decomposition of Multi-Region Population Activity Using Data-Driven Recurrent Neural Network Models

Matthew G. Perich, Camille Spencer-Salmon, Tyler Benster, Karl Deisseroth, Kanaka Rajan*

Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: The behavior of even small animals is governed by a remarkably complex and interconnected circuitry of neurons with distinct anatomical organization, often separated into distinct "brain regions". Recent advances in technologies such as optical calcium imaging and high-density silicon electrophysiology probes have provided unprecedented access to large numbers of neurons from many brain regions simultaneously. However, identifying meaningful interactions between neurons at such a scale poses a challenge. Recent studies have begun to characterize the interactions between pairs of brain regions using analytical methods based on correlations or regression, but these methods are difficult to scale beyond two regions. In particular, they are unable to assign directionality to the interactions uncovered and cannot account for "common inputs" driving both regions simultaneously. CURBD (CURrent-Based Decomposition) is our unique approach to addressing this bottleneck.

Methods: CURBD employs recurrent neural networks (RNNs) directly constrained, from the outset, by time series measurements acquired experimentally, such as calcium imaging or electrophysiological data. Once trained, these data-constrained RNNs allow us to infer matrices quantifying the interactions between all pairs of modeled units. We call these "directed interaction matrices". Importantly, by constraining the RNNs to match the entire neural population's dynamics simultaneously, we can account for common inputs and assign both magnitude and directionality to all the interactions responsible for the observed dynamics. We can then use the model-inferred directed interaction matrices to separately compute excitatory and inhibitory input currents that drive a given neuron from all other neurons. Therefore, we can de-mix the different current sources - either within the same region or from other regions, brain-wide - which collectively give rise to the population dynamics observed experimentally. Source de-mixed currents obtained through CURBD therefore allow us an unprecedented view into brain-wide interactions inaccessible from measurements alone.

Results: First, we validated CURBD using simulated datasets from multiple interacting RNNs representing interconnected brain regions. We found that CURBD could effectively separate the activity of one RNN into its constituent input currents. We then applied the CURBD technique to understand the brain-wide flow of information leading to behavioral state transitions in larval zebrafish (data from an ongoing collaboration with Deisseroth lab, Stanford) [Andalman et al Cell, 2019]. The fish were exposed to a behavioral challenge protocol consisting of repeated, mild shocks. Initially, when shocked, the fish showed vigorous tail movements, indicating an active coping (adaptive) strategy. As the inescapable stressful stimuli persisted, fish entered into a passive coping (maladaptive) state characterized by a reduction in tail movements, reminiscent of learned helplessness in depression. We sought to characterize the multi-region neural dynamics underlying this transition in behavioral state. Throughout the experiment, whole-brain neural activity was recorded using two-photon calcium imaging. We applied CURBD to understand the input currents driving the habenula, a brain region found in previous work to be crucial for this behavioral state transition. We trained multi-region RNNs to replicate the data recorded from the habenula, as well as from neurons in the raphe nucleus and telencephalon. We found that immediately upon receiving shocks, habenula was driven by currents specifically from the raphe nucleus. Later, as the fish lapsed into passivity, there was a gradual ramping of currents from within the habenula and from the telencephalon. We then analyzed the directed interaction matrices inferred from the model, both within and across different regions, to understand how changes in synaptic connectivity could be driving the altered habenular currents. We found that during early shocks (active coping), no change in connectivity was observed, while specific changes emerged within the habenula and between the habenula and raphe during the transition to passive coping.

Conclusions: This work helps uncover the complex, multi-dimensional dynamics governing the interactions between brain regions to control behavior and guide transitions between brain states. Our results illustrate a possible switch in mechanisms between the two coping strategies - a faster mechanism mediated by immediate changes in input currents from the raphe, and a slower mechanism driven by changes in the magnitude and direction of the effective interactions within individual brain regions as well as inter-area projections. We are currently using this approach to decipher mechanisms from data collected in other animal models, e.g., mice and macaques, as well as from human patients. Taken together, our work could uncover unifying theories of minimal circuit motifs underlying inter-area communication in the brain.

Keywords: Neural Network Connectivity, Neural Modelling, Computational Neuroscience, Zebrafish, Diverse Animal Models

Disclosure: Nothing to disclose.

W70. Relations Among Individualized Brain Systems Patch Sizes and Diagnosis, Symptoms, and Treatment Outcomes in Major Depressive Disorder: Preliminary Results

Matthew Sacchet*, Diego Pizzagalli

McLean Hospital - Harvard Medical School, Belmont, Massachusetts, United States

Background: Major depressive disorder (MDD) is associated with the largest global burden of disease of any illness. Clearly continued research on MDD is warranted. Extant research has relied on group-level information to map individual brains. This group-level information is problematic as individual brains differ considerably in their functional organization. Advancing individualized brain mapping into a clinical framework will provide a significant advance for neural models of, and treatments for, MDD, and thus address important unmet needs. New developments in computational neuroimaging facilitate new precision in neural characterization at the person-specific level. Here, we describe preliminary results that link the size of specific nodes of large-scale functional brain systems that have been defined at the individual level – "individualized brain systems" – to MDD, including in relation to diagnosis, symptom severity, and treatment outcomes. Our investigation targeted brain systems previously implicated in MDD, including the affective and frontoparietal brain systems.

Methods: Adults diagnosed with MDD (N = 15) and healthy controls (HC; 9) were randomly identified (and matched based on age and gender) from the Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care for MDD (EMBARC) trial. 33 min of fMRI data per participant were analyzed. FMRI data were preprocessed using common approaches into MNI surface space and subsequently processed in a series of steps for the identification of individualized brain systems. These steps included systematically and iteratively labeling specific vertices according to their signal similarity with reference brain system signals computed for the entire brain system. Reference brain systems were initiated using a functional brain system atlas and were repeatedly increasingly refined by including additional information from person-specific fMRI data. Upon algorithmic

convergence, this resulted in individualized brain systems defined for each participant. The size of core nodes of the affective and frontoparietal brain systems were then computed as the number of region-specific adjacent (or close) vertices within a given brain system (i.e., brain system "patches"). Given the small sample size non-parametric statistical tests were used to compare patch sizes between groups (Wilcoxon rank sum test) and in relation to symptom severity and treatment-related symptom change (Spearman correlation). Symptom measures included anhedonia and anxious arousal as measured by the Mood and Anxiety Symptom Questionnaire [MASQ] Anhedonic Depression subscale and Anxious Arousal subscale, respectively. Symptom change as a result of sertraline (selective serotonin reuptake inhibitor [SSRI]) treatment was computed as baseline to 8-week change in scores of the Hamilton Rating Scale for Depression (HRSD) clinician interview.

Results: Our preliminary analyses suggest (1) smaller patch sizes of the frontoparietal brain system dorsolateral prefrontal cortex (DLPFC) in the MDD group compared to HC group ($r = 0.40$, $p = 0.053$); (2) negative relations between patch size of the affective brain system orbitofrontal cortex (OFC) and anhedonia ($r = -0.62$, 0.018); and that (3) response to sertraline is predicted by larger pre-treatment frontoparietal brain system DLPFC patch size ($r = -0.72$, 0.004).

Conclusions: The current study provides preliminary evidence that features of individualized brain systems relate to core clinical features of MDD including diagnosis, symptom severity, and treatment prediction. Specifically, we found relations between diagnosis and patch size of frontoparietal brain system DLPFC, anhedonia and patch sizes of the affective brain system OFC, and outcomes to treatment with sertraline and patch sizes of the frontoparietal brain system DLPFC. These regions have been previously linked to cognitive and emotional processes in MDD and the current results provide new evidence for their role in depressive psychopathology. In conclusion, the results of the current study provide promising evidence that individualized brain system features will inform new neural models of MDD and ultimately contribute to a brain-based mechanistic understanding of this condition that directly informs its treatment.

Keywords: Major Depressive Disorder (MDD), Human Neuroimaging, Computational Psychiatry, Individual Differences, Treatment Prediction

Disclosure: Nothing to disclose.

W71. Machine Learning Prediction of Treatment Response in Late-Life Depression

Adrienne Grzenda*, William Speier, Prabha Siddarth, Helen Lavretsky

David Geffen School of Medicine at UCLA, Los Angeles, California, United States

Background: Prediction of treatment response in late-life depression is fraught with difficulties. Numerous studies have evaluated potential predictive biomarkers—from clinical characteristics to genotypes—with variable success. Recent evidence suggests that improved predictive capability can be achieved by machine learning (ML) models that integrate multiple sources of clinical and biological data. In the current study, clinical and structural MRI data were evaluated with multiple ML models to predict treatment response in late-life depression.

Methods: Data were combined from two clinical trials conducted with depressed adults aged 60 and older. The first (N=28, NCT01902004) assessed response to escitalopram/placebo. The second (N=35, NCT02460666) compared Tai Chi versus health education. Remission was defined as a score of 6 or less on the 24-item Hamilton Rating Scale for Depression (HAMD) at the end of 24 weeks of treatment in both studies. Gray matter volumes (GMVs) were extracted using Freesurfer.

Features subsets were constructed from baseline clinical features, GMVs, or a combination of both. The three features subsets of interest were reduced to the top ten most informative features as determined by the ReliefF algorithm. The feature subsets were then evaluated using four classification algorithms: least squares (LS), linear discriminant analysis (LDA), support vector machine (SVM), and random forest (RF). Ten-fold cross-validation was employed to avoid model over-fitting. The best performing feature subset was further evaluated in a simple linear regression model to predict post-treatment HAMD score.

Results: The feature subset that combined baseline clinical features and GMVs outperformed either of the other subsets alone for all classifiers tested. The LS classifier predicted treatment response most accurately (Accuracy 0.686, Precision 0.630, Recall 0.809, Area under the ROC Curve/AUC 0.721). Predicted post-treatment HAMD scores using the combined feature subset correlated strongly with true measurements (R=0.55). The combined feature subset included: baseline HAMD, education, baseline well-being (Short Form-36) score, baseline disability score (Cumulative Illness Rating Scale), sex, left anterior cingulate, Rright posterior cingulate, right paracentral, right orbitofrontal, and left temporal pole GMVs.

Conclusions: This preliminary exploration into the use of ML methods to identify predictors of treatment response in late-life depression indicates that integration of clinical and structural MRI significantly increases predictive capability. Due to the limited sample size, findings could not be validated in an external sample. The identified features are among those previously implicated in geriatric depression, encouraging future work in this arena.

Keywords: Machine Learning, Predictor of Treatment Response, Late-Life Depression

Disclosure: Nothing to disclose.

W72. Interim Results From a Longitudinal Clinical and Biomarker Study in Offspring of Bipolar Parents: The Role of Impaired Global Functioning in Early Onset

Gahan Pandina*, Giacomo Salvadore, Ziad Saad, Stephanie Rassnick, Solanki Bhavna, Carla Canuso, Wayne Drevets

Johnson & Johnson, Pennington, New Jersey, United States

Background: The strong genetic risk for bipolar disorder (BD) affords an opportunity to prospectively examine risk factors in offspring of adults with BD. Retrospective studies of young adults with BD, and studies of subjects at high risk for BD suggest that premorbid clinical characteristics include anxiety and depressive symptoms, and greater functional impairment predate onset of diagnosis. The Aretaeus Study, initiated by Janssen Research & Development, uses a longitudinal observational design to characterize psychosocial and biological predictors of BD in youth with high familial risk for BD. It is unclear whether earlier functional impairment presages worse clinical course, or more likely conversion to BD. The current analysis examines whether functional status in youth with familial risk for BD relates to clinical outcomes at one year.

Methods: Participants (aged 15 to 25 years) who are offspring of parents with BD were assessed every 3 months (by phone, or 6-month clinic visits) for up to 24 months. Participants were either drug-naïve or on stable treatment (no mood stabilizers/or antipsychotics) for >4 weeks, with no BD or psychosis. Global Assessment of Function (GAF) was assessed at all timepoints by trained clinicians using the modified GAF scale (Hall, 1995), scored from 1 to 100 (higher scores = better functioning). Participants were stratified by screening GAF as functionally impaired or not impaired (GAF ≤70 or >70). Clinical diagnostic and symptom

rating scales assessed mania and depression using the Longitudinal Interval Follow-Up Evaluation (LIFE) Psychiatric Status Rating (PSR) score for DSM-IV psychiatric disorders, and the General Behavioral Inventory. Patient-reported outcomes of depression, mania, sleep, anxiety, and other domains were collected via smart-phone.

Results: Baseline data were available at interim analysis for N=223 participants; N=193 had 12-month clinical data. The Low GAF group was slightly younger (17.9 years vs. 19.3 years), more female (66.7% vs. 59.0%), with similar race / ethnicity as the high GAF group (82.0% White and 89.9% Not Hispanic/Latino vs. 78.6% White and 85.7% Not Hispanic/Latino). At baseline, the Low GAF group had slightly lower IQ (K-BIT; 103.6 vs. 99.6) versus the High GAF group. Low GAF (vs. High GAF) participants had more frequent prior antidepressant (29.8% vs. 14.4%) and prior anxiolytic use (6.0% vs. 1.4%) at baseline, and were twice as likely to start a new concomitant psychiatric medication after study start (36.9% vs. 17.3%), or require a dose increase (10.7% vs. 2.2%).

Both clinician- and participant-reported clinical symptoms were generally worse in the Low vs High GAF group. Number of weeks (past 6 months) with subthreshold or diagnostic level symptoms of a Major Depressive Episode on the LIFE (PSR score >3) was greater for Low vs. High GAF group (25.8+33.96 weeks vs. 6.5+20.36 weeks): number of weeks (6 months prior to baseline) with Mania/Hypomania was comparable between groups (Low GAF=0.4 [+1.98 SD] weeks vs. High GAF 0.5+3.13 weeks). Low GAF vs. High GAF participants had higher baseline BPSS-P Index score for both Mania (9.3+9.00 vs.3.9+5.41) and Depression (20.7+14.93 vs. 9.0+12.65).

Patient reported outcomes of depression and anxiety, but not mania, were worse at baseline in the Low vs High GAF group; depression – Quick Inventory of Depressive Symptoms-16 (8.4+5.11 vs. 4.3+3.43), anxiety - General Anxiety Disorder-7 (6.4+5.30 vs. 2.5+3.17), and manic symptoms - Altman Self-Rating Mania (2.4+2.60 vs. 1.6+2.16).

The lifetime MINI was used to confirm parental BD diagnosis. Parents of Low vs. High GAF group had earlier age of first manic/hypomanic episode (22.1+8.77 vs. 25.5+10.27) and earlier age of BD diagnosis (31.7+10.52 vs. 34.7+9.86). Parents of Low GAF participants reported a greater frequency of alcohol dependence (past 12 months; 16.9% vs. 3.0%), reported more current depression (MDE past two weeks; 33.9% vs. 24.0%), and had higher rates of current suicidality (past month; 62.7% vs. 44.0%) vs. parents of High GAF participants. Rates of GAD, ADHD, and mania were comparable across parents of both groups.

Participant-reported adverse events were comparable (46.4 % vs. 48.9%, respectively) in the Low and High GAF groups, as were psychiatric AEs (13.1% vs. 10.1%). Serious psychiatric AEs were higher in the Low (n=2 SAEs of major depression, n=5 suicide attempts) vs. High GAF group (n=1 patient reported new SAE of depression).

Prospective data on the LIFE at 12 months confirmed baseline differences: weeks spent with LIFE-PSR MDE scores of >3 (past 6 months) were significantly greater ($p<0.05$) in the Low (n=64; 25.8+33.96 weeks) vs. High GAF group (N=129; 9.8+20.36 weeks). Proportion of time manic/hypomanic was not significant (both groups had PSR >3 for <1 week, past 6 months; p=0.58).

Conclusions: Functional impairment in youth with familial risk for BD (but without a BD diagnosis) was associated with greater clinical symptoms of depression and anxiety, but not mania, at baseline. Low GAF subjects were younger, and more likely to have been prescribed psychiatric medication. Over a year, Low GAF participants spent significantly more time in clinically relevant depression, not mania, vs. High GAF participants, suggesting functional impairment may be a marker for poorer clinical course. Parental characteristics may contribute to poor prognosis in offspring, and predict later parental symptoms/comorbidities (depression, suicidality, and alcohol use disorder).

Keywords: Bipolar Disorder, Genetic Risk Factor, Depression and Anxiety

Disclosure: Janssen Research & Development: Employee (Self)

W73. Identification of High Impact Gene-Drug Pairs for Pharmacogenetic Testing in Alberta, Canada

Abstract not included

W74. Combinatorial Pharmacogenomic Testing Outperforms Individual Pharmacokinetic Gene Guidelines When Predicting Blood Levels of Psychotropic Medications and Clinical Outcomes in Patients With Depression

Anthony Rothschild*, Sagar Parikh, Daniel Hain, Rebecca Law, Michael Thase, Boadie Dunlop, Charles DeBattista, Charles Conway, Brent Forester, Richard Shelton, Matt Macaluso, Krystal Brown, David Lewis, Alexander Gutin, Michael Jablonski, John Greden

University of Massachusetts Medical School, Worcester, Massachusetts, United States

Background: There are many available options for pharmacogenomic testing, and it is important that tests be rigorously evaluated to ensure appropriate clinical use and patient management. We evaluated the clinical validity of a combinatorial pharmacogenomic test and single-gene Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines against patient outcomes and medication blood levels to assess their ability to appropriately inform prescribing in major depressive disorder (MDD).

Methods: All patients were enrolled in the Genomics Used to Improve DEpression Decisions (GUIDED) randomized-controlled trial, had a diagnosis of MDD, and ≥1 prior medication failure. All patients received combinatorial pharmacogenomic testing that included 8 pharmacokinetic and pharmacodynamic genes. Medications were categorized according to the level of gene-drug interactions predicted by a combined, weighted assessment of individual phenotypes. CPIC single-gene guidelines with Level A or Level B evidence were also evaluated, with actionable recommendations defined as selecting a different medication or a dose change of 50%. Individual gene phenotypes used in these single-gene guidelines were obtained as part of the combinatorial pharmacogenomic testing.

The ability to predict medication blood levels was evaluated according to predicted changes in metabolism (significant increase in metabolism, significant decrease in metabolism, no/moderate change in metabolism) for the combinatorial pharmacogenomic test and single-gene guidelines. Significant changes in metabolism were related to having significant gene-drug interactions identified by the combinatorial pharmacogenomic test or having actionable recommendations from single-gene guidelines. All blood levels analyses were adjusted for age, sex, and smoking status.

The ability to predict patient outcomes at week 8 was assessed according to whether prescribed medications were congruent with the combinatorial pharmacogenomic test or single-gene guidelines recommendations. Medications were considered congruent with the combinatorial pharmacogenomic test if they had no or moderate gene-drug interactions. Medications were considered congruent with the single-gene guidelines if there were no actionable recommendations. Patient outcomes were evaluated using the 17-item Hamilton Depression Rating Scale and included symptom improvement (% decrease in HAM-D17 from baseline), response (≥50% decrease in HAM-D17 from baseline to week 8), and remission (HAM-D17 ≤7 at week 8).

All analyses were performed for all eligible medications (outcomes N=1,022, blood levels N=1,034) and the subset of medications with single-gene guidelines (outcomes N=584, blood levels N=372). Eligible medications were those included on the combinatorial pharmacogenomic test report at the time of analysis.

Results: There was a significant correlation between patient outcomes at week 8 and changes in medication congruence from baseline to week 8 with the combinatorial pharmacogenomic test but not with congruence with single-gene guidelines. In multivariate analysis for all eligible medications, the combinatorial pharmacogenomic test was the only significant predictor of patient outcomes (symptom improvement p=0.002, response p=0.036, remission p=0.025). Predicted changes in metabolism were correlated with medication blood levels for both the combinatorial pharmacogenomic test and single-gene guidelines. For all eligible medications, both the combinatorial pharmacogenomic test (p=7.55x10-8) and single-gene guidelines (p=0.01) were significant predictors of blood levels when evaluated separately; however, only the combinatorial pharmacogenomic test remained significant when both were included in the multivariate model (p=6.71x10-7). There were no substantial differences when the subset of medications with single-gene guidelines were evaluated separately. Multivariate analysis that included both combinatorial pharmacogenomic testing and single-gene guidelines showed that only the combinatorial pharmacogenomic test predicted patient outcomes (symptom improvement p=0.005, response p=0.041, remission p=0.044) and medication blood levels (p=2.64x10-6) in the subset of medications with single-gene guidelines.

Conclusions: This study shows that only the combinatorial pharmacogenomic test was significantly associated with improved patient outcomes. In addition, the combinatorial pharmacogenomic test was a superior predictor of medication blood levels across a larger group of medications relative to guidelines focused on only CYP2C19 and CYP2D6. These data suggest that the superior ability of combinatorial pharmacogenetic testing to predict variation in medication blood levels may result in improved patient outcomes.

Keywords: Pharmacogenomic-Guided Treatment Recommendations, Major Depressive Disorder (MDD), Antidepressants

Disclosure: Allergan: Grant (Self), Assure Rx: Grant (Self), Janssen: Grant (Self), Takeda: Grant (Self), Alkermes: Advisory Board (Self), Glaxo Smith Kline: Consultant (Self), Janssen: Advisory Board (Self), Myriad Neuroscience: Advisory Board (Self), Sage Therapeutics: Advisory Board (Self), Up-to-Date: Honoraria (Self)

W75. Large-Scale Lesion Symptom Mapping of Depression: Anatomy of Risk and Resilience

Nicholas Trapp*, Joel Bruss, Ossama Abu-Halawa, Shuwen Li, Fatimah Albazron, Kenneth Manzel, Jordan Grafman, Daniel Tranel, Aaron Boes

University of Iowa Carver College of Medicine, Stanford University, Iowa City, Iowa, United States

Background: Depression is the leading cause of disability worldwide and the leading cause of disease burden in the U.S. At the level of the brain, symptoms of depression are mediated by neural networks. To understand depression and devise more effective treatment options we need to know how different brain networks are causally related to symptoms. Most methods to investigate the regional anatomy of depression rely on correlational data, such as structural and functional imaging or electrophysiological correlates. This correlational data implicates various brain regions in the prefrontal cortex and limbic system. Given the multitude of regions implicated in depression it is increasingly important to identify those regions most causally associated with symptoms of depression that could serve as effective treatment targets.

The lesion method of localizing anatomical regions associated with specific post-lesion symptoms has been successfully used to elucidate the anatomical sites most critical in supporting a number of functional processes. This includes symptoms relevant for psychiatry, such as executive dysfunction, hallucinations, and emotion regulation. There is also a fairly extensive literature dedicated to lesion studies of depression symptoms.

Lesion studies of depression have had largely inconclusive findings to date, with prominent long-standing controversies that have emerged, such as inconsistent results on the role of lesions to the left prefrontal cortex, or whether there is any significant lesion localization at all. Here, we attempt the largest lesion-symptom mapping study of depressive symptoms to date, utilizing a single depression measure across all patients for consistency. We employ multivariate lesion-symptom mapping, which has advantages over a mass univariate approach when multiple nodes of a network may be critical for the expression of symptoms, as is likely to be the case for depression.

Methods: Participants included 526 individuals who met study criteria, selected from the Patient Registry of the Division of Behavioral Neurology and Cognitive Neuroscience at the University of Iowa Department of Neurology (Iowa cohort) (n=330) and the Vietnam Head Injury Study (VHIS cohort) (n=196). For the University of Iowa registry, inclusion criteria were the presence of an acquired focal brain lesion that was stable. Each participant was enrolled in the chronic epoch, more than three months after the lesion onset. Exclusion criteria for the Patient Registry included a history of significant alcohol or substance abuse, psychiatric disorder prior to the brain lesion, medically intractable epilepsy, or other neurologic disorder unrelated to the lesion. The VHIS cohort included male veterans who had a penetrating brain injury. All patients had neuroimaging obtained >3 months post-injury, as well as a Beck Depression Inventory obtained in the chronic phase.

Each participant included in the analysis had a focal brain lesion with visible boundaries evident from research-quality structural imaging from T1 and T2 sequences on MRI. CT scans were also used in the Vietnam Head Injury Study and in rare cases in the Iowa registry when MRI was contraindicated. The anatomical segmentation of lesion borders was traced manually for each subject and brought to a common template space for statistical analyses. Lesion-behavior mapping analyses were performed on the mood rating scale results using sparse canonical correlation analysis (SCCAN) as implemented in LESYMAP, a package available in R.

Results: LESYMAP results demonstrate several brain regions that are significantly associated with higher and lower levels of depression severity (p=0.0064). "Risk" regions include portions of the bilateral insula, the right middle frontal gyrus, the left superior frontal gyrus, the right medial temporal cortex, the left dorsomedial prefrontal cortex and peri-cingulate region, and the left frontal white matter tracts. The strongest "risk" findings were in the insula. The "resilience" regions, where a brain lesion is associated with lower depression scores, include the bilateral orbitofrontal cortex (OFC) and ventromedial prefrontal cortex (vmPFC), the right inferolateral temporal cortex, the right dorsomedial prefrontal/frontopolar cortex, and the right inferior parietal lobule. The cross-validated correlation value, a measure of the strength of the correlation, for this dataset is 0.12.

Conclusions: The brain regions which pose the greatest risk of resulting in depressive symptoms when lesioned are the bilateral anterior insula and left frontal white matter. The brain regions which result in relatively lower depressive symptoms when lesioned are the right OFC, right vmPFC, and right inferolateral temporal lobe. Interestingly, the "risk" regions incorporate several nodes of the salience network of the brain. The "resilience" regions

include primarily nodes of the default mode network. These results demonstrate that large samples of lesion patients are needed to see significant findings in lesion symptom mapping of depression, likely due to the complex and heterogeneous nature of the illness. Future studies will focus on lesion symptom mapping of specific depression subtypes or symptom categories. Targets identified in this study could be candidates for neuromodulation studies of depression and other mood disorders. Limitations of this study include the inclusion of patients across two different sites with different demographics and lesion etiologies.

Keywords: Lesion, Depression, Human Neuroimaging

Disclosure: Nothing to disclose.

W76. Neural Antidepressant Expectancies Moderate Antidepressant Treatment Response in Major Depression

Abstract not included.

W77. Integrity and Segregation of Macroscale Cerebral Functional Networks Correlate With Plasma Psilocin Level and Psychedelic Experience

Gitte Knudsen*, Martin K. Madsen, Dea S. Stenbæk, Albin Arvidsson, Sophia Armand, Maja Marstrand-Joergensen, Sys S. Johansen, Kristian Linnet, Brice Ozenne, Patrick Fisher

Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

Background: Consciousness-altering effects of psychedelics such as psilocin are suggested to occur because of increased entropy, reduced functional connectivity (FC) within networks (reduced integrity)— including the default mode network (DMN)— and increased FC between networks (reduced segregation). Here we investigate effects of a psychoactive dose of psilocybin on temporal trajectories and interrelations of plasma psilocin level (PPL), subjective drug intensity (SDI) and FC as measured with fMRI over a five-hour period after high doses of oral psilocybin.

Methods: Fifteen healthy volunteers (mean age ± SD 34.3 ± 9.8 years; six females) participated. Blood-oxygen-level-dependent (BOLD) rs-fMRI data (10 min) was acquired using a Siemens 3T Prisma scanner (TR/TE=2000/30ms, voxel-size: 3.6x3.6x3.0mm) before and app. 40, 80, 130 and 300 minutes after oral psilocybin (dose: 0.2 (n=4) and 0.3 (n=11) mg/kg). Imaging data was processed in SPM12 and CONN, including denoising, using a principal-component-based procedure (aCompCor), and motion and spike regression. An SDI rating ("How intense is your experience right now?", 0-10 Likert scale: 0 = "not at all intense", 10 = "very intense"), and a blood sample were obtained immediately after each scan acquisition. PPL was determined from the blood samples using ultra-performance liquid chromatography and tandem mass spectrometry. Average within- and between-network RSFC was estimated for seven networks from Fisher-transformed r-to-z values (DMN, dorsal attention, executive control, salience, auditory, visual, and sensorimotor). Associations were estimated using linear mixed effects modelling (multiple comparisons correction: Bonferroni-Holm).

Results: PPL and SDI correlated positively and exhibited similar time courses. We found a statistically significant negative correlation of DMN FC with PPL ($R = -0.26$, pFWER = 0.04) and SDI ($R = -0.33$, pFWER = 0.007), suggesting reduced integrity of the DMN. We also observed that average between-network FC increased as a function of PPL ($R = 0.30$, pFWER = 0.01) and SDI ($R = 0.43$, pFWER = 0.0001), suggesting reduced network segregation.

Conclusions: These findings demonstrate that psilocin critically shapes the time course and magnitude of changes in the cerebral functional architecture and subjective experience after psilocybin and implicate the expression of network integrity, including DMN, and network segregation as important for both the psychedelic experience and for consciousness.

ClinicalTrials.gov Identifier: NCT03289949

Keywords: Psilocybin, Resting-State fMRI, Plasma Psilocin

Disclosure: Nothing to disclose.

W78. FKBP5 Regulates HPA Axis Activity by Fine-Tuning the Mineralocorticoid:Glucocorticoid Receptor Balance in the Hippocampus

Jakob Hartmann*, Thomas Bajaj, Claudia Klengel, Christos Chatzinakos, Nina Dedic, Kenneth McCullough, Roy Lardenoije, Marian Joëls, Onno Meijer, Katharine McCann, Serena Dudek, Angela Sarabdjitsingh, Nikolaos Daskalakis, Torsten Klengel, Nils Gassen, Mathias V Schmidt, Kerry Ressler

McLean Hospital, Harvard Medical School, Belmont, Massachusetts, United States

Background: An imbalance between central mineralocorticoid (MR) and glucocorticoid (GR) receptor signaling is proposed to underlie hypothalamic-pituitary-adrenal (HPA) axis dysregulation observed in stress-related psychiatric disorders; however, the underlying molecular mechanisms remain unclear. Here we show that FK506-binding protein 51 (FKBP5) plays a critical role in fine-tuning MR:GR balance in the hippocampus.

Methods: We utilized a number of analytic and causal approaches across species – biotinylated-oligonucleotide-mediated chromatin immunoprecipitation (oligoChIP) in mouse primary hippocampal neurons, single-cell RNA sequencing data, human postmortem brain tissue expression analyses, pharmacological approaches as well as region- and cell type-specific GR and MR knockout mice.

Results: OligoChIP in primary hippocampal neurons revealed that MR-, rather than GR-binding to the Fkbp5 gene regulates FKBP5 expression (one-way ANOVA: $F3, 8 = 7.578$, $p < 0.01$) during baseline activity of glucocorticoids. Notably, in mouse and human hippocampus, similar mRNA and protein expression patterns were observed for FKBP5 and MR, which were distinct from that of GR. Finally, pharmacological inhibition, region- and cell-type specific receptor deletion in mice demonstrated that lack of MR decreases hippocampal Fkbp5 levels (two-way ANOVA: genotype x hippocampal sub-region interaction $F3, 88 = 77.2$, $p < 0.0001$; $n = 10$ (ctrl) and $n = 14$ (Forebrain-specific MR knockout (MR-CKOCamk2α-Cre)) and dampens the stress-induced increase in glucocorticoid levels (two-way ANOVA: genotype x condition interaction $F1, 19 = 6.228$, $p < 0.05$).

Conclusions: Overall, our results demonstrate that MR-dependent changes in baseline Fkbp5 expression modify GR-sensitivity to glucocorticoids to ultimately alter HPA axis activity during acute stress. This provides additional insights into the molecular mechanisms underlying the MR:GR balance hypothesis. Such findings suggest that therapeutic targeting of MR, GR, and FKBP5 may be complementary in manipulating CNS and peripheral regulation of stress homeostasis. Our data further underline the important, but largely unappreciated role of MR signaling in stress-related psychiatric disorders.

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

Disclosure: Nothing to disclose.

W79. Genomic Signatures of Corticosterone in Dorsal and Ventral Hippocampus of Males and Females

Salvatore Caradonna, Huzefa Khalil, Nathan Einhorn, Eleonora Gatta, Vikram Saudagar, Francis Lee, Huda Akil, Bruce McEwen, Jordan Marrocco*

The Rockefeller University, New York, New York, United States

Background: Dorsal and ventral hippocampus are functionally distinct brain structures because of their unique anatomical connectivity and their role in distinct biological functions. The dorsal hippocampus is primarily implicated in spatial memory while the ventral hippocampus mostly encodes memories associated with emotions. Curiously, gene expression in the dorsal hippocampus also correlates with that in cortical regions involved in exploratory behavior and locomotion, whereas genes expressed in the ventral hippocampus correlate with those in limbic regions involved in emotion and stress. Whole-genome analysis of discrete cell populations has markedly advanced the dissection of diverse brain circuits. More importantly, within the same brain areas, females and males may trigger distinct sets of genes in response to similar stimuli. We studied the transcriptomic profiling of dorsal and ventral hippocampus in male and female mice maintained on chronic low-dose oral corticosterone (CORT), a mouse model that shows disruption of the hypothalamic-pituitary-adrenal axis associated with a blunted response to stress.

Methods: Wild-type (WT) mice or heterozygous BDNF Val66Met (BDNF Het-Met) male and female mice were maintained on chronic oral CORT (25ug/ml) or vehicle for six weeks prior to dissection of the ventral (vHPC) and dorsal hippocampus (dHPC). Three biological replicates were used per experimental group, comprising of RNA pooled from hippocampi from two animals. The cDNA libraries were sequenced on an Illumina NextSeq 500 using 75-bp single-end reads. 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 < 0.05 were calculated. Microsoft Excel (Microsoft, USA) was used to obtain gene expression profiles by sorting genes based on fold change > 1.3. Differences in integrated read density were visualized using the heat map tool Multi Experiment Viewer. GO categories were manually curated from results of the Database for Annotation, Visualization and Integrated Discovery (DAVID) functional annotation cluster tool.

Results: We examined the number of differentially expressed genes (DEGs) of both vHPC and dHPC and found that CORT induced a greater number of DEGs in the vHPC than in the dHPC. After chronic CORT, WT males showed 184 DEGs in the vHPC and 119 DEGs in the dHPC. This difference was more marked in BDNF Het-Met male mice, that showed 493 DEGs in the vHPC and 178 DEGs in the dHPC. Overall, CORT induced a lower number of DEGs in females than in males. Curiously, WT females showed more DEGs in the dHPC (151) than in the vHPC (54) when treated with CORT. However, CORT had only a mild effect on DEGs in BDNF Het-Met female mice. CORT-regulated DEGs of the vHPC were processed and grouped according to their GO, which showed unique gene sets in males, including metabolism and cell signaling, and females, where CORT mainly induced immediate early genes, such as Arc and Fos. By inputting in the analysis a selection of epigenetic modifiers involved in DNA methylation, chromatin remodeling, and histone methyltransferases, we found that in males CORT induced epigenetic DEGs that matched across genotypes, while in females epigenetic DEGs were regulated in opposite directions in WT and BDNF Het-Met mice.

Conclusions: Together, we found that gene sets underlying the response to chronic CORT are not only unique in males and females as a function of the BDNF Val66Met genotype, but are also distinct in the dHPC and vHPC. Greater number of DEGs in the vHPC compared to the dHPC are consistent with the functionally distinct role of the hippocampal regions along its dorsoventral axis. The profiling of sex- and genotype-specific DEGs in the vHPC suggests that chronic CORT recapitulates the characteristics of stress-related disorders, in which involvement of the vHPC is predominant. Finally, there were sex differences in the epigenetic signatures after chronic CORT, warranting the investigation of separate epigenetic mechanisms in males and females in response to similar stimuli.

Keywords: Corticosterone, Hippocampus, Sex Differences, RNA-Sequencing, BDNF Val66Met

Disclosure: Nothing to disclose.

W80. Early Life Stress-Induced Vulnerability to Postpartum Mental Disturbance: Prolonged Dysregulation of the HPA Axis

Minae Niwa*, Sedona Lockhart, Daniel Wood, Kun Yang, Jennifer Payne, Akira Sawa

University of Alabama at Birmingham, Birmingham, Alabama, United States

Background: Early life stress, such as psychosocial stress during adolescence, increases the risk for postpartum mental disturbance. Nonetheless, the mechanistic role of adverse events in early life in the pathogenesis of postpartum mental disturbance has not been well examined. In the present study, we have built a novel platform to study the biological mechanisms underlying the effects of adolescent stress on postpartum behaviors in first-time dams and to investigate a new avenue of pharmacological treatments for postpartum mental disturbance. We also assessed the significance of a history of major mental illnesses in the hypothalamic-pituitary-adrenal (HPA) axis and postpartum mental conditions in humans.

Methods: Healthy virgin C57BL/6J female mice were exposed to mild isolation stress during late adolescence (from 5 to 8 weeks of age), which alone caused no endocrine or behavioral changes. Each mouse was then mated with a C57BL/6J male and gave birth to pups. Behavioral tests [tail suspension (TST), forced swim (FST), and three-chamber social interaction tests (SIT)] and measurement of hormone levels in plasma were performed at 0-week, 1-week, and 3-weeks postpartum. Dams were treated with a glucocorticoid receptor (GR) antagonist C113176, a serotonin reuptake inhibitor (SSRI) fluoxetine, or a GABAA receptor modulator ganaxolone from postpartum day 0 to 24 hour prior to the behavioral test. Human cortisol levels in plasma from participants with a history of major mental illnesses at 2nd trimester, 3rd trimester, 2-week postpartum, and 6-week postpartum were examined by ELISA.

Results: Adolescent social isolation augmented the vulnerability to long-lasting postpartum behavioral deficits in TST, FST, and SIT at one-week postpartum, but not immediately after delivery. The behavioral deficits in dams exposed to adolescent social isolation (stressed dams) were aberrantly prolonged for at least three weeks after delivery. The onset of behavioral deficits during the postpartum period in stressed dams matched the sustained elevation of corticosterone, but not estradiol, progesterone, oxytocin, and prolactin. The behavioral deficits in stressed dams were ameliorated by 1-week post-delivery treatment of a GR antagonist C113176, but not a SSRI or a GABAA receptor modulator. We suggest that the prolonged HPA axis dysregulation in stressed dams may underlie the long-lasting behavioral deficits in the postpartum period. In the human study, postpartum-onset major depressive disorder (PP MDD) patients with a history of

mental illnesses exhibited both elevated and sustained levels of plasma cortisol until at least six weeks postpartum.

Conclusions: Our present preclinical study showed that adolescent social isolation elicits aberrantly prolonged dysregulation of the HPA axis, which in turn results in long-lasting postpartum behavioral deficits. Our clinical data mirrored the preclinical findings and showed a link between pre-partum adverse events, sustained increase in glucocorticoid signaling, and long-lasting postpartum mood disturbance. Together, these data suggest that adverse events in early life result in long-lasting changes in HPA axis function, which in turn augment the vulnerability to PP MDD. Our mouse model may be a promising model to study pathological mechanisms underlying postpartum mental disturbance and to explore novel therapeutic strategies for it.

Keywords: Adolescent Stress, HPA axis, Glucocorticoids, Postpartum Depression

Disclosure: Nothing to disclose.

W81. Alterations in Estradiol and Inflammatory Cytokines From Pregnancy to Postpartum in Women Veterans With Depression: A Pilot Investigation

Mercedes Szpunar*, Anne Malaktaris, Selena Baca, Richard Hauger, Ariel Lang

VA San Diego Healthcare System, San Diego, California, United States

Background: The population of reproductive-age women veterans is growing, and as a result, more women veterans are requiring perinatal care. Women veterans are twice as likely as their non-pregnant counterparts to have mental health issues, such as depression, anxiety, and posttraumatic stress disorder. Women are known to have significant alterations in hormonal and inflammatory signaling during both pregnancy and postpartum, also known as the peripartum period. Achtyes and colleagues recently determined significantly increased interleukin-6 (IL-6), modestly increased in tumor necrosis factor-α (TNF-α), and no change in interleukin-1β (IL-1β) in women with postpartum depressive symptoms compared to healthy controls. We sought to investigate changes in these inflammatory cytokines in the peripartum period in relation to estradiol and depression.

Methods: We recruited 28 pregnant women veterans to complete a psychological assessment battery, including the Edinburgh Postnatal Depression Scale (EPDS) to assess for depressive symptoms (2). We assessed the women during the 3rd trimester of pregnancy (mean 36 weeks gestation, range 31-40 weeks) and in the early postpartum period (mean 7 weeks, range 5-14 weeks). We obtained serum for determination of estradiol by enzyme-linked immunosorbent assay (ELISA). IL-1β, IL-6 and TNF-α were determined by the MesoScale Discovery (MSD) platform.

Results: As anticipated, we observed a significant decrease in estradiol (6666.04 pg/mL, versus 114.86 pg/mL) from pregnancy to postpartum. However, we did not determine any differences from pregnancy to postpartum in the means of IL-6 (0.722 pg/mL, versus 0.739 pg/mL), IL-1β (0.0344 pg/mL, versus 0.0424 pg/mL), and TNF-α (1.61 pg/mL, versus 1.78 pg/mL). Mean IL-6 and estradiol levels among pregnant women were significantly positively correlated, $r = .656$, $p = .008$, large effect size (ES), after controlling for number of weeks pregnant at time of assessment. Furthermore, there was a significant positive correlation between mean estradiol during pregnancy and mean IL-6 postpartum, $r = .648$, $p = .023$. Larger increases in IL-1β from pregnancy to postpartum trended towards higher postpartum depression scores, $r = .535$, $p = .09$, large ES, although this finding was not statistically significant. Similarly, larger increases in TNF-α from pregnancy to postpartum trended towards higher postpartum depression scores, $r = .501$, $p = .08$, large ES, although not statistically significant.

Conclusions: During pregnancy and postpartum, women veterans are at higher risk compared to their civilian counterparts to develop mental health symptoms, including depression. The results presented here add to our currently limited understanding of changes in estradiol and inflammatory cytokines during these unique reproductive phases. Although no mean difference was determined in IL-6 from pregnancy to postpartum, positive correlations were determined between IL-6 and estradiol during pregnancy as well as postpartum IL-6 with estradiol during pregnancy. Both IL-1β and TNF-α trended towards positive correlations with postpartum depressive symptoms. These results must be interpreted with caution due to the small sample size, but they add to our current understanding of hormonal and inflammatory changes in relation to depression during the peripartum period. Further investigation is required to confirm theses preliminary findings.

Keywords: Postpartum Depression, Estradiol, Inflammatory Cytokines

Disclosure: Nothing to disclose.

W82. Race, Cytokines and Depression

Olusola Ajilore*, Anand Kumar, April Thames

University of Illinois at Chicago, Chicago, Illinois, United States

Background: Previous studies have demonstrated that racial discrimination and the perceived stress of racism have been associated with elevations in pro-inflammatory cytokines, which in turn may serve as a biological risk factor for disparities in COVID-19 cases and deaths in the US. Given the well-documented relationship between pro-inflammatory cytokines and major depression, the goal of the present study was to examine whether there were racial differences in the relationship between inflammation and major depression using a secondary analysis of an ethnically diverse dataset.

Methods: 121 subjects were recruited as part of a larger program of research investigating major depressive disorder (MDD) at the University of Illinois at Chicago (UIC). 46 were African American (21 healthy comparison subjects, 25 with MDD) and 75 were Caucasian (34 healthy comparison subjects, 41 with MDD). The final inclusion criterion for depressed subjects was a score of ≥15 on the Hamilton Rating Scale for Depression. Severity of depression was also assessed using the Center for Epidemiological Studies – Depression scale. In addition to C-reactive protein (CRP), levels of pro-inflammatory cytokines were determined in plasma/serum aliquots by enzyme-linked immunosorbent assay (ELISA) using commercially available Quantakine® kits (R & D Systems, Inc., Minneapolis, MN) for human IL-1β, IL-6, and TNF-α. Between-group differences in CRP and serum cytokine levels were investigated using an ANCOVA adjusting for age, education, hemoglobin A1c, body mass index and diastolic blood pressure. Bivariate Pearson's correlation analyses using two-tailed tests were performed to examine associations between serum cytokines and depression severity.

Results: In the non-depressed sample, African American participants had significantly higher levels of CRP and IL-6 than Caucasian participants (CRP: $F = 4.48$, $p = .04$, $df = 1, 46$;IL-6: $F = .9.33$, $p = .004$, $df = 1, 52$). In the context of major depression, there was no difference between groups (CRP: $F = .001$, $p = .973$, $df = 1,58$); IL-6: $F = .097$, $p = .757$, $df = 1, 64$). Furthermore, IL-6 was not correlated with depression severity in African American participants ($r = .14$, $p = .369$) but was in Caucasian participants ($r = .38$, $p = .001$).

Conclusions: The well-known relationship between pro-inflammatory cytokines and major depression may be moderated

by race due to high baseline levels of cytokines in African Americans. Based on the known literature, these elevations may be related to the stressful life events due to discriminatory experiences.

Keywords: Pro-Inflammatory Cytokines, Major Depression, Racial Differences

Disclosure: Keywise Inc.: Stock / Equity (Self), Embodied Labs: Advisory Board (Self), Blueprint Health: Advisory Board (Self)

W83. Long-Term Symptom Relief in Severe Treatment-Resistant Depression Using Subcallosal Cingulate DBS

Paul Holtzheimer*, Patricio Riva Posse, Mustafa Husain, Helen Mayberg, Andres Lozano, Kopell Brian, Peter Giacobbe, Anthony D'Agostino, Konstatin Slavin, Raymond Lam, Theodore Kolivakis, Andrea Crowell, Stephan Taylor, Renana Eitan, Keyoumars Ashkan, Robert Gross, Julie Pilitsis, Ananda Pandurangi, Georgios Petrides, Clement Hamani, Edward Karst, Binith Cheeran, Gabriela Molnar, Sidney Kennedy

Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire, United States

Background: Major depressive disorder affects 17% of Americans over their lifetime and confers excess risk of suicide and all-cause mortality. Electroconvulsive therapy can be effective for drug-resistant patients, but relapse is common and side effects may hinder continued use. Transcranial magnetic stimulation appears more suited for moderate depression, and ketamine protocols are still in nascent stages of development. Initial open-label studies demonstrated positive results from Deep Brain Stimulation (DBS) of the subcallosal cingulate region for treatment-resistant depression (TRD), but a randomized controlled trial (BROADEN) comparing active to sham DBS at 6 months was halted by the sponsor after a futility analysis. Reconciling these discrepant results requires long-term effectiveness data from larger patient cohorts.

Methods: The pooled analysis combines open-label follow-up data from studies using Abbott (St. Jude Medical) DBS devices to stimulate the subcallosal cingulate region in patients with TRD: a randomized, double-blind sham-controlled multi-center trial with a 6-month primary endpoint (BROADEN, N=90), a randomized, double-blind sham-controlled crossover study at University of Toronto (N=31), an open-label study at Emory University (N=21), an open-label three-site pilot study in Canada (N=21), and a double-blind randomized trial evaluating the effect of stimulation frequency at 3 centers in Europe (N=9) . Since studies had different experimental designs in the first 6 months, results are reported from the time of stimulation activation. Based on change in Hamilton Depression Rating Scale (HDRS-17), we report rates of response (≥50% reduction in HDRS-17) and remission (HDRS-17 score ≤7) at 6-month intervals for up to 5 years.

Results: There were 172 subjects (48±7 years, 58% female) in 5 independent studies who received subcallosal cingulate DBS for depression from 2005-2016. Length of illness averaged 26 years, including 8.1 years in the current major depressive episode. All subjects failed ≥4 adequate antidepressant trials, and 88% had previous electroconvulsive therapy. In BROADEN, subjects averaged 14 failed treatments in the current episode. Baseline HDRS-17 for the 172 implanted subjects was 22.8±4.3 and decreased by 40% to 13.7±7.7 at 12 months and 46% to 12.2±7.2 at 24 months. At 12 months, 38% of subjects were responders, and 54% responded at 24 months. At least 50% of subjects were responders at each time point from 24 to 60 months. Remitter rate was 26% at 12 months and remained above 25% at all subsequent time points. Between 66% and 87% of the 12-month responders were responders at every subsequent follow-up visit. In 117 subjects with at least 2 years of data, 81% achieved response at least once within 5 years of follow-up and 61% experienced remission of depression symptoms. There were 3 suicides and 13 suicide attempts in a total of 523 patient-years. The event rate for suicide was 0.55 events per 100 patient-years (95% confidence interval 0.18-1.71); for suicide attempt, the rate was 2.39 (95% confidence interval 1.39-4.12). Infection classified as a serious adverse event occurred in 7 subjects, and there were no intracranial hemorrhages.

Conclusions: In subjects with depressive episodes averaging 8 years duration, at least 50% achieved ≥50% reduction in symptoms at each time point from 2 to 5 years. Open-label data suggest DBS can be a safe, durable, and effective treatment for severe chronic depression, and response rates appear to improve over time.

Keywords: Depression, Deep Brain Stimulation, Subcallosal Cingulate

Disclosure: Abbott: Consultant (Self)

W84. Ventromedial Prefrontal Cortex fMRI Neurofeedback Training Focused on Positively-Valenced Future Thinking for Individuals Experiencing Suicidal Thoughts

Robin Aupperle*, Tsuchiyagaito Aki, Tim McDermott, Elisabeth Akeman, Jessica Santiago, Namik Kirlic, Danielle DeVille, Jared Smith, Martin Paulus, Jerzy Bodurka

Laureate Institute for Brain Research, Tulsa, Oklahoma, United States

Background: There is a need to identify novel and fast-acting interventions to reduce suicidal thoughts and likelihood of future attempts. Hopelessness is a cardinal symptom associated with suicide, while greater levels of hope or optimism have been found protective of future suicidal ideation and attempts. Hope involves expectation of positive future outcomes and both depressed and suicidal patients show less specificity and positivity when imagining future events (termed "episodic future thinking" [EFT]). Other psychological constructs thought to contribute to the likelihood of suicide attempts include impulsivity and poorer decision-making. Engaging in EFT decreases impulsivity and shifts the balance of decision-making more towards delayed, long-term outcomes. EFT activates a prefrontal-temporal-parietal network centered upon the ventromedial PFC, which is thought to be crucial for the valuation of future events and incorporating a sense of "self". We hypothesized that enhancing vmPFC neural activity during EFT may have therapeutic potential for improving outcomes related to suicidal thoughts and behaviors. As an initial step toward this long-term objective, we conducted a proof-of-concept feasibility study using real-time functional magnetic resonance imaging neurofeedback (rtfMRI-nf) to enhance vmPFC activity during positively-valenced future thinking, and present pilot results from for individuals with suicidal thoughts.

Methods: Participants included six individuals with major depressive disorder and current suicidal intent or plan. Each participant identified future positive events likely to occur within six months. The three events with the highest valence ratings were selected for use during rtfMRI-nf and participants were asked to identify details (sensory details, emotions) related to the future events to aid in vividly imagining the events during scanning. The rtfMRI-nf experiments were conducted on a GE MR750 3T MRI scanner (parameters: TR/TE=2000/30ms, EPI SENSE acceleration R=2, matrix 96×96, 34 axial slices, 1.9×1.9×2.9 mm3 voxels). EEG 32-channel data were recorded simultaneously using Brain Product MR-compatible system. The vmPFC region-of-interest (7mm sphere, Talairch coordinates = −2, 35, −3) was selected based on meta-analyses of positive valence processing. Each of three rtfMRI-nf runs started with a 40s "Rest" block, followed by alternating blocks of "Count" (backward from 300 by one) or "Imagine" conditions (40s each). For the latter, participants were asked to imagine future positive events while upregulating vmPFC

activity represented by the height of a red bar (Imagine vs Count). To reduced fluctuations in the bar height, a moving average of the two preceding percent signal change values were used for computation. After rtfMRI-nf runs, there was a "Transfer" run in which no neurofeedback signal was provided. Participants completed the Montgomery-Åsberg Depression Rating Scale (MADRS) and Profile of Mood States (POMS) before and after scanning, and also completed a feedback questionnaire created for the purposes of this study.

Results: Participants exhibited vmPFC linear signal increase, including a 52% increase from the first to the last neurofeedback run, and a 56% increase from the first neurofeedback run to the transfer run. Subjects reported a 34% average decrease in the POMS depression score (from M = 16.9 at pre to 11.1 at post-scan) and four of the six participants reported decreases on the suicide item of the MADRS. On the feedback questionnaire, participants indicated the neurofeedback session was challenging (M = 6.17 on a scale of 1-10, with 10 being the most challenging) but reported relatively high success in imagining future positive events (M = 7.33 on 1-10 with 10 being "extremely successful") and modulating brain activation (M = 6.33 on scale of 1-10). Participants also indicated that the neurofeedback session would be moderately to extremely (M = 7.2 on a scale of 1-10) helpful for their mental health treatment.

Conclusions: Our results provide: initial evidence of 1) safety and feasibility of vmPFC rtfMRI-nf training utilizing positively-valenced future thinking and 2) capacity to increase vMPFC neural activity for individuals with current suicidal thoughts. Research in larger samples is warranted to determine (a) whether vmPFC rtfMRI-nf during positive future thinking will lead to statically and clinically significant changes in brain activation, (b) to confirm specificity by comparing to control or sham neurofeedback and (c) to examine the potential efficacy of such interventions for reducing severity of suicidal thoughts or future attempts. Neurofeedback interventions may be particularly relevant for populations at acute suicide risk, given the need for efficacious treatments and that treatment often occurs in inpatient hospital settings with access to MRI.

Keywords: Suicide, Real-Time fMRI Neurofeedback, Positive Emotion, Hopelessness, Ventromedial Prefrontal Cortex

Disclosure: Nothing to disclose.

W85. Efficacy of Amygdala rtfMRI Neurofeedback for Treatment Resistant Depression

Laurie Compere, Scott Barb, Sarah Lazzaro, Gia Canovali, Marlene Strege, Theodore Huppert, Greg J. Siegle, Kymberly Young*

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States

Background: There is a need to develop novel therapeutics for treatment resistant depression (TR-MDD) and to improve the effectiveness of non-invasive treatments. Here we apply an intervention that involves real-time functional magnetic resonance imaging neurofeedback (rtfMRI-nf), which we've successfully applied in MDD, to a treatment resistant population.

Methods: In this pilot study, 12 participants with TR-MDD, defined as having not responded to two previous antidepressant treatments, received amygdala rtfMRI neurofeedback training (NCT03428828). This training involved increasing the amygdala's response during positive autobiographical memory recall. Five neurofeedback sessions were provided. Depressive symptoms (as measured by the BDI-II) were measured at each neurofeedback sessions and for 12-weeks post intervention.

Results: Overall, participants were able to significant increase their amygdala response to positive autobiographical memories with rtfMRI neurofeedback training. The average increase from baseline to the final transfer run was 0.31%, which was significantly different from 0 ($t(11)=2.47$, $p=0.03$, $d=0.93$). In the entire group, BDI scores decreased by an average of 47% at the 12-week follow up period ($t(11)=5.21$, $p=0.002$, $d=1.32$). Five of the 12 participants met criteria for remission. There was a marginally significant correlation ($r=-0.48$, $p=0.10$) between neurofeedback success and improvement in BDI scores at the 12-week follow up, supporting the conclusion that learning to increase the amygdala response to positive stimuli is associated with clinical improvement.

In contrast to our previous studies with MDD participants, only half of the participants learned to regulate their amygdala activity, and these were all female participants. Indeed, the main effect of sex on neurofeedback success was highly significant ($F(1,11)=16.6$, $p<0.001$), with females having greater neurofeedback success than males.

There was a significant sex difference at baseline, with males showing significantly higher amygdala activity when recalling positive memories prior to training relative to females ($t(10)=4.05$, $p=0.002$, $d=2.38$, Male mean = 0.25+/- 0.26, Female mean = -0.43 +/- 0.31).

Conclusions: Our real-time fMRI neurofeedback intervention appears to be effective at reducing symptoms of TRD-MDD, but only in women. Male participants were less able than female participants to increase their amygdala response and derived less clinical benefit from the intervention. The male participants had a significantly increased baseline amygdala response relative to the female participants. This suggests that our deficit targeting intervention is only effective in those who present with this deficit and that men with TRD MDD may have different mechanisms underlying their depression and that different interventions are needed. This neurofeedback intervention does, however, show promise for treating severely ill depressed females.

Keywords: Real-Time fMRI Neurofeedback, Treatment Resistant Depression, Amygdala, Autobiographical Memory

Disclosure: Nothing to disclose.

W86. Low Frequency Right Sided and High Frequency Left Sided Repetitive Transcranial Magnetic Stimulation for Depression: The Evidence of Equivalence

Yosef Berlow*, Amin Zand Vakili, Noah Philip

Alpert Medical School, Brown University, Barrington, Rhode Island, United States

Background: Multiple studies comparing the efficacy of low-frequency (1 Hz), right-sided, repetitive transcranial magnetic stimulation (LFR-TMS) and high-frequency (≥ 5 Hz), left-sided, transcranial magnetic stimulation (HFL-TMS) have failed to detect differences in these approaches. While the failure to detect differences in these studies using null hypothesis significance testing is insufficient to establish that LFR-TMS is as efficacious as HFL-TMS, formal equivalence testing of this same data can provide quantitative evidence for the absence of a clinically meaningful difference between these treatment protocols. This study aims to perform equivalence testing of the pooled efficacy outcomes from LFR-TMS and HFL-TMS treatments for depression.

Methods: Study Selection:

Randomized controlled trials of LFR-TMS and HFL-TMS for patients with major depressive episodes were identified through Pubmed following the methods described by Cao et al. (Front Psychiatry. 2018;9:413), but extending to June 21, 2020, and defining low-frequency stimulation as less than 5 Hz. Treatment response was defined as a 50% reduction in depression rating scales; remission was defined using established values on standardized rating scales.

Data Analysis:

Pooled odds ratios for response and remission rates were calculated using the Mantel-Haenszel fixed-effect method (R news. 2007;7:40-45.). Odds ratios were converted to effect size (Cohen's d) estimates (Stat Med. 2000;19:3127-3131.). Equivalence testing for meta-analysis was completed using the two one-sided test for meta-analysis (Soc Psychol Personal Sci. 2017;8:355-362.). The equivalence margin was set at d=0.25, following previous meta-analytic methods for equivalence testing of depression treatments (Am J Psychiatry. 2017;174:943-953). Additional analyses were performed using alternative study selection to assess the robustness of these findings.

Results: Twelve randomized controlled trials were included in the analysis, with a total of 627 subjects with major depressive episodes treated with either LFR-TMS (n=320) or HFL-TMS (n=307). Forty-five percent (144/320) of subjects treated with LFR-TMS and 48% (148/307) of subjects treated with HFL-TMS were classified as responders. The pooled odds ratio was 1.12 (90% CI: 0.85-1.48), corresponding to an effect size of d = 0.064 (SE=0.09) nominally favoring HFL-TMS. Equivalence testing was significant (Z=-2.02, p=0.022), indicating evidence to reject the difference hypothesis for treatment response. A subset of six of these studies (n=431) reported remission rates in addition to response rates, with 26% (57/217) of patients randomized to LFR-TMS achieving remission compared to 25% (54/214) in the HFL-TMS groups. The pooled odds ratio was 0.92 (90% CI: 0.64-1.33), nominally in favor of LFR-TMS. Equivalence testing with the same margin was significant (Z=1.687, p=0.046), indicating equivalence. Additional sensitivity analyses, excluding three studies in which bipolar depression made up the majority of subjects, yielded similar results supporting equivalence for response rates (pooled OR = 1.10, 90% CI: 0.82-1.49; equivalence testing Z=-1.94, p=0.026 given margin of d=0.25) and modest results for remission rates (pooled OR = 0.87, 90% CI: 0.59-1.28; equivalence testing Z=-1.35, p=0.088).

Conclusions: This study provides statistical evidence of the equivalence of LFR-TMS and HFL-TMS efficacy when used to treat major depressive episodes. If a true difference between LFR-TMS and HFL-TMS efficacy existed larger than the tested equivalency margin (d=0.25), the current findings of equivalence for response and remission rates would be unlikely (p<0.05). LFR-TMS protocols present many potential advantages over HFL-TMS, including safety, tolerability, and costs (Brain Stimul. 2020;13:1296-1297.). While low-frequency, right-sided stimulation protocols have not been studied to the same extent as HFL-TMS, the current findings of equivalent efficacy support a reappraisal of this approach for the treatment of depression.

Keywords: Repetitive Transcranial Magnetic Stimulation, Meta-Analysis, Depression, Statistical Methods, Patient Outcomes

Disclosure: Nothing to disclose.

W87. The Rapid Mood Screener: A Novel and Pragmatic Screener Tool for Bipolar I Disorder

Abstract not included.

W88. Vital Sign Changes During Intravenous Ketamine Infusions for Depression: An Exploratory Study of Prognostic Indicators

Katherine Thomashow*, Cynthia Arfken, Nicholas Mischel

Wayne State University School of Medicine, Detroit, Michigan, United States

Background: Depression is a common medical problem, with 30-40% of patients not achieving remission with first or second-line therapies. A new class of medications, rapid-acting antidepressants (RAADs), have been developed to address this issue. Among RAAD options now available in the community are intravenously infused racemic ketamine and intranasal esketamine. Throughout treatment with ketamine, vital signs and subjective mental states are routinely monitored for safety, and treatments may be titrated based on subjective response. It is not known if vital sign responses constitute an indicator of treatment efficacy.

Methods: Patients were treated in a clinical setting with racemic ketamine infusions within the period of 10/2018 – 05/2020. All patients were screened for severity of depression and common comorbidities using the nine-question Patient Health Questionnaire (PHQ9) and seven-question Generalized Anxiety Disorder questionnaire (GAD7). The PTSD Checklist (PCL), and the Yale-Brown OCD Scale (YBOCS) were additionally used to track symptoms in patients with PTSD and OCD, respectively. Blood pressure and heart rate were recorded at resting baseline and every 5-10 minutes during 6 to 8 biweekly infusions in a treatment course.

Results: Patients (n=144) presented in a unipolar (n=129) or bipolar (n=15) depressive episode, 56.3% were female. Nearly all patients were severely depressed, as mean PHQ9 Score=19.24 (95% CI 18.55, 19.93) and anxious, mean GAD7=14.03 (95% CI 13.26, 14.81). The percentage of patients who did not show clinically relevant changes were in mean arterial pressure was 21.5%, heart rate 52.8%, and rate-pressure product 34.7%. Preliminary independent samples t-tests showed PHQ9 was significantly higher both at baseline and after the first infusion in patients showing a decrease in HR of 5 bpm or greater compared to all other patients. However, there was no significant difference in the PHQ9 change when comparing these two groups.

Conclusions: In general, patients experienced a significant decrease in symptoms with ketamine treatment that was not associated with a certain pattern of vital sign response to the first infusion. This may indicate that physiologic responses during ketamine are spurious, nonindicative of a treatment effect. It is also possible that prognostic physiologic responses to ketamine infusions are not reflected in the gross measurement of MAP and HR and rather require a more detailed approach to elucidate.

Keywords: IV- Ketamine, Depression, Autonomic Nervous System, Neuropharmacology, Blood Pressure

Disclosure: Nothing to disclose.

W89. Stress During Puberty Exerts Sexually Dimorphic Effects on Antidepressant-Like Behaviour and Monoamine Neurotransmitters in Adolescence and Adulthood

Erin Harris*, Francisca Villalobos-Manriquez, Gerard Clarke, Olivia O'Leary

University College Cork, Cork, Ireland

Background: Puberty is a period characterized by dramatic physical, hormonal, and psychological changes. During this critical window, the brain is particularly sensitive to stressful events. Stress is a major risk factor for the development of psychiatric disorders including major depression. Stress-related psychiatric disorders are twice as prevalent in women compared to men and this difference only emerges after the onset of puberty, suggesting that puberty may be a sensitive period during which sex-dependent vulnerability to depression might become established. However, no studies have yet investigated whether stress occurring specifically during the pubertal window is responsible for this sex difference in depression vulnerability.

Methods: Male and female Sprague Dawley rats were exposed to a three-day stress protocol during puberty (postnatal days 35-37 in females, 45-47 in males) consisting of a 10-minute forced

swim, elevated platform stress, and 2-hour restraint stress. Stressed rats and non-stressed control rats then underwent behavioural tests in adolescence (n=13-15 per sex per group) or adulthood (n=11-13) measuring anhedonia, anxiety-like behaviour, locomotor activity and antidepressant-like behaviour. Brainstem tissue was also collected from a separate cohort of behaviour-naïve rats as adolescents (n=8-12) or adults (n=4-5, preliminary) to evaluate monoamine neurotransmitter levels by HPLC. Two-way ANOVA (Pubertal stress x Sex) was used to assess the statistical significance of results.

Results: Pubertal stress reduced antidepressant-like behaviour (increased immobility) in the forced swim test (FST) in both adolescents [$F(1,52)=14.1$, $p<0.001$] and adults [$F(1,45)=13.8$, $p<0.001$]. Interestingly, this effect was manifested via different behavioural strategies in a sex-dependent manner. Pubertal stress decreased climbing behaviour in adolescent males [Stress x sex interaction: $F(1,52)=6.8$, $p<0.01$] and decreased swimming behaviour in adolescent females [Stress x sex interaction: $F(1,52)=5.2$, $p<0.05$]. However, in adults, climbing was decreased in both sexes [$F(1,45)=26.9$, $p<0.0001$] while swimming was unaffected [$p>0.05$]. Pubertal stress also impacted head shake frequency in the FST, a behaviour with a robust sex difference where males exhibit more head shakes than females. In adolescents, pubertal stress reduced head shakes in males compared to controls [Stress x sex interaction: $F(1,52)=5.2$, $p<0.05$] but not in adults [$p>0.05$].

Previous studies have implicated monoaminergic signalling in forced swim test behaviours. Pubertal stress decreased noradrenaline levels in adolescent brainstem [$F(1,33)=5.9$, $p<0.05$], which was more pronounced in females [Fisher's LSD, $p<0.05$], whereas there was no effect of pubertal stress in adult brainstem [$F(1,14)=2.5$, $p>0.05$]. Serotonin (5-HT) turnover, as measured by metabolite 5-HIAA/5-HT, was affected by pubertal stress in a sex-specific manner [Stress x sex interaction: $F(1,38)=20.1$, $p<0.0001$], where stress decreased turnover in males and increased turnover in females. However, in adult brainstem, pubertal stress lead to decreased serotonin turnover in both sexes [$F(1,14)=5.3$, $p<0.05$]. In the saccharin preference test, a measure of anhedonia, there was a male-specific effect of pubertal stress in adults where stressed males had a lower saccharin preference compared to controls [Stress x sex interaction: $F(1,45)=6.6$, $p<0.05$]. Pubertal stress did not impact anxiety-like behaviour the open field test (OFT). However, there was a significant sex difference where females were more active (total distance traveled) and exploratory (rearing behaviour) than males in the OFT both as adolescents [$F(1,52)=7.5$, 4.4; $p<0.01$, 0.05, respectively] and adults [$F(1,45)=9.1$, 14.1; $p<0.01$, 0.001, respectively]. In addition, females stressed during puberty were less exploratory than control females in adulthood in the OFT as measured by total rearing behaviours [Stress x sex interaction: $F(1,45)=6.6$, $p<0.05$].

Conclusions: Pubertal stress increased immobility in FST and rats stressed during puberty used sex-specific strategies of escape-oriented behaviours in adolescence and adulthood. Pubertal stress also increased anhedonia in adult males and decreased exploratory behaviour in adult females. These behavioural changes were accompanied by differences in brainstem levels of noradrenaline and serotonin. Taken together, these data suggest that stress during puberty exerts sex-specific effects on stress-related behaviours possibly through discrete neurotransmitter systems.

Keywords: Pubertal Stress, Sex Differences, Depression, Serotonin

Disclosure: Nothing to disclose.

W90. Depressive Symptoms – Could It Be Bipolar Depression? An Overview for Primary Care

Abstract not included.

W91. Clinical Global Impression Scores and Number Needed to Treat Outcomes in Patients With Postpartum Depression Treated With the Oral Neuroactive Steroid Zuranolone

Kristina Deligiannidis*, Brian Werneburg, Ming-Yi Huang, Ellison Suthoff, Robert Lasser, Handan Gunduz-Bruce, Sarah Acaster, Moshe Fridman, Vijayveer Bonthapally, Stephen J. Kanes

Zucker Hillside Hospital, Feinstein Institute for Medical Research; Zucker School of Medicine at Hofstra/Northwell, Glen Oaks, New York, United States

Background: Postpartum depression (PPD) is one of the most common medical complications during and after pregnancy. In the United States, approximately 13.2% of mothers experience symptoms of PPD, varying by state from 9.7% to 23.5%. Under- or untreated PPD is associated with negative short- and long-term consequences for the mother, infant, and family. PPD has also been associated with altered functional connectivity of the default mode network, salience, and central executive networks. Enhancing GABAergic inhibition may restore excitatory/inhibitory balance to regulate brain network activity, which has been proposed to reduce depressive symptoms. Zuranolone (ZRN; SAGE-217) is an investigational oral neuroactive steroid GABAA receptor positive allosteric modulator. Neuroactive steroid GABAA receptor positive allosteric modulators activate both synaptic and extrasynaptic GABAA receptors to produce phasic and tonic inhibitory currents to potentially enhance GABAergic inhibition. ZRN was evaluated in a double-blind, randomized, placebo-controlled Phase 3 trial in adults with PPD (NCT02978326). In this trial zuranolone met the primary endpoint, reducing depressive symptoms assessed by change from baseline (CFB) in the 17-item Hamilton Rating Scale for Depression total score (HAMD-17) at Day 15 versus placebo (CFB ±SE: ZRN: -17.8±1.04, placebo: -13.6±1.07, $p=0.0028$). The most common AEs (≥5%) in the zuranolone group were somnolence, headache, dizziness, upper respiratory tract infection, diarrhea, and sedation. Post hoc analyses examined patients achieving categorical response and remission as measured by the Clinical Global Impression-Improvement (CGI-I) and Clinical Global Impression-Severity (CGI-S) scales were examined. Number needed to treat (NNT) outcomes for CGI-I and CGI-S response and remission were also explored.

Methods: In this Phase 3 study, women (N=151) ages 18-45, ≤6 months postpartum, with PPD (a major depressive episode beginning in the third trimester or ≤4 weeks postpartum), and a qualifying HAMD-17 total score ≥26, were randomized 1:1 to receive either zuranolone 30 mg or placebo for 14 days, with 4 weeks follow-up. Patients were not permitted to breastfeed from just prior to receiving study drug until 7 days after the last dose. Psychotropic medications were permitted if initiated ≥30 days prior to Day 1 and taken at a stable dose until after Day 15 assessments. Secondary endpoints included CGI-I scores, which measure overall improvement post-treatment, and CGI-S scores, which reflect severity at the time of assessment. Post hoc analyses assessed response and remission rates defined as scores ≤2 ('much improved'/'very much improved' for CGI-I; 'borderline mentally ill'/'normal, not at all ill' for CGI-S) and scores ≤1 ('very much improved' for CGI-I, 'normal, not at all ill' for CGI-S), respectively, for both the CGI-I and CGI-S scales. NNT was calculated using the proportion of CGI-I and CGI-S responders or remitters in each treatment arm at Days 15 and 45. CGI-I and CGI-S response and remission rates were assessed using generalized estimating equations models for repeated measures, adjusting for baseline covariates. Secondary endpoints and post hoc analyses were not adjusted

for multiplicity. Adverse events (AEs) were assessed throughout the study.

Results: 76 and 74 patients from the ZRN and placebo treatment arms were included in these post hoc analyses, respectively. At Day 15 a significantly higher proportion of ZRN-treated patients compared with placebo achieved CGI-I response (71.6% versus 52.1%, p=0.0276, NNT=6), CGI-S response (54.1% versus 30.1%, p=0.0070, NNT=5) and CGI-S remission (29.7% versus 11.0%, p=0.0117, NNT=6). At Day 45 a significantly higher proportion of ZRN-treated patients compared with placebo achieved CGI-S response (63.0% versus 42.6%, p=0.0334, NNT=5), CGI-S remission (42.5% versus 11.8%, p=0.0007, NNT=4), and CGI-I remission (53.4% versus 29.4%, p=0.0111, NNT=5).

Conclusions: Zuranolone administration in women with PPD has previously been shown to provide rapid (Day 3 HAMD-17 total score) and sustained improvement in depressive symptoms (HAMD-17 total score at all measured time points up to Day 45) compared to placebo. In addition, these secondary and post hoc analyses also show that zuranolone achieved significantly greater improvements and rates of response and remission as measured by CGI-I (at both Day 15 and Day 30, respectively) and CGI-S (at both Day 15 and Day 30, respectively) in women with PPD. Single-digit NNTs support the robustness of these effects compared to placebo.

Keywords: Postpartum Depression, Zuranolone, Number Needed to Treat

Disclosure: Sage Therapeutics, Inc.: Consultant (Self)

W92. Insomnia Symptom Response in Patients With Postpartum Depression Treated With the Neuroactive Steroid Brexanolone Injection

Samantha Meltzer-Brody*, Brian Werneburg, Robert Riesenberg, C. Neill Epperson, Kristina M. Deligiannidis, David R. Rubinow, Kemi Bankole, Ming-Yi Huang, Haihong Li, Stephen J. Kanes

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

Background: Postpartum depression (PPD) is one of the most common medical complications during and after pregnancy. Approximately 13.2% of mothers experience symptoms of PPD in the US, and difficulty sleeping including insomnia is a common symptom in PPD. Gamma-aminobutyric acid (GABA) is associated with sleep and wake cycle regulation. In addition to other mechanisms, dysfunctional GABA signaling and hormonal changes during pregnancy have been implicated in PPD. Neuroactive steroid GABAA receptor positive allosteric modulators have a pharmacological profile distinct from benzodiazepines, activating both synaptic/extrasynaptic GABAA receptors to produce phasic/tonic inhibitory currents, enhancing GABAergic inhibition. This may restore excitatory/inhibitory balance to regulate brain network activity, and has been proposed to reduce depressive symptoms. Circulating allopregnanolone levels, an endogenous neuroactive steroid and GABAA receptor positive allosteric modulator, rise throughout pregnancy and drop rapidly after childbirth. Brexanolone injection (BRX) is a first-in-class, intravenous formulation of allopregnanolone, FDA-approved for the treatment of PPD in adults. In a prespecified integrated analysis of three pivotal trials in PPD, BRX met the primary endpoint of a significant reduction in depressive symptoms, assessed by change from baseline (CFB) in the 17-item Hamilton Rating Scale for Depression total score (HAMD-17) at Hour 60 (CFB±SE: BRX90: -16.95±0.74, placebo: -12.83±0.71; p<0.0001). In addition, CFB using the predefined Bech-6 subscale of HAMD-17, comprised of the core symptoms of depression, favored BRX90 compared with placebo beginning at Hour 24 (CFB±SE: BRX90: -30.06±1.98, placebo: -24.07±1.92; p=0.0173) and at all measured timepoints to Day 30 (BRX90: -38.47±2.11, placebo: -30.63±2.02; p=0.0301). AEs occurring in ≥5% of BRX and at ≥2x the rate of placebo included sedation and/or somnolence, dry mouth, loss of consciousness, and flushing/hot flush. Post hoc analyses of the integrated dataset assessed the effect of BRX on insomnia symptoms in women with PPD, using the HAMD-17 insomnia subscale (HAMD-17-Ins).

Methods: Women (N=247) ages 18-45, ≤6 months postpartum, with PPD (defined as a major depressive episode with onset in the 3rd trimester or ≤4 weeks postpartum), and a qualifying HAMD-17 score (Studies A and B: HAMD-17 ≥26; Study C: HAMD-17 20-25) were enrolled. Patients received a 60-hour infusion of placebo or BRX titrated to 60 μg/kg/h (BRX60) or 90 μg/kg/h (BRX90), with follow-up through Day 30. Post hoc analyses included the CFB in HAMD-17-Ins (sum of individual items for early, middle, and late insomnia). HAMD-17-Ins response was defined by either a ≥50% or ≥70% reduction in baseline score. Patients with HAMD-17-Ins scores of ≥1 were defined as having insomnia of "any" severity and those with scores ≥4 were defined as having "high" insomnia. Exploratory subgroup analyses of patients with "any" or "high" insomnia at baseline were assessed for a categorical response to below those values. CFB assessments utilized mixed-effect models for repeated measures Response assessments utilized the generalized estimating equation or logistic regression approaches. Post hoc analyses were not adjusted for multiplicity. Adverse events (AEs) were assessed throughout the study.

Results: 102 and 107 patients from the BRX90 and placebo treatment arms were included in these post hoc analyses, respectively. At baseline, the majority of patients reported insomnia of "any" severity (98% in both groups) and the mean (±SD) HAMD-17-Ins scores were high (BRX90: 4.39±1.50, and placebo: 4.58±1.47). Patients receiving BRX90 achieved significantly greater reductions in HAMD-17-Ins from baseline at Hour 24 (-0.95 ±0.24, p=0.0001), at all measured timepoints to Day 14 (-0.84±0.33, p=0.0122), and at Day 30 (-0.65±0.29, p=0.0245). A significantly greater proportion of BRX90 patients achieved HAMD-17-Ins ≥50% response compared with placebo at Hour 24 (BRX90: 61.4%, placebo: 41.5%, p=0.0007) and at all measured timepoints to Day 7 (BRX90: 70.0%, placebo: 44.3%, p<0.0001). A significantly greater proportion of BRX90 patients also achieved HAMD-17-Ins ≥70% response compared with placebo at Hour 24 (BRX90: 35.6%, placebo: 16.0%, p=0.0006) and at all measured timepoints to Day 30 (BRX90: 52.1%, placebo: 40.0%, p=0.0375). In BRX90 patients with "any" degree of insomnia, a significantly greater proportion achieved a score of 0 compared with placebo at Hour 24 (BRX90: 29.3%, placebo: 9.6%, p=0.0004) at all measured timepoints to Day 3 (BRX90: 41.2%, placebo: 17.1%, p=0.0001), and at Day 30 (BRX90: 43.5%, placebo: 29.1%, p=0.0254).

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

Keywords: Postpartum Depression, Insomnia, Brexanolone Injection

Disclosure: Sage Therapeutics: Grant (Self), Janssen: Grant (Self), WebMD: Consultant (Self), Cala Health: Consultant (Self)

W93. One-Week Escitalopram Intake Alters Excitation-Inhibition Balance in Healthy Female Participants:

Implications for Neuroplasticity and Individual SSRI Responsivity

Rachel Zsido*, Eóin Molloy, Elena Cesnaite, Gergana Zheleva, Ulrike Scharrer, Regenthal Ralf, Arno Villringer, Vadim Nikulin, Julia Sacher

Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

Background: Over half of patients with major depressive disorder have inadequate responses to initial antidepressant therapy, leading to weeks of trial and error in an attempt to find the right medication. Neuroplasticity models of depression propose that dysfunctional neural networks underlie depression and that antidepressants may reduce symptoms via enhanced synaptic plasticity. Recent findings suggest that selective serotonin reuptake inhibitors (SSRIs), a class of antidepressants upregulating serotonergic transmission, induce plasticity via alterations in cortical excitation-inhibition balance. These findings, however, are mostly based on a single SSRI dose or animal models; empirical evidence from human research remains lacking, particularly during steady state SSRI intake. Given the highly variable response rates to SSRIs as well as the increased risk for depression in women and current lack of female samples in neuroscience research, there is a critical need to (I) understand the neurophysiological mechanisms underlying SSRI action in women and (II) identify a non-invasive biomarker to predict individual responsivity to treatment and treatment-effect monitoring.

Methods: We administered a commonly-prescribed and clinically-relevant dose of 20 mg escitalopram (fastest-acting SSRI) for seven days. Given known sex differences in response to SSRIs as well as the current lack of female samples in neuroscience research, we chose to use an all-female sample using oral contraceptives (to downregulate endogenous hormonal fluctuations). Fifty-nine participants (N = 28 escitalopram, 31 placebo) underwent resting-state electroencephalography at three assessments: before randomization (baseline), after a single dose of administration (single dose), and after one week of daily administration (time when steady state is achieved). We estimated the aperiodic component (1/f slope) of the power spectral density per channel, shown to reflect excitation-inhibition balance. We then subtracted 1/f decay from the original power spectral density and calculated power of alpha oscillations to assess oscillatory activity independent from aperiodic activity.

Results: Linear mixed modeling showed no differences in mean alpha power, but significant effects of time ($p<0.001$, R2c=0.39), group ($p=0.030$, R2c=0.39), and an interaction ($p<0.001$, R2c=0.41) for 1/f slope. Post-hoc cluster-based permutations tests showed widespread decreases in 1/f slope (i.e., increases in excitation-inhibition balance) in the escitalopram group at single dose ($p=0.002$, mean zelectrode=-2.85) and steady state administration ($p=0.026$, mean zelectrode=-2.47) as compared to placebo. Within the escitalopram group, 1/f slope changed across time: decreases from baseline to single dose ($p<0.001$, mean zelectrode=-3.14) and baseline to steady state ($p=0.004$, mean zelectrode=-2.70), and increases from single dose to steady state ($p<0.001$, mean zelectrode=2.77). Regression analyses showed that 1/f slope at baseline ($p=0.005$, R2adj=0.24) and at single dose ($p<0.001$, R2adj=0.46) predicted steady state response, and taking into account escitalopram plasma levels strengthened this relationship across the drug administration week (interaction: $p=0.040$, $\beta=0.40$, $t24=2.16$, BBCI 0.002 to 0.071).

Conclusions: By combining a novel measure to assess cortical excitation-inhibition balance and a rigorously-controlled interventional study design in health, we present first longitudinal evidence for increases in excitation-inhibition balance in response to one week of escitalopram intake. As both baseline and single dose 1/f slope signals were associated with steady state signal, and that taking into account escitalopram plasma levels strengthened this relationship, we propose that 1/f slope may serve as a mechanistic biomarker for predicting individual neural responsivity to SSRIs. With the continuously rising number of prescribed antidepressants and increased risk of depression in women, as well as the proposed role of excitation-inhibition balance in neuropsychiatric disorders, establishing this model in healthy women provides a well-timed conceptual framework to clarify the effects of SSRIs on human cortical excitation-inhibition balance. Our findings provide a platform for future translational research in clinical populations, such as depression and other neural plasticity-related disorders, to inform pharmacological treatment strategies at a single patient level.

Keywords: Selective Serotonin Reuptake Inhibitors (SSRIs), Women's Mental Health, EEG Biomarkers, Cortical Excitation-Inhibition Balance, Escitalopram

Disclosure: Nothing to disclose.

W94. Correction of Circadian Rhythm Abnormalities in Bipolar Depression is Specifically Associated With Lithium-Responsiveness

Monica Federoff*, Michael McCarthy, John Kelsoe

UCSD School of Medicine, San Diego, California, United States

Background: Bipolar disorder is characterized by episodes of depression and mania causing social impairment, disability and increased risk for suicide in affected patients. Other clinical features of BD include disruptions in circadian rhythms that negatively impact upon sleep, daytime activity, positive affect and cognition. These disruptions may be present at any phase of bipolar disorder, impacting depression, mania and long-term maintenance. Lithium is an effective therapy for BD, relieving the symptoms of depression and mania, and maintaining stability between episodes, but only 30-40% of patients are fully lithium responsive. Preclinical models show that lithium regulates the stability of "clock proteins" such as REV-ERBα and others that regulate circadian rhythms. However, it is unknown if the role of lithium on circadian rhythms is essential to the therapeutic effects of the drug and/or if it affects particular phases of bipolar disorder preferentially.

Methods: The pharmacogenetics of bipolar disorder (PGBD) was a 11-site, prospective, non-randomized open trial of lithium monotherapy for BD I. In a secondary analysis, we examined the relationships among lithium, circadian rhythm disruption and clinical response to lithium. Using items collected from standardized instruments (QIDS-SR16, CARS-M, TEMPS), we characterized baseline levels of circadian rhythm disturbances and trait morningness among 386 subjects in the PGBD cohort with varying past exposures to lithium. Next, we tracked mood symptoms over 12 weeks in a subset of 88 BD patients treated with lithium for the first time. After 12 weeks of treatment, patients were deemed lithium responsive (Li-R) or non-responsive (Li-NR). Mood symptoms were examined in detail to extract total scores for depressive and manic symptoms, as well as "circadian" symptoms of depression/mania limited to sleep and activity. For comparison, "affective" symptoms of depression/mania limited to positive and negative mood states were extracted. Changes in each symptom category over time was analyzed using analysis of covariance (ANCOVA) to adjust for age and sex.

Results: Over the 12-week treatment period, both Li-R and Li-NR subjects demonstrated a significant reduction in total and affective symptoms of depression. Only Li-R patients showed improvement in circadian symptoms of depression. For manic symptoms, Li-R and Li-NR improved to a similar extent in each symptom category.

Conclusions: Collectively, these data suggest that any exposure to lithium, even in patients not fully responsive to the drug, is associated with greater morningness and a reduced burden of circadian disruption. In patients with no prior treatment history with lithium, mood stability was selectively associated with the reduction of circadian depressive symptoms. Our results provide evidence that stabilization of circadian symptoms of depression may be an essential feature of lithium's therapeutic effects in BD I patients.

Keywords: Circadian Rhythms, Bipolar I Disorder, Lithium Response, Chrono-Pharmacology

Disclosure: Nothing to disclose.

W95. Age Affects Temporal Response, but not Durability, to Serial Ketamine Infusions in Veterans With Treatment Refractory Depression

Anusha Badathala, Brian Roach, Steven Pennybaker, Art Wallace, Daniel Mathalon, Tobias Marton*

UCSF and San Francisco VA Medical Center, San Francisco, California, United States

Background: Ketamine is a rapid-acting, poor durability, treatment for patients with treatment refractory depression (TRD). Little is known about which patient characteristics are associated with faster or more durable ketamine responses. We therefore investigated the interaction between age and the speed and durability of ketamine's antidepressant effects in veterans receiving serial intravenous ketamine infusions for TRD.

Methods: Beck Depression Inventory (BDI-II) scores from 49 veterans receiving six ketamine infusions (2x weekly) were examined. Percent change in BDI-II scores across six infusions were assessed with respect to patient age using a mixed-linear model. Follow-up analyses examined the age x time interaction at each infusion. To assess durability, percent change in BDI-II three weeks following the sixth infusion was examined with respect to age.

Results: There was a significant age x time interaction ($F=3.01$, $p=.0274$) across the six infusions. Beta estimates at each infusion showed a significant effect of age at infusion #4 ($B=.88\% +/-.29\%$, $t=3.02$, $p=.004$) and a trend towards significance at infusion #5 ($B=.62\% +/-.31\%$, $t=1.95$, $p=.057$). There was no correlation between percent change in BDI-II and age at three-week follow-up.

Conclusions: Older age is associated with a slower antidepressant response across six serial ketamine infusions, with a model-predicted difference of 8.8% in BDI-II score for each decade in age at infusion #4. Further, antidepressant durability at three weeks was not correlated with age. Age is an important moderating factor to consider in assessing patient response to ketamine, and differing mechanisms may underlie speed and durability of ketamine's antidepressant activity.

Keywords: IV- Ketamine, Late Life Depression, Treatment-Refractory Depression

Disclosure: Nothing to disclose.

W96. Discovery and Development of SEP-4199 and Characterization of its Enantiomer-Specific Pharmacology

Seth Hopkins*, Scott Wilkinson, Taryn J. Corriveau, Antony Loebel, Kenneth Koblan*

Sunovion Pharmaceuticals, Inc., Marlborough, Massachusetts, United States

Background: We report here the discovery that the pharmacology of amisulpride is enantiomer-specific with antagonist activity at the 5-HT7 receptor residing in the R-enantiomer. This discovery permitted development of SEP-4199, a non-racemic ratio of the R-enantiomer (aramisulpride) and the S-enantiomer (esamisupride) that has been engineered to minimize dopamine D2 blockade-related adverse effects while enhancing 5-HT7 receptor activity. Serotonin 5-HT7 receptors are highly distributed in the suprachiasmatic nucleus, hippocampus, cortex, thalamus and raphe nuclei and are involved in the central regulation of sleep and circadian rhythms, mood, and cognition. Compounds with selective 5-HT7 receptor antagonist activity have been shown to modulate rapid eye movement (REM) sleep and to have antidepressant effects in mouse models. We summarize here results of three studies conducted in discovering the ratio of aramisulpride and esamisupride that optimized the antidepressant efficacy of the compound for the treatment of severe forms of depression (eg, bipolar disorder, mixed mood/psychotic states).

Methods: Single isomers of amisulpride were synthesized and affinities were measured in radioligand binding assays. Study 101 then evaluated D2 and D3 receptor occupancies for a range of single oral doses of esamisulpride in healthy subjects using positron emission tomography (PET) and the 11C-PHNO radiotracer. Study 102 evaluated the antagonist activity of the aramisulpride at the 5-HT7 receptor in healthy subjects ($N=33$) using polysomnographic (PSG) assessment of rapid eye movement (REM) sleep suppression as a proxy measure. The study utilized a single-blind, placebo-controlled, 2-stage, 2-way, crossover design to evaluate the REM suppression effects of 2 single oral doses (340 mg; 600 mg) of the R-amisulpride enantiomer. The optimal ratio was the dose range of esamisulpride that achieved a low level of D2 occupancy (30-50%), combined with an amount of aramisulpride that maximized the 5-HT7 effect (detected by REM suppression) but with a combined dose that was ≤400 mg (the starting dose for racemic amisulpride). Study 103 was an open-label, single dose, PET study (11C-PHNO radiotracer) in healthy subjects ($N=11$) designed to measure the D2 receptor occupancy of oral doses of the selected non-racemic ratio SEP-4199.

Results: Aramisulpride and esamisulpride demonstrated 2 distinct stereoselective pharmacological profiles. In vitro radioligand binding studies demonstrated that esamisulpride binds with higher affinity to dopamine D2 receptors, with approximately 20-fold difference in affinity compared to aramisulpride (IC50 of 3.28 ± 0.77 nM and 80.0 ± 4.0 nM, respectively), whereas 5-HT7 receptor antagonism of amisulpride unexpectedly resides almost exclusively in aramisulpride (IC50 of aramisulpride 110 ± 6 nM is approximately 300-fold more potent than esamisulpride). In Study 101, a reliable dose-occupancy relationship was described by a 50% level of D2 occupancy at 92 mg (with a narrow 95% CI of 75.1 to 108.4 mg). In Study 102, aramisulpride doses of 340 mg and 600 suppressed REM time (LS mean reductions of 18 and 31 minutes, respectively) and REM percent (-4 and -6 points, respectively) compared to placebo. In Study 103, SEP-4199 (in a ratio of 85% aramisulpride to 15% esamisulpride) was generally well-tolerated, and exhibited linear and dose-dependent exposure parameters (Cmax, AUC[0-12h]) at single doses of 200-700 mg. For single SEP-4199 doses of 200-400 mg, peak (Cmax) D2 receptor occupancy ranged from 25% to 38%. The 50% receptor occupancy (RO50) dose for SEP-4199 was in the range of 600-900 mg.

Conclusions: The results of these three studies suggest that doses of 200 and 400 mg/d of SEP-4199 (in a ratio of 85% aramisulpride to 15% esamisulpride) will provide clinically significant 5-HT7 receptor antagonist activity, based on REM suppression as a proxy measure. In addition, this dose range provides a significant reduction in D2 receptor occupancy (peak range, 25% to 38%) which is expected to minimize dopamine blockade-related adverse effects. SEP-4199 is expected to have antidepressant and antimanic effects and is currently being developed for the treatment of bipolar depression.

Keywords: Bipolar I Depression, Atypical Antipsychotics, Positron Emission Tomography Imaging, Polysomnography

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

W97. What Does the Sucrose Preference Test Tell Us About Reward Behavior? An Analysis of Licking Behavior in the SPT

Andreas Wulff*, Scott Thompson

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: Depression is a mental illness expressed through cognitive, metabolic and affective symptoms. Rodent models for anhedonia have been widely used to understand the mechanisms underlying depression and in the development of efficacious and fast-acting antidepressants. Mice subjected to chronic stress will lose preference for a slightly sweet 1% sucrose solution, this is further restored following administration of antidepressants. However, it is uncertain how accurately the classical overnight sucrose preference test measures hedonic state or what the immediate experience of a rewarding sweet solution is. Lickometry allows the analysis of licking behaviors by recording individual licks as the mice drink. Aspects of the licking microstructure has previously been related to hedonic experiences. Here we use lickometry during the sucrose preference test to determine how it relates to hedonic experiences. We further test how this is changed in chronically stressed mice with or without antidepressant treatment. Finally, we combine lickometry with a conditioned place preference test to start separating rewarding and memory components of contextual reward memories.

Methods: We used lickometry to analyze the licking microstructure of animals during a classical sucrose preference test where they had the choice between drinking water or a 1% sucrose solution before and after corticosterone and psilocybin treatment. We further tested the scalability of the sucrose preference by letting the mice choose between a 1% and a 10% sucrose solution while recording the licking microstructure. Finally, we put the mice through a conditioned place preference task to test if they could remember the context of receiving a 10% sucrose solution before and after corticosterone and psilocybin treatment. We further recorded the licking microstructure during the conditioning phase to determine the relationship between the rewarding experiences and the following contextual memory.

Results: Mice exhibited a preference for 1% sucrose over water when measuring total number of licks as well and number of drinking bouts but not licks/bout. However, mice exhibited a preference for 10% sucrose over 1% sucrose when looking at total licks, number of drinking bouts, and licks/bout. Our analyses also suggest that the preference for the sweeter solution is only experienced in the first 3-4 hours of the test. During the conditioning phase of the conditioned place preference test, mice exhibited a clear preference for 10% sucrose over water when measuring total licks, number of drinking bouts, and licks/bout. The 10% sucrose further produced a lasting memory of the context, as evidenced by a subsequent small but robust preference for the sucrose-paired chamber. Experiments are underway to determine how chronic stress and psilocybin, a drug showing promise as a fast-acting antidepressant, change the licking microstructure and conditioned place preference at different levels of sucrose concentrations.

Conclusions: Here we show that lickometry can be used as a measurement of rewarding experiences and to test anhedonia and antidepressant efficacy. Moreover, we have shown that by combining lickometry and conditioned place preference, we will be able to distinguish how the neural substrates for reward and memory are affected by chronic stress and antidepressant treatment.

Keywords: Anhedonia, Depression, Reward Memory, Antidepressants, Psilocybin

Disclosure: Nothing to disclose.

W98. Protection From the Neurodevelopmental Consequences of Prenatal Stress

Rachel Schroeder*, Andrew Pieper, Hanna Stevens, Lynn Nguyen, Alexandra Loren

University of Iowa Carver College of Medicine, Iowa City, Iowa, United States

Background: Depression during pregnancy is difficult to treat because both antidepressant medicines and the illness itself can deleteriously impact the neurodevelopment of offspring. Therefore, new and effective treatments for maternal depression are needed. P7C3 compounds rescue depressive-like phenotypes in multiple rodent models. Additionally, when administered to mouse dams during gestation and nursing, no negative impacts on embryonic or early postnatal development are observed. We hypothesized that P7C3 compounds would protect offspring from the impacts of maternal chronic stress during pregnancy in a mouse model.

Methods: We examined offspring at both embryonic day 18 (E18) and as adults. In the latter group, offspring were weaned on postnatal day 21 (P21) and then group-housed by sex. To produce both groups, primiparous pregnant CD1 dams (n=6-8) were subjected to restraint stress under bright lights (45 min, 3x daily) from E5 to E18. P7C3-A20, a potent, well-studied analog of the P7C3 compound, or vehicle was administered to the dam via oral gavage twice daily from E5 through E18 or P21. In E18 offspring brain, changes in gene expression, levels of nicotinamide adenine dinucleotide, and markers of oxidative stress were measured. In adult offspring, elevated plus maze, repeated open field test, tail suspension, forced swim test, pre-pulse inhibition, and accelerating rotarod were used to measure learning and affective behavior changes. At least two weeks after completion of behavior assays, offspring brain was collected for immunohistochemistry and quantitative PCR assays. One male and one female from each litter were randomly selected for each assay, and 3-way ANOVA was used to screen for for sex differences. If no sex effects were found, data were pooled. Two-tailed, a priori t-tests were used to assess data for differences between stressed and non-stressed groups, and two-way ANOVA was used to detect main effects and interactions of stress and P7C3-A20 treatment, as well as post-hoc pairwise comparisons.

Results: Our results show that prenatal chronic stress caused both general and sex-specific learning deficits, and that learning deficits in offspring were rescued by treatment of pregnant dams with P7C3-A20. We also found independent effects of prenatal chronic stress and P7C3-A20 administration on affective-like behaviors. We identified changes of prefrontal and hippocampal GABAergic systems in offspring by prenatal chronic stress via stereological counts and GABA-related gene expression. Finally, in E18 brain, we found that prenatal stress disrupted the expression of NAD-producing enzymes, an effect that maternal P7C3-A20 rescued.

Conclusions: This study is the first report of a pharmacologic treatment that protects adult offspring from cognitive impairment after prenatal chronic depression-like stress. This protection is associated with rescue of aberrant expression of enzymes important in NAD synthesis, as well as restoration of normal GABAergic systems.

Keywords: GABAergic Interneurons, Pregnancy, Behavior, NAD + and NADH

Disclosure: Nothing to disclose.

W99. Harnessing Psilocybin: Antidepressant-Like Behavioral and Synaptic Actions of Psilocybin are Independent of 5-HT2R Activation in Mice

Natalie Hesselgrave, Timothy Troppoli, Andreas Wulff, Anthony Cole, Scott Thompson*

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: Faster, more effective antidepressant treatments are sorely needed. Recent open label trials indicate that psilocybin exerts a rapid and persistent antidepressant action in patients with treatment resistant depression. However, psilocybin induces strong 5-HT2R-dependent alterations of perception, limiting its clinical utility. Advancement of psilocybin as an antidepressant would be facilitated by a better mechanistic understanding of its therapeutic effects. The therapeutic efficacy of psilocybin has not been studied in well validated preclinical animal models of neuropsychiatric disorders. We asked whether psilocybin exerts an antidepressant-like behavioral and synaptic actions in mice subjected to chronic stress induced anhedonia. We further asked whether these beneficial actions would be observed when the 5-HT2ARs receptors responsible for the psychedelic effects are blocked.

Methods: Chronic multimodal stress was applied to male C57 mice daily for 14-21 days. Hedonic state was assayed using the sucrose preference and female urine sniff tests. Brain slices containing the hippocampus were obtained before and after stress and/or drug treatment. Extracellular recording was used to assay synaptic strength, quantified as changes in AMPA:NMDA ratios. Statistical comparisons were made using one-, two- and three-way repeated measure ANOVAs, followed by post-hoc tests.

Results: Chronically stressed mice displayed an anhedonic loss in preference for both sucrose and female urine. Injection of psilocybin (1mg/kg, ip) restored preferences for both sucrose and female urine 24 – 48 hrs after psilocybin injection, but vehicle injection did not. Hippocampal slices prepared from these mice revealed that AMPA:NMDA ratios were significantly higher in psilocybin-injected mice compared to vehicle controls. 5HT2Rs mediate the psychedelic response in humans. Neither the behavioral nor the synaptic responses to psilocybin were impaired by pretreatment with the 5HT2R antagonist ketanserin. Ketanserin significantly reduced head twitch responses after psilocybin injection, providing a positive control for its efficacy. Psilocybin also induced a decrease in local field potential oscillations in the delta frequency band, similar to EEG changes seen in human studies, and this effect was also blocked by ketanserin.

Conclusions: We present the first evidence that psilocybin exerts a rapid beneficial action in a well-studied and well-validated model of chronic stress-induced deficits in depression-relevant hedonic behaviors. We further suggest that, like other fast- and slow-acting antidepressants, psilocybin can restore stress-impaired excitatory synapses in key nodes of the cortico-mesolimbic reward circuitry, providing a neurobiological substrate for lasting improvements in psychological processing. Finally, we suggest that 5-HT2Rs, and thus psychedelic responses, may not be required for an antidepressant behavioral and synaptic response to psilocybin. The combination of psilocybin and a 5-HT2R antagonist may offer a safe and effective means to eliminate, attenuate, or shorten the duration of psilocybin-induced alterations of perception while retaining the therapeutic benefits, thus increasing the availability of this promising therapeutic approach.

Keywords: Psychedelics, Antidepressant, Depression, Synaptic Function, Chronic Stress

Disclosure: University of Maryland: Patent (Self)

W100. Using an Acute Drug Challenge to Predict Memantine Therapeutic Effects in Alzheimer's Disease: A Preliminary Proof of Concept and Feasibility

Neal Swerdlow*, Juliana Kotz, Jo Talledo, Joyce Sprock, Branko Guarte, Steven Huege, Jairo Romero, Michael Walsh, Lisa Delano-Wood, Gregory Light

University of California, San Diego, La Jolla, California, United States

Background: The NMDA receptor antagonist, memantine (MEM), is approved for treatment of moderate-to-severe Alzheimer's Disease (AD). While studies confirm MEM's effectiveness in slowing the progression of cognitive and behavioral disturbances in AD, the clinical response to MEM is modest, short-lived and highly heterogeneous. MEM also has acute effects on neurophysiological measures of early auditory information processing (EAIP), including the auditory steady state response (ASSR), mismatch negativity (MMN) and prepulse inhibition of startle (PPI): a single, 20 mg dose of MEM enhances EAIP assessed via these electro-encephalographic (EEG) and -myographic (EMG) measures. These acute effects of MEM on EAIP measures might serve as "biomarkers" of MEM sensitivity, since presumably, changes in auditory-evoked EEG and EMG responses reflect the action of MEM within the brain. We hypothesize that sensitivity to MEM effects on EAIP after an acute MEM challenge will identify AD patients who are most vs. least likely to experience clinical gains from MEM. To our knowledge, this "drug-challenge" approach towards assessing drug sensitivity via putative EAIP "biomarkers" in AD has not been studied. We now present preliminary (n=6) data from an ongoing study as a proof of concept and feasibility for this approach.

Methods: MEM-naïve patients with a confirmed AD diagnosis (n=6; ages 68-80y; M:F = 2:4) were carefully screened for comorbid medical, neurologic and psychiatric illness. Neurocognitive testing included the Mini-Mental State Examination (MMSE; range 10-24) and/or the Montreal Cognitive Assessment (MoCA; range 6-21) to establish study eligibility, a broader neurocognitive battery to characterize subjects and "baseline" tests for primary and secondary outcome measures: the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog; primary), the Neuropsychiatric Inventory Questionnaire (NPI-Q; secondary) and the Geriatric Depression Scale (GDS; secondary). Eligible subjects returned for 2 test days, 1 week apart. On test days, subjects were administered a pill (either placebo or MEM 20 mg) in a double-blind, order-balanced design. Laboratory measures included ASSR (Phase Locking (PLF), Power (GEP)), MMN, P3a amplitude and PPI, as well as the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Vital signs and subjective self-ratings were taken across each test day. One week after the 2nd test day, subjects were titrated to the target dose of MEM (10 mg bid); 1 subject subsequently reduced her dose to 15 mg/d due to unsteady gait. Outcome measures and a physical examination were completed 8 and 16 weeks after reaching the target dose (24-week data pending); subjects with outcome measures at week 8 were considered "completers", with an intention-to-treat, carry-forward design. Blinded data ("Pill A vs. B", effect sizes (Cohen's d)) from this preliminary cohort are presented to demonstrate feasibility of this "drug-challenge" strategy.

Results: Subjects completed assessments of acute MEM effects on EAIP measures and follow-up outcome measures after 8 (n=6) and 16 (n=4) weeks of MEM treatment. Pills A vs. B produced no differential autonomic and minimal subjective differences; differential effects on EAIP ranged from small (GEP, d = 0.10) to large (PPI (60 ms), d = 0.92). Clinical response to MEM (total of 8 performance items from ADAS-cog at the latest available treatment week vs. baseline) was compared between groups with the most (n=3) vs. least (n=3) robust response to acute drug challenge (difference score, Pill (A – B)) on "test day" measures.

Treatment effect sizes (d) were 0.21, 0.21, 1.54, 0.36, 4.25 and 0.96, based on most vs. least robust responses to PLF, GEP, MMN, P3a, PPI (60 ms) and RBANS, respectively. Similar analyses conducted for secondary outcome measures revealed effect sizes ranging from 0.00 – 1.26 for NPI-Q and 0.0 – 3.54 for GDS. Analyses based on autonomic and subjective effects of acute MEM challenge will also be presented.

Conclusions: It is feasible to assess EAIP responses to a single "challenge" dose of MEM, and to identify the relationship between these "biomarker" responses and subsequent clinical responses, in patients with AD. Specific logistical constraints of individual EAIP measures will be discussed. Our ongoing trial will apply more sophisticated analytic strategies in larger samples to determine whether MEM effects on these measures can serve as meaningful biomarkers that predict an individual AD patient's sensitivity to the clinical benefits of MEM. Supported by R01-AG059640 and P30-AG062429.

Keywords: Alzheimer's Disease, Memantine, Biomarker, Electroencephalography, Experimental Medicine

Disclosure: Nothing to disclose.

W101. Key Insights Gained From the Balovaptan Clinical Development Program

Suma Jacob*, Evdokia Anagnostou, Eric Hollander, Roger Jou, Nora McNamara, Linmarie Sikich, Russell Tobe, Declan Murphy, James McCracken, Elizabeth Ashford, Chris Chatham, Susanne Clinch, Janice Smith, Kevin Sanders, Lorainne Murtagh, Jana Noeldeke, Jeremy Veenstra-VanderWeele

University of Minnesota, Minneapolis, Minnesota, United States

Background: Balovaptan, a vasopressin 1a receptor antagonist, has been evaluated for effects on social communication and interaction in autism spectrum disorder (ASD). Here, we reflect on key insights from the balovaptan clinical development program.

Methods: VANILLA (phase 2, 12-week; NCT01793441), V1aduct (phase 3, 24-week; NCT03504917), and aV1ation (phase 2, 24-week; NCT02901431) were double-blind randomized controlled trials (RCTs) that enrolled 223 adult male, 305 adult, and 339 pediatric participants, respectively. Participants had ASD diagnosis confirmed by the Autism Diagnostic Observation Schedule, IQ ≥70, and Social Responsiveness Scale, 2nd edition (SRS-2) t score ≥66. VANILLA primary and secondary endpoints were change from baseline (Cfb) on SRS-2 and Vineland™-II Adaptive Behavior Scale (VABS), respectively. V1aduct and aV1ation primary endpoint was Cfb in VABS 2 Domain Composite (2DC; Social and Communication domains) score at week 24. In aV1ation and V1aduct, VABS interviews were audio recorded and co-scored by an independent centralized third-party rater for quality control.

Results: VANILLA did not meet its week 12 primary endpoint but did show clinically and statistically significant dose-dependent improvements in VABS. VABS 2DC primary endpoint was not met in V1aduct or aV1ation and no signals of efficacy were observed on secondary endpoints. A substantial placebo response was observed across different endpoints and informants. Placebo response varied by site type; in V1aduct, private research sites had greater improvements in VABS 2DC in the placebo group than academic research centers (mean [SD] improvement at week 24: 7.44 [13.13] vs 2.53 [7.94]). Sites with no prior experience of balovaptan clinical trials also had greater improvements in VABS 2DC in the placebo group versus those with prior experience of balovaptan trials (mean [SD] improvement at week 24: 7.43 [14.21] vs 5.07 [9.85]) in V1aduct. Placebo response was also greater with increased baseline severity, varied by outcome measure, and was greater for the Aberrant Behavior Checklist Lethargy/Social Withdrawal subscale than for VABS 2DC. Balovaptan demonstrated safety.

Conclusions: While balovaptan did not show efficacy in V1aduct and aV1ation, these trials span a broad age range and are the largest biomedical RCTs in ASD. The placebo response reflects likely treatment expectation, while repeat administration of VABS and differences in the experience level of administering raters may have influenced outcomes. This highlights an ongoing need to develop robust, objective outcome measures for sensitive assessment in this heterogeneous population, and the importance of reliably replicating positive trial results. Insights derived from this dataset should enhance future research in ASD across the lifespan.

Keywords: Autism Spectrum Disorder, Balovaptan, VANILLA, V1aduct, aV1ation

Disclosure: NIH: Grant (Self), Roche: Grant (Self), Roche: Advisory Board (Self), Fraser Community Clinics: Advisory Board (Self), Minnesota Independence College & Community: Advisory Board (Self)

W102. Methyl-Cpg-Binding Protein 2 Mediates Overlapping Mechanisms Across Brain Disorders

Snow Bach, Niamh Ryan, Paolo Guasoni, Aiden Corvin, Albert Sanfeliu, Daniela Tropea*

Trinity College Dublin, Dublin, Ireland

Background: MECP2 and its product, Methyl-CpG binding protein 2 (MeCP2), are mostly known for their association to Rett Syndrome, a rare neurodevelopmental disorder. Additional evidence suggests that MECP2 may underlie other neuropsychiatric and neurological conditions, and perhaps modulate common presentations and pathophysiology across disorders.

Methods: We established a procedure to quantify MeCP2 binding in silico using the combination of a position weight matrix (PWM) and DNA sequence GC content. Using the latest genetic studies, we use our procedure to identify the genes recognized by MeCP2 and associated to several neurological and neuropsychiatric disorders. We then use bioinformatics programs to uncover the mechanisms and the pathways modulated by MeCP2. Last, we use transcriptomic analysis in a mouse mutant for the MeCP2 gene, and permutation analysis to functionally validate our findings.

Results: We find that the genes controlled by MeCP2 are involved in three main processes: neuronal transmission, immunoreactivity, and development. Also, while the nervous system is the most relevant in the pathophysiology of the disorders, additional systems may contribute to MeCP2 action through its target genes. We validate our results with transcriptome analysis on MeCP2-null models, confirming that the genes identified by our procedure are directly modulated by MeCP2.

Conclusions: The study shows that MeCP2 may modulate similar mechanisms in several neurological and psychiatric conditions, suggesting that treatments for one condition may be effective for related disorders.

Keywords: MeCP2, GWAS, Biological Pathway Analysis

Disclosure: Nothing to disclose.

W103. Epigenome Wide Association Study of Disease Duration Prior to Trial Entry (DDPTE), a Quantitative Measurement for Alzheimer's Disease Continuum

Qingqin Li*, Timothy Schultz, Vaibhav Narayan

Johnson & Johnson Pharmaceutical, Titusville, New Jersey, United States

Background: Recently, an epigenome wide association study was conducted using the largest number of samples collected from

ADNI and differentially methylated positions (DMPs) associated with clinical diagnosis status (CN, MCI, vs AD) were reported.

Methods: In this study, we took into account that disease stage is a continuum and not a sudden transition from one clinical stage to another and use disease duration prior to trial entry (DDPTE) as a measurement for disease continuum and aimed to identify DMPs associated with the continuous measurement of disease continuum.

Results: We identified 128 DMPs associated with DDPTE with p-values passing Bonferroni correction threshold. Gene set enrichment analysis revealed significant enrichment (adjusted p-value < 0.05) for MAPK signaling pathway, calcium signaling pathway, neuroactive ligand-receptor interaction, endocytosis, axon guidance, focal adhesion/ECM-receptor interaction/adherens junction, and regulation of actin cytoskeleton. Over representation analysis also revealed gene sets involved in biological processes such as anterograde trans-synaptic signaling (FDR adjusted p-value = 7.77x10-8) and learning (FDR adjusted p-value = 0.01) being enriched.

Conclusions: A continuous measurement of AD disease continuum may represent a more powerful endpoint in epigenome wide association study in identifying DMPs.

Keywords: EWAS, Alzheimer's Disease, ADNI

Disclosure: Johnson & Johnson: Stock / Equity (Self)

W104. BPN14770 Alleviates Cognitive Impairment and Amyloid Pathology in APP/PS1/hPDE4D Mice

Jinpeng Lyu, Ying Xu, Shichao Gao, Mark Gurney, James ODonnell*

University at Buffalo, SUNY, Buffalo, New York, United States

Background: Alzheimer's disease (AD) is characterized clinically by progressive cognitive decline and pathologically by the accumulation of amyloid-β (Aβ) in the brain. Type 4 phosphodiesterase (PDE4) inhibitors such as rolipram could ameliorate cognitive deficits by upregulating intracellular cAMP level and activating downstream cAMP-PKA-dependent neuroprotective signaling. Although conventional PDE4 inhibitors have benefits on memory enhancement in preclinical and clinical studies, they were prevented to be developed as a novel class of memory-enhancing drugs due to the adverse effects such as gastrointestinal irritation. BPN14770, a novel allosteric inhibitor of PDE4D, exhibits high selectivity on PDE4D based on its close interaction with a single amino acid present at primate PDE4D.

Methods: In the present study, the humanized PDE4D (hPDE4D) mice crossed with APP/PS1 transgenic mice to generate APP/PS1/hPDE4D mice, which not only provide a unique and powerful genetic tool for assessing the PDE4D target engagement, but also decipher whether and how BPN14770 could enhance memory in AD mouse model.

Results: The results suggested that BPN14770 improved both early stage of memory impairment in the novel object recognition task, and mid- or late-stage of memory deficits in the Morris water maze and step-down passive avoidance tests. Particularly, there was an approximate 30-fold difference between doses of BPN14770 that induce behavioral changes between APP/PS1/hPDE4D and APP/PS1 mice, suggesting that BPN14770 was more potent in different stages of memory impairment, in APP/PS1/hPDE4D mice than those of APP/PS1 mice. Moreover, BPN14770 reduced AD-related soluble and insoluble Aβ levels and amyloid plaque burden in the cortical cortex and hippocampus of APP/PS1/hPDE4D mice.

Conclusions: These findings provide the useful evidence that BPN14770 has ideal binding sequence for subtype specific PDE4 inhibition with high efficacy for treatment of AD symptoms.

Keywords: Phosphodiesterase-4 (PDE4), Memory, Transgenic Mice

Disclosure: Nothing to disclose.

W105. TRV045, a Novel, Selective S1P1 Receptor Modulator is Efficacious in Rodent Models of Epilepsy, Without Affecting Lymphocyte Trafficking

Stefania Risso, Michael Kramer, Michael Fossler, Mark Demitrack*

Trevena, Inc., West Chester, Pennsylvania, United States

Background: Sphingosine 1-phosphate (S1P) acts on five distinct G-protein-coupled receptors (S1P1-5) to evoke responses including calcium mobilization, adenylate cyclase inhibition, and mitogen activated protein kinase activation. In the CNS, S1P 1, 2, 3, and 5 receptor subtypes are expressed on neurons, astrocytes, microglia, macrophages, and oligodendrocytes to influence cell survival, proliferation, migration, differentiation, and morphological changes. S1P1 receptors in particular are highly expressed on astrocytes and neurons and are important in brain development and perpetuation of brain inflammation, as well as in the formation and preservation of the blood brain barrier. Because of their functions and localization in the brain, S1P1 receptors should be investigated as a potential target for the treatment of epilepsy.

Fingolimod (FTY720), a non-selective S1P modulator is approved for the treatment of relapsing forms of multiple sclerosis. FTY720 has also been shown to demonstrate antiepileptogenic effects in both the rat lithium-pilocarpine model of status epilepticus and the pentylenetetrazole (PTZ)-induced kindling mouse model. Early long-term treatment of WAG/Rij rats with FTY720, before absence seizure onset, has shown antiepileptogenic and antidepressant-like effects. Although FTY720 has provided evidence that targeting S1P receptors has potential benefits as an epilepsy therapeutic, FTY720 is not selective for S1PR subtype 1, and importantly, affects lymphocyte trafficking, limiting its application in an epilepsy population.

Here, in collaboration with the NINDS Epilepsy Therapy Screening Program (ETSP), we report the antiepileptic properties of a new chemical entity, TRV045, in well-established rodent models of epilepsy. We also report evidence that TRV045 potently and selectively activates S1P1R, while having no effect on lymphocyte trafficking in rodents and non-human primates.

Methods: In the corneal kindled seizure model, male C57Bl/6 mice (18-25 g) were kindled electrically with corneal electrodes to a criterion of 5 consecutive stage 5 seizures (facial clonus and head nodding progressing to forelimb clonus, and finally rearing and falling accompanied by a generalized clonic seizure). Fully kindled mice were then stimulated every-other day until all mice within each group reached the criterion of 5 consecutive stage 5 seizures. Testing of TRV045 commenced 5-7 days after the last stimulation. Mice were stimulated on the day prior to TRV045 evaluation to ensure that all mice to be used in the drug study will present with a Stage 5 seizure. TRV045 was initially dosed at 10 mg/kg s.c. (n=8/group) and evaluated 1 and 2 hr after dosing to determine time of peak effects (TPE). An ED50 dose was then determined by dosing mice at 1, 2.5, 5, 7.5 or 10 mg/kg TRV045 s.c. (n=8/group) and an individual seizure score was determined 2 hr after dosing.

In a test based on maximal electroshock (MES) convulsions, 60Hz of alternating current (150 mA) was delivered in male SD rats for 0.2 seconds by corneal electrodes. Rats were tested to determine TPE combined with an initial assessment of tolerability at 0.25, 0.5, 1, 2, and 4 hr (n= 4/group) following the s.c. administration of 10 mg/kg TRV045. An animal was considered

protected from MES-induced seizures upon abolition of the hindlimb tonic extensor component of the seizure.

Results: TRV045 was efficacious in protecting mice against chronic secondarily generalized focal seizures in the corneal kindled mouse model. In the initial screen, 10 mg/kg TRV045 s.c. protected 2 out of 8 mice at 1 hr and 7 out of 8 mice at 2 hr, with average seizure scores of 2.38 and 0.63, respectively. In the full dose-response study, 1, 2.5, 5, 7.5 and 10 mg/kg TRV045 s.c. protected 1, 2, 3, 3 and 7 out of 8 animals at 2 hr post-dose, respectively. TRV045 produced a dose-dependent effect on seizure scores, ranging from 3.25 (1 mg/kg) to 0.63 (10 mg/kg), resulting in a calculated ED50 of 6.2 ± 0.7 mg/kg. Importantly, no adverse effects were observed in kindled animals at the doses tested.

TRV045 prevented seizures in the rat MES model of generalized tonic-clonic seizures. TRV045 dosed at 10 mg/kg s.c. prevented generalized seizures in 1, 2, 2, 1 and 0 out of 4 rats when tested 0.25, 0.5, 1, 2, and 4 hr post-dose, respectively. No adverse effects were observed at the 10 mg/kg dose level. Determination of the TRV045 ED50 dose when tested 1 hr post-dose is currently ongoing.

Conclusions: We have shown that the S1P1R¬selective compound TRV045 prevented generalized seizures in a rat MES model and demonstrated anti-convulsant activity in a corneal kindled mouse model. These data suggest that the novel mechanism of action of TRV045 may provide a new therapeutic option for the treatment of epilepsy.

Keywords: Sphingosine-1-Phosphate, Epilepsy, Animal models

Disclosure: Trevena, Inc.: Employee (Self)

W106. Global and Local Network Efficiency Abnormalities in Veterans With Mild Traumatic Brain Injury

Elias Boroda, Casey Gilmore, Randy Kardon, Alicia Fenske, Bryon Mueller, Timothy Hendrickson, Michael Armstrong, Kelvin Lim*

University of Minnesota, Minneapolis, Minnesota, United States

Background: In mild traumatic brain injury (mTBI), diffuse axonal injury results in disruption of functional networks in the brain and is thought to be a major contributor to cognitive dysfunction. This study utilized a graph theoretical approach to investigate alterations in global and local network efficiency based on resting-state functional connectivity (RSFC) in Veterans with mTBI compared to healthy Veterans. Our long-term objective is to identify biomarkers that can be used for early identification of those Veterans at risk for future functional decline.

Methods: 42 veterans with mTBI (mean age = 50.9 years; 5 women) and 33 age-matched control veterans (mean age = 47.7 years; 9 women) underwent two functional magnetic resonance imaging scans 18 months apart. Functional connectomes were extracted from RSFC data using a whole brain parcellation scheme composed of 17 distinct cortical networks. Graph theory functions were used to quantify network efficiency measures on a per subject basis. Global efficiency was characterized by computing characteristic path length, a measure of information transfer across the network. Clustering coefficient, a measure of functional segregation, was used to infer local efficacy. Hierarchical linear mixed models were used to examine longitudinal change in network efficiency. Models accounted for main effects of group (healthy vs mTBI), time (timepoint 1 vs timepoint 2), and their interaction

Results: Contrasts revealed that at timepoint 1, veterans with mTBI exhibited lower local efficacy ($t=3.57$, $p=.003$) and reduced global efficiency ($t=-3.30$, $p=.008$) compared to age-matched healthy veterans. Interestingly, our model revealed significant interactions between group and time for both global ($t=-3.65$, $p<.001$) and local network efficiency ($t=4.06$, $p<.001$). In the mTBI group, there was a significant reduction in characteristic path length ($t=5.34$, $p<.001$) across time, representing improved global information transfer. There was also a significant increase in clustering coefficient ($t=5.34$, $p<.001$), implying enhanced local efficiency across the 18-month timespan. In contrast, there were no significant changes across time in the healthy Veterans.

Conclusions: We found significant differences in global and local network efficiency on a whole brain basis between Veterans with mTBI and age-matched healthy Veterans. Veterans with mTBI presented with reduced network efficiency at timepoint 1 compared to healthy controls. These findings are consistent with disruption of long-range fibers as a result of traumatic axonal injury as revealed in diffusion MRI studies, which show that patients with greater white matter damage exhibit reduced network efficiency.

Keywords: TBI, Resting State Functional Connectivity, Graph-based Analysis, Veterans

Disclosure: Nothing to disclose.

W107. Dorsolateral Prefrontal Cortex Metabolites and Their Relationship With Plasticity in Alzheimer's Disease

Abstract not included.

W108. Haloperidol Does Not Affect Dynamic Structural Changes, Differentiation Efficiency or Cell Attachment in Monocyte-Derived-Neuronal-Like Cells (MDNCs) From Healthy Individuals

Anjali Iyer, Alonso Cortez-Resendiz, Alfredo Bellon*

Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania, United States

Background: Despite intense research, the pathophysiology of neurodevelopmental illnesses such as autism and schizophrenia remains obscure. Among the main obstacles is the lack of adequate models that: 1) Carry the genetic susceptibility to such illnesses, and 2) Replicate essential neurodevelopmental processes. We have recently developed a protocol to transdifferentiate blood circulating monocytes into neuronal-like cells in only 20 days and without reprograming. Monocyte-Derived-Neuronal-like cells (MDNCs) express several neuronal markers and present spontaneous action potentials as well as postsynaptic inhibitory and excitatory currents. Moreover, MDNCs delivers reproducible results in sequential samples from the same donors. We have also shown that these cells structurally resemble human developing neurons after five days in culture. 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. These structural dynamic changes are crucial developmental steps neurons undertake to establish connections with appropriate targets. Before studying dynamic structural changes in MDNCs from patients, we aim to determine whether MDNCs from healthy individuals treated with blood circulating levels of haloperidol present deficits in such structural changes.

Methods: Human monocytes were transdifferentiated following our published protocol. On day 4 of the transdifferentiation protocol a group of MDNCs were treated with 20ng/ml of haloperidol dissolved in methanol. Another group of MDNCs were treated with methanol only. The rest of MDNCs and the rest of the transdifferentiation protocol was performed as usual. On day 20, light microscopy pictures were taken using a Nikon Eclipse Ti-S/L 100 inverted microscope equipped with a CoolSNAP Myo, 20 MHz, 2.8 Megapixel, 4.54 x 4.54 µm pixels camera at time zero (T0) and then after an hour of incubation (T1hr). Random fields where

identified via a micro-ruled coverslip (Cellattice CLS5-25D, Nexcelom Bioscience). The exact same group of MDNCs were photographed at T0 and then at T1hr for all three conditions (control, vehicle and haloperidol). Each possible structural change was given a predetermined value. The sum of all structural changes is reported as a Structural Dynamic Index (SDI). Experiments were conducted with blood samples from 3 healthy individuals (2 men and 1 women). This study was approved by the Penn State Hershey Medical Center IRB (00006911). Statistical analysis was conducted via One-way ANOVA. P values of < 0.05 were considered significant.

Results: First we determined whether haloperidol altered cell differentiation via phenotype as previously described. MDNCs cultured under control conditions presented a differentiation average of 7.5 ± 0.9% (mean ± SEM, n = 1543), while the vehicle showed 10.2 ± 2.7% (mean ± SEM, n = 1500) and haloperidol 9 ± 1.4% (mean ± SEM, n = 2531). No statistical differences were encountered [$F(2, 11) = 0.466$, $P = 0.63$]. We then studied if there were differences in the number of MDNCs detached after one hour of incubation. Control conditions led to 0.029 ± 0.01 detached cells (mean ± SEM, n = 96), whereas the vehicle resulted in 0.0067 ± 0.006 of detached cells (mean ± SEM, n = 116) and haloperidol, 0.019 ± 0.006 (mean ± SEM, n = 186). Again, no statistical differences were identified [$F(2, 11) = 1.1$, $P = 0.36$]. Finally, we assessed whether haloperidol or methanol impacted MDNCs structural dynamic changes. MDNCs that received our standard differentiation protocol showed an SDI of 5.25 ± 0.6 (mean ± SEM, n = 96), while those cells cultured with methanol had 6.3 ± 0.8 (mean ± SEM, n = 116) and MDNCs treated with haloperidol presented an SDI of 5.1 ± 0.4 (mean ± SEM, n = 186). There were no statistical differences between the three groups [$F(2, 11) = 1.0$, $P = 0.38$].

Conclusions: Blood circulating levels of haloperidol does not affect dynamic structural changes, differentiation efficiency or cell attachment in MDNCs from healthy individuals. These results open the possibility to study dynamic structural changes in MDNCs from patients with neurodevelopmental illnesses even if they are treated with this first-generation antipsychotic.

Keywords: Stem Cells, Dendrites, Axons, Neurodevelopmental Disorders, Monocytes

Disclosure: Nothing to disclose.

W109. Transcriptome-Scale Spatial Gene Expression in the Human Dorsolateral Prefrontal Cortex

Kristen Maynard*, Leonardo Collado-Torres, Lukas Weber, Cedric Uytingco, Brianna Barry, Stephen Williams, Joseph Catallini, Matthew Tran, Zach Besich, Madhavi Tippani, Jennifer Chew, Yifeng Yin, Joel E. Kleinman, Thomas M. Hyde, Nikhil Rao, Stephanie Hicks, Keri Martinowich, Andrew Jaffe

Lieber Institute for Brain Development, Baltimore, Maryland, United States

Background: The spatial organization of the brain is fundamentally related to its function. This structure-function relationship is especially apparent in the context of the laminar organization of the human cerebral cortex, where cells residing within different cortical layers show distinct gene expression patterns and exhibit differing patterns of morphology, physiology, and connectivity. To the extent that structure entrains function, understanding normal brain development as well as disorders of the central nervous system will require identifying the cell types that make up the brain, and ultimately linking functional correlates of individual cell classes with structural architecture. Advanced approaches like spatial transcriptomics can quantify RNA transcripts within tissue architecture thereby retaining both anatomical and transcriptome-scale molecular information. 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 refine cell type-specific molecular signatures in the human brain.

Methods: To further our understanding of gene expression within the context of the spatial organization of the human cortex, we used the recently-released, 10x Genomics Visium platform, a novel barcoding-based transcriptome-wide spatial transcriptomics technology, to generate spatial maps of gene expression in the six-layered dorsolateral prefrontal cortex (DLPFC) of the adult human brain (n=3 neurotypical donors).

Results: Using Visium technology, we identified extensive layer-enriched gene expression signatures, and refined associations to previous laminar marker genes. We overlaid our laminar expression signatures onto large-scale sn-RNAseq data, enhancing spatial annotation of expression-driven clusters. By integrating neuropsychiatric disorder gene sets, we showed differential layer-enriched expression of genes associated with schizophrenia and autism spectrum disorder, highlighting the clinical relevance of spatially-defined expression. We then developed a data-driven framework to define unsupervised clusters in spatial transcriptomics data, which can be applied to other tissues or brain regions where morphological architecture is not as well-defined as cortical laminae. We lastly created a web application for the scientific community to explore these raw and summarized data to augment ongoing neuroscience and spatial transcriptomics research (http://research.libd.org/spatialLIBD).

Conclusions: Our study demonstrates that the Visium spatial transcriptomics platform is capable of analyzing gene expression with high spatial resolution within the existing architecture of the human DLPFC. We demonstrate the ability to integrate Visium with snRNA-seq data for spatial registration, further increasing the utility in discovering patterns of gene expression within spatially defined cell populations in the normal brain as well as the brains of individuals with neuropsychiatric disorders. We provide analytical resources and proof of concept examples for how this data can be used to understand human brain function and disease.

Keywords: Transcriptomics, Single-cell RNA Sequencing, DLPFC

Disclosure: Nothing to disclose.

W110. Effect of Treatment With Paliperidone Palmitate Versus Oral Antipsychotics on Frontal Lobe Intracortical Myelin Volume in Patients With Recent-Onset Schizophrenia: Magnetic Resonance Imaging Results From the Dream Study

Todd A. Tishler*, Benjamin M. Ellingson, Giacomo Salvadore, Pamela Baker, Ibrahim Turkoz, Amy O'Donnell, Kenneth L. Subotnik, Camilo de la Fuente-Sandoval, Keith H. Nuechterlein, Larry Alphs

UCLA, Los Angeles, California, United States

Background: White matter abnormalities in patients with schizophrenia have been extensively reported in the literature from postmortem and imaging studies. These abnormalities are present early in the course of illness, and their progression may be prevented by consistent treatment with atypical antipsychotics. Intracortical myelin (ICM) is found predominantly in the deep layers of the cortex and is a key determinant of neuronal synchrony and plasticity. Studies conducted in the past two decades suggest that intracortical myelination is disrupted early in the course of schizophrenia and shows progressive decline over time. Longitudinal analyses in a small randomized controlled trial suggest that treatment with long-acting injectable atypical antipsychotics may reduce or prevent these abnormalities compared with treatment with atypical oral antipsychotics (OAPs). In this study, we investigated the changes in brain ICM volume in

the frontal lobe following 9 months of treatment with paliperidone palmitate (PP) long-acting injection compared with 9 months of treatment with an OAP in participants with recent-onset schizophrenia. The imaging findings reported here are part of a large prospective randomized study that compared the effects of PP with those of OAPs.

Methods: The Disease Recovery Evaluation and Modification (DREaM: NCT02431702) study was a prospective, matched-control, randomized, open-label, active-controlled, flexible-dose, multicenter study that assessed time to first treatment failure and measured changes in cognition, functioning, and ICM volume in participants treated with PP versus an OAP (oral paliperidone extended-release [ER] or risperidone). Treatment with aripiprazole, haloperidol, olanzapine, perphenazine, or quetiapine was permitted in cases of inadequate response to paliperidone ER or risperidone. All participants had a diagnosis of schizophrenia or schizophreniform disorder and onset of psychosis within 24 months of screening. The DREaM study had 3 phases: Part I was a 2-month oral run-in to assess the tolerability of oral paliperidone or risperidone; Part II was a 9-month treatment phase in which one-third of participants were randomized to PP and two-thirds to OAP; Part III was an additional 9 months of treatment in which participants receiving PP continued on PP (PP/PP) and participants receiving OAP were rerandomized to either PP (OAP/PP) or OAP (OAP/OAP). The study was conducted at 34 sites in the United States, Brazil, and Mexico and enrolled 273 participants. Magnetic resonance imaging (MRI) scans were optional and were planned in approximately half of enrolled participants. Scans were performed at baseline, day 92, and day 260 (9 month time point) in Part II. Three sites were selected to perform the MRI brain imaging component of the study. All images were sent to a central site for analysis. Brain ICM volume was measured through a subtraction technique using inversion recovery and proton density MRI sequences focused on the frontal lobe. The imaging raters at the central site were blinded to the treatment assignment and the clinical and demographic characteristics of the participants. Frontal ICM volume was quantified as a fraction of total intracranial volume.

Results: In the MRI intent-to-treat (ITT) analysis population (71 participants: PP, 23; OAP, 48), the mean (SD) age was 22.9 (3.66) years in the PP group and 22.7 (3.89) years in the OAP group. Most participants were aged 20 to 24 years (PP, n=12 [52.2%]; OAP, n=26 [54.2%]) and were male (PP, n=18 [78.3%]; OAP, n=40 [83.3%]). Baseline-adjusted ICM fraction values did not differ between groups (PP, 0.057; OAP, 0.058). By day 92, the adjusted ICM fraction in the OAP group had decreased significantly (-0.002, P=0.001), whereas the adjusted ICM fraction remained unchanged in the PP group (-0.000, P=0.80). Although the change from baseline did not differ significantly between treatment groups (P=0.147), the sample was small. A medium effect size in the predicted direction was observed (Cohen's d=0.50). At day 260, the directional difference between groups was similar and again of medium effect size (Cohen's d=0.52).

Conclusions: The present findings extend prior results and suggest that abnormalities in frontal ICM may be an early biomarker of the progression of schizophrenia. In recent-onset schizophrenia, changes appear to progress more in participants treated with an OAP compared with those treated with PP. The primary MRI variable, ICM fraction, decreased significantly during OAP treatment, whereas it was preserved at baseline levels during PP treatment. The differences in effects on ICM fraction between treatments were of medium effect size but were not statistically significant; however, the study sample for MRI was small. This study suggests that treatment with long-acting injectable antipsychotics may preserve ICM in individuals with recent-onset schizophrenia. These findings need to be replicated in further studies with a larger sample size.

Keywords: Schizophrenia, Paliperidone Palmitate Long-Acting Injection, Intracortical Myelin

Disclosure: Nothing to disclose.

W111. Poster Withdrawn

W112. The Effects of Prebiotic Treatment in Schizophrenia: Results of a Pilot Study

Deanna Kelly*, Bhuvaneswari Kotnana, Maureen Kane, Claire M. Fraiser, McKenzie Sayer, Silvia Beurmann, Gopal R. Vyas, Charles M. Richardson, Fang Liu, Robert Buchanan

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: Emerging research suggests that disruptions of the normal flora in the gut microbiota may affect brain development and function and play a role in psychiatric disorders. Butyrate is one of the three major short chain fatty acids (SCFA) that are produced by bacterial fermentation in the gut and plays a critical role in maintaining the integrity of the gut/blood barrier and in several aspects of brain development, including cognitive function. People with schizophrenia are characterized by marked cognitive impairments and currently no known treatments are available to improve cognitive performance. In this study, we examined the effects of oligosaccharide-enriched inulin treatment (OEI, Prebiotin®), a prebiotic that is taken up by the gut bacteria to produce butyrate, on psychiatric symptoms and cognitive changes.

Methods: In this 2-week open-label pilot study, we enrolled participants with a DSM-5 diagnosis of schizophrenia or schizoaffective disorder, between the ages of 18-64 years, and who were hospitalized for at least 7 days, were treated with an antipsychotic without dose changes in the last 14 days and had no antibiotic, prebiotic or anti-inflammatory treatments within the last 3 months. All participants received 2 weeks of OEI (4 grams three times daily) with standard inpatient meals. We measured butyrate levels at baseline and end of study both pre- and post-dose (2 hour) of OEI and food (i.e., pre- and post-prandial). Psychiatric symptoms were evaluated with the Brief Psychiatric Rating Scale (BPRS), the Schedule for Assessment of Negative Symptoms (SANS), the Clinical Global Impression Scale (CGI), and the MATRICS Consensus Cognitive Battery (MCCB). We also collected stool samples nearest baseline and endpoint. Descriptive statistics are included for other variables due to the small sample size and only changes >15% are noted. Changes in butyrate levels were evaluated using Cohen's D effect size measurements for pre- to post-changes at baseline and endpoint.

Results: We enrolled five participants: all of whom were taking olanzapine or clozapine and smoked cigarettes. Three of the five participants were female and four of five were African American. The mean age of illness onset was 18.8 ± 7.0 years and mean age at the time of study was 38.1 ± 8.5 years. We observed a 28% increase in the MCCB composite score over the 2 weeks of the study; in particular, there was a 28% increase in visual learning, a 31% increase in processing speed and a 16% increase in attention performance. On the BPRS, we observed a 15% decrease in psychosis and a 23% decrease in hostility symptoms. We observed little to no change in SANS total score or on other BPRS symptom dimensions. Only 3 participants had baseline and endpoint pre- and post-prandial butyrate levels and stool samples. In the absence of OEI treatment, there was essentially no change in serum butyrate levels pre- to post-prandial (change in serum butyrate = -0.95 μg/ml (0.87% decrease). In contrast, after two weeks of OEI treatment, the pre- to post-prandial change in

serum butyrate levels was 25.3 µg/ml, a 24.0% increase (Effect size= 1.32). Microbiome data shows that OEI treatment caused a change in the microbiome diversity, associated with increased relative abundances of known butyrate producing bacteria, such as Bifidobacteria, Roseburia and Coproccus. Side effects were negligible.

Conclusions: Based on our preliminary results, OEI treatment in people with schizophrenia has a promising therapeutic potential. Future studies should replicate these initial findings and OEI may be an effective treatment for cognitive dysfunction. We are currently conducting an NCCIH funded R61/33 clinical trial to examine the effects of OEI on butyrate in a double blind randomized clinical trial.

Keywords: Schizophrenia, Butyrate, Prebiotic Treatment in Schizophrenia, Schizoaffective Disorder

Disclosure: Alkermes: Consultant (Self)

W113. Efficacy of Add-On Sulforaphane for Improving Symptoms and Cognition in Schizophrenia: A Randomized Double-Blind Study

Faith Dickerson*, Robert Yolken

Sheppard Pratt Health System, Baltimore, Maryland, United States

Background: The consumption of cruciferous plants such as broccoli and cauliflower has been associated with a reduced risk of cancer and other chronic diseases. This beneficial effect has been ascribed largely to the plants' high content of glucosinolates which are converted to isothiocyanates such as sulforaphane. Sulforaphane crosses the blood brain barrier and has antioxidant and anti-inflammatory activities. A previous trial in males with autism found that adjunctive sulforaphane was associated with improvements in some indicators of social functioning and aberrant behavior. The primary aim of the current study was to evaluate the safety and efficacy of an adjunctive sulforaphane nutraceutical for individuals with schizophrenia in a placebo-controlled, randomized double blind trial.

Methods: : Individuals with schizophrenia or schizoaffective disorder, most of whom had long-standing illness and who had residual psychotic symptoms of at least moderate severity were randomized to receive 6 tablets per day of 16 mg of glucoraphanin, which is metabolized following ingestion yielding approximately 100 micromoles of sulforaphane, or identical-appearing placebo added to usual psychiatric medications. The study duration was 16 weeks following a 2-week placebo run-in. The primary outcome was change in the severity of psychiatric symptoms, measured biweekly by the Positive and Negative Syndrome Scale (PANSS) over the double-blind phase. The secondary outcome was change in cognitive functioning, measured by the MATRICS Consensus Cognitive Battery (MCCB), from the beginning to the end of the trial. Mixed effects models were used to evaluate the relationship between the administration of the sulforaphane precursor and change in symptoms or cognitive functioning during the study period. Exploratory analyses were performed to examine the association between levels of the sulforaphane metabolite, dithiocarbamate, in urinary samples and changes in the outcome measures.

Results: A total of 64 participants were randomized (mean age 44.0 (±12.0) years); 58 participants, 29 in the active arm and 29 in the placebo arm, completed the 18 weeks of the trial. There were no significant differences in the change of positive, negative, general, or total PANSS symptom scores between groups including all of the randomized participants or the subgroup of individuals who completed the study. There was also no significant improvement in MCCB total or domain scores by treatment group in the entire cohort. However, there was a significant association between glycophorin treatment and improvement in the MCCB working memory domain in individuals with urine concentrations of dithiocarbamate of > 1 mmol/L . Reasons for the differences in sulforaphane metabolism are not known with certainty but may be related to host genetics, the composition of the gastrointestinal microbiome, or medication compliance. The study medication was well tolerated with no significant difference in the number of adverse events between groups.

Statistics: From mixed effects models for the primary outcome: for the PANSS total symptoms, Coefficient = .7754, 95% CI -5.304, 6.855, p=0.803; for PANSS Positive symptoms, Coefficient = .0003, 95% CI -2.551, 2.552, p=1.0; for PANSS negative, Coefficient = .9197, 95% CI -1.259, 3.099, p=0.408; for PANSS General symptoms, Coefficient = -.0311785, 95% CI .1320388, .0696817, p=0.545. For the change in MCCB Working Memory domain by urinary DTC: Wald Test, $F(2, 47) = 4.35$. $p = 0.0185$

Conclusions: Anti-oxidant agents may improve some aspects of cognitive functioning in individuals with schizophrenia

Keywords: Dietary Supplement, Cognitive Functioning, Clinical Trial

Disclosure: Nothing to disclose.

W114. A Linear Model of Subjective Value Best Captures Effort Discounting in Schizophrenia Patients With Motivation Impairment

Greer Prettyman*, Paige Didier, Sheila Shankar, Theodore D. Satterthwaite, Christos Davatzikos, Warren Bilker, Mark Elliott, Kosha Ruparel, Joseph Kable, Daniel Wolf

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Negative symptoms of schizophrenia including impaired motivation are a significant driver of disability and constitute a major unmet therapeutic need. Progress toward novel therapeutics for negative symptoms necessitate a greater understanding of the pathophysiology of amotivation using behavioral and neural techniques. We applied an fMRI Effort Discounting Task (EDT) that quantifies motivation using a neuroeconomic decision-making approach, capturing the degree to which effort requirements produce reductions in (discount) the subjective value (SV) of monetary reward. Much existing neuroeconomic discounting literature postulates a hyperbolic discounting function, but we consider a linear model of effort discounting to be theoretically superior for modeling effort discounting behavior and thus hypothesized it would prove empirically superior as well. We also hypothesized that brain motivation circuitry including ventral striatum (VS) and anterior cingulate (AC) would encode SV, integrating reward and effort costs. We expected reduced task effort and impaired SV encoding in schizophrenia (SZ) that correlates with dimensional severity of clinical amotivation.

Methods: A sample of 22 patients with SZ (stable/medicated) and 23 group-matched controls (CT) performed an EDT during 3T fMRI. In each of 200 trials, subjects chose between higher-effort/higher-reward (HARD) and lower-effort/lower-reward (EASY) options. The required effort involved repetition of easy but attention-requiring trials (choosing which of 2 numbers is larger). The reward and effort magnitudes for the HARD option were parametrically and independently varied across trials. Behavioral analysis of EDT applied a quantitative neuroeconomic model capturing how the SV of a decision to perform an effortful task incorporates the tradeoff between monetary reward and effort costs, using the linear equation SV=A-B*E. This equation describes how the SV of a particular monetary reward amount (A) is reduced or "discounted" as the effort cost (E) needed to obtain it increases. Higher values of the estimated free parameter B indicate a stronger negative impact of effort on subjective value, and hence lower motivation. We compared this linear model to a hyperbolic

model SV=A/(1-B*E) and a parabolic model SV=A-B*E^2. fMRI analysis focused on VS, AC, vmPFC, and associated valuation and decision-making circuitry using both ROI and voxelwise analyses. The primary clinical outcome was amotivation on the CAINS negative symptom scale.

Results: As expected, EDT choices were accurately predicted by the linear model, but poorly predicted by the parabolic model and by the hyperbolic model used in nearly all temporal discounting studies and a significant proportion of prior effort discounting work. Of the 45 participants, behavior was best fit by a linear model for 26, parabolic for 12, and hyperbolic for 7. All 7 of the participants whose behavior was best described with a hyperbolic model were healthy controls. Participants best fit by the linear model had significantly greater amotivation than those fit best by the other models. ($p < .05$). Quality of model fit (Akaike Information Criterion) and estimated individual discounting parameters for each model did not differ based on participants' percent of hard options selected or overall cognitive ability. VS and AC, as well as a broader cortico-limbic network, were activated during the decision task and this activation correlated positively with SV using the linear discounting model. The correlation with SV reflected both a strong inverse relationship with parametric effort variation, and a weaker positive relationship with parametric variation in monetary reward. VS activation to task decisions (independent of parametric variation in reward and effort) was selectively reduced in SZ, and VS hypofunction was correlated with amotivation in both SZ and controls (small-volume cluster-corrected p's<0.05).

Conclusions: Findings demonstrate that VS and AC, together with a broader cortico-limbic network, integrate effort costs and reward benefits into subjective values that drive decision-making, consistent with prior work in healthy individuals as well as animal models. We found evidence that a linear model of effort discounting best represents calculation of SV. None of the SZ patients' behavior was best described by the commonly used hyperbolic model, suggesting that a linear model may be especially advantageous for correctly modeling effort discounting in clinical populations with amotivation. A linear model also makes better theoretical sense for effort-based decisions since a high effort choice could have a negative subjective value, in contrast to temporal discounting decisions which would plateau at an SV of zero. This distinction will be particularly relevant for individuals with low motivation who are expected to have negative SV for high effort options, and for tasks like ours that incorporate a wide range of effort requirements. The fMRI abnormalities we observed in SZ were selective for VS, and within this region correlated with severity of amotivation. This adds to growing evidence that VS is a critical region for motivation impairment in SZ as well as other psychiatric conditions. Accurately modeling decision behavior in psychiatric populations will help to elucidate neural computation of subjective value and the role of VS in amotivation.

Keywords: Functional MRI (fMRI), Schizophrenia (SCZ), Neuroeconomics

Disclosure: Nothing to disclose.

W115. Adolescent Social Isolation Alters Reward-Seeking Behavior and Increases ΔFosB Expression in Mice

Michael Noback*, Gongliang Zhang, Noelle White, Gregory Carr, James Barrow

Johns Hopkins School of Medicine, Baltimore, Maryland, United States

Background: Social isolation (SI) during adolescence is a risk factor for psychiatric illness in adulthood. Rodent models of SI have shown various behavioral and neurological abnormalities, but so far there has been no conclusive mechanism found linking SI to the observed phenotype. A potential mediator of SI is the transcription factor ΔFosB, a truncated isoform of FosB which accumulates following chronic stress. Increases in ΔFosB have also been observed in response to chronic drug exposure, and have been linked to increases in reward-seeking behavior in rodents.

Human studies of SI have shown a phenotype of impaired cognition and an increased risk of substance use disorders. The continuous performance task (CPT) is a touchscreen-based task that is sensitive to changes in sustained attention and vigilance, and is translatable between humans and rodents. In this study, we investigated changes in ΔFosB expression in the brain of SI mice, and administered the CPT to SI mice to further clarify the behavioral phenotype associated with isolation. We also tested mice in a fixed ratio/progressive ratio paradigm to assay reward-seeking behavior.

Methods: Mice were socially isolated during adolescence (days 21-35) and then re-housed with a littermate until adulthood, when testing was conducted. Molecular screening consisted of Western blots for ΔFosB and FosB in the medial prefrontal cortex, hippocampus, and striatum ($n = 10$ group-housed males, 10 group-housed females, 8 SI males, 9 SI females). Behavioral testing consisted of the CPT ($n = 6$ group-house males, 6 group-housed females, 7 SI males, 8 SI females), as well as a fixed ratio/progressive ratio task ($n = 12$ group-housed males, 14 SI males).

Results: Molecular screening revealed increases in ΔFosB in several regions of the brain, most notably the medial prefrontal cortex ($p < 0.01$) and hippocampus ($p < 0.05$). FosB increases were also observed in female mice ($p < 0.05$).

Behavioral testing showed a significant increase in the sensitivity (d') of SI male mice in the CPT compared to control ($p < 0.05$). Female SI mice did not show changes in CPT scores compared to control. As the SI mice were hypothesized to have lower scores than control, further testing in the form of a fixed ratio/progressive ratio task was conducted to investigate changes in reward-seeking behavior due to SI. Reward-seeking behavior as measured by progressive ratio break point was significantly increased in SI male mice ($p < 0.05$).

Conclusions: The increases in ΔFosB, as a known marker of chronic stress that is also associated with reward-seeking behavior, are a promising step towards elucidating the mechanism translating the experience of SI into the observed phenotype. Sex-based differences in the ΔFosB/FosB expression profiles also provide valuable information about the manifestation of the SI phenotype. The CPT and progressive ratio results highlight an interesting interaction between the effects of SI on attention and its effects on reward-seeking behavior. The reward-seeking behavior changes in particular are notable because of the potential application to the susceptibility to substance use disorders seen in humans.

Keywords: Social Isolation, Chronic Stress, Touchscreen, Continuous Performance Task, Reward-Seeking Behavior

Disclosure: Nothing to disclose.

W116. Reduced EEG Alpha Peak Frequency and its Relationship With Cognition in Individuals With Psychosis Compared to Healthy Controls

Ian Ramsay*, Peter Lynn, Brandon Schermitzler, David Leipold, Scott Sponheim

University of Minnesota, Minneapolis, Minnesota, United States

Background: Individual alpha peak frequency (IAPF) is the frequency of the strongest alpha oscillation observed by electroencephalography (EEG) during a resting state, and has been shown to be low in psychotic disorders such as schizophrenia. In

healthy individuals, higher IAPF has been shown to be predictive of cognitive abilities, while reduced IAPF reflected impaired cognition in developmental and attention disorders. While cognitive deficits are consistently observed in psychosis, the relationships between IAPF and cognition remain relatively unexplored. We examined the relationships between IAPF and cognition during resting EEG to establish the functional relevance of aberrations in this neural marker of individual differences in resting brain state.

Methods: Patients with psychosis (N=160) and healthy controls (N=114) drawn from 4 separate studies all underwent 4 minutes of eyes open (EO) and eyes closed (EC) resting EEG. Data from 62 electrodes was resampled (524 hz), epoched (250 ms), and submitted to an ICA procedure to remove artifactual signals before undergoing power spectral density analysis. IAPF was identified as the frequency with the highest spectral power between 7 and 13 hz. At each electrode site, we compared IAPF between groups and examined relationships between IAPF and cognition measured by the Wechsler Abbreviated Scale of Intelligence (WASI). We also examined the relationship between positive and negative symptoms and IAPF in the psychosis group alone. All analyses controlled for age, gender, and study, and were corrected for multiple comparisons using the false-discovery rate (FDR).

Results: IAPF was reduced in the psychosis group compared to healthy controls at all electrode sites in the EC condition, while differences in the EO condition were most prominent in frontal and central electrodes (FDR $p<.05$). Across groups and electrode scalp locations, EC IAPF related to processing speed, perceptual reasoning, and verbal reasoning (FDR $p<.05$), while no relationships were found with working memory. A similar but more restricted pattern was observed in the EO condition. Neither EC or EO IAPF related to positive or negative symptoms in the psychosis group.

Conclusions: We observe a widespread reduction IAPF in the psychosis group compared to controls in both EC and EO conditions. Reduced IAPF related to impaired perceptual and verbal reasoning and slower processing speed, but did not relate to working memory or psychotic symptoms. Reduced resting peak alpha frequencies may serve as a relevant index of cognitive dysfunction and a viable treatment target for interventions targeting these deficits in psychosis.

Keywords: EEG Biomarkers, Resting State, Alpha Oscillations, Cognition, Psychosis

Disclosure: Nothing to disclose.

W117. The Association Between the Neural Response to Psychosocial Stress and Affective Reactivity to Real-Life Stressors in Early Stages of Psychosis

James Waltz*, Thomas Vaessen, Evelyne van Aubel, Ulrich Reininghaus, Inez Myin-Germeys

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: Stress plays an important role in the development of psychotic illness. Everyday stressful situations have consistently been shown to elicit larger and longer-lasting effects on affect in individuals with psychosis than in healthy individuals. This seems to be especially true of individuals at ultra-high risk (UHR) for psychotic illness, in the prodromal phase of psychotic illness, or in the first episode of psychosis (FEP). At the same time, the physiological stress response appears to be blunted in psychosis. On the neuronal level, patients with schizophrenia, individuals at familial risk for psychosis, and individuals with negative symptom schizotypy all show a blunted response or greater deactivation to stress in limbic and frontal areas when compared to healthy controls. No study to date has investigated whether stress-related activity in these areas is associated with affective reactivity to daily-life stressors in early stages of psychosis.

Methods: We recruited 28 individuals in the early stages of psychotic illness (sex: male = 15; female = 13; status: UHR = 10; FEP = 18), who were administered a modified version of the Montreal Imaging Stress Task (MIST) in conjunction with functional magnetic resonance imaging (fMRI). Before and throughout the task, participants rated their current stress level. All participants also provided 6 days of ecological momentary assessment (EMA) data on their mood and activities in their everyday environment. Functional imaging data from individual subjects were preprocessed with AFNI, using standard steps (including spatial normalization, volume registration, and spatial filtering) and then subjected to group-level whole-brain and regions-of-interest (ROI) analyses, using ROIs from the literature, such as amygdala, hippocampus, anterior insula (AI), anterior cingulate cortex (ACC), inferior frontal gyrus (IFG), and superior frontal gyrus (SFG). Multilevel models, controlling for gender and clinical status, were used to estimate whether affective responses to daily stressors were moderated by neural responses to acute psychosocial stress in our ROIs.

Results: During performance of the MIST, participants reported significantly higher levels of stress, relative to baseline. Moreover, a higher increase in reported stress levels during the MIST was significantly associated with a larger momentary increase in negative affect in response to stressful activities in daily life, as measured by EMA. Whole-brain analyses revealed significant stress-related activation in several areas, including the right SFG, right middle frontal gyrus, and right anterior insula. Significant stress-related deactivations were observed in ventromedial prefrontal cortex (vmPFC), ACC, and hippocampus. Stress-related activity (i.e., stress vs control activation contrasts) in the anterior insula significantly moderated daily-life affective stress reactivity, such that greater stress-evoked neural activity in AI was associated with a stronger affective response to daily-life stressors. By contrast, greater stress-evoked deactivation of vmPFC was associated with a stronger affective response to daily-life stressors.

Conclusions: Our results show that brain signals associated with the experience of acute psychosocial stress in a laboratory environment are predictive of affective reactivity to everyday stressful activities. While the anterior insula is well-established as a hub of brain salience networks, vmPFC has been implicated in cognitive control over emotions through its connectivity with limbic structures, such as the hippocampal formation. The identification of neural correlates of real-world stress reactivity provides clues as to how stress reactivity influences psychopathology in daily life.

Keywords: Clinical High Risk For Psychosis, Ecological Momentary Assessment, Insula, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

W118. Frontal Intracortical Myelin Volume is Related to Speed of Processing in Patients With First-Episode Schizophrenia Assessed With the MATRICS Consensus Cognitive Battery (MCCB)

Keith Nuechterlein*, Todd Tishler, Benjamin Ellingson, Joseph Ventura, David Williamson, Ibrahim Turkoz, Richard Keefe, Amy O'Donnell, Larry Alphs

UCLA, Los Angeles, California, United States

Background: Changes in intracortical myelin (ICM) volume have been documented in patients with first-episode schizophrenia (FES). While white matter integrity has been found to be related to some aspects of cognition in normal samples, specific

investigation into the relationship of ICM volume and cognition in patients with FES has yet to be reported. Myelination increases the speed of axonal conduction and thereby aids the timing of arrival of signals from multiple axons. We hypothesized that higher ICM volume would be associated with faster speed of processing (SoP) in cognitive performance. In this post hoc analysis of 2 studies of antipsychotic medications that included measures of ICM and cognition, we examined whether ICM volume in the frontal cortex correlates with SoP measured with the MATRICS Consensus Cognitive Battery (MCCB).

Methods: This is a post hoc analysis of 2 randomized, controlled trials designed to compare long-acting injectable (LAI) antipsychotics with their respective oral antipsychotic (OAP) formulations (risperidone [NCT00330551] and paliperidone [DREaM: NCT02431702], respectively) for the treatment of symptomatic patients with schizophrenia within 2 years of their first episode. In the risperidone trial, magnetic resonance imaging (MRI) and MCCB were performed at baseline and 6 months, and MCCB was performed again at 12 months. The DREaM trial had 3 phases: a 2-month oral run-in (Part I), a 9-month disease progression phase (Part II: LAI or OAP), and 9 months of additional treatment (Part III: LAI/LAI; OAP re-randomized: OAP/OAP or OAP/LAI). LAI/LAI and OAP/OAP comprised the 18-month extended disease progression phase. Within this paradigm, we analyzed MRI and MCCB data collected at Part II baseline (day 57), Part II end point/Part III baseline (day 260 of Part II), and Part III end point (day 260 of Part III). ICM volume in the frontal lobe was quantified using a previously described method that subtracts a proton density image from an inverse recovery image, as the latter is more sensitive to myelin. Our focus in this post hoc analysis was on the fundamental relationship between frontal ICM volume and SoP rather than on group differences, as we had no a priori reason to hypothesize that this relationship would vary across groups. Consequently, we combined the respective LAI and OAP samples within each study. Pearson correlations between ICM volume and SoP were obtained.

Results: In the earlier risperidone trial, 22 patients had MRI data at baseline and complete MCCB data at baseline, 6 months, and 12 months. A positive relationship was present between baseline ICM volume and SoP at baseline ($r = 0.44$, $P < 0.05$), 6 months ($r = 0.38$, $P = 0.08$), and 12 months ($r = 0.53$, $P < 0.02$). In DREaM, the numbers of patients with MRI and MCCB data at baseline, day 260 of Part II, and day 260 of Part III were 64, 42, and 35, respectively. In this trial, there was a significant positive correlation between baseline ICM volume and SoP at baseline ($r = 0.294$, $P < 0.05$) and at 9 months ($r = 0.316$, $P < 0.05$). The relationship remained positive but was weaker at 18 months ($r = 0.215$, $P = 0.216$).

Conclusions: Within the context of 2 independent treatment trials, frontal ICM volume at baseline has an enduring relationship with SoP performance that spans at least the first 12 months of treatment in symptomatic persons with recent-onset schizophrenia. The significant relationship between ICM volume and the speed with which cognitive processing occurs supports the functional significance of ICM volume, given that SoP is a consistent predictor of daily function in persons with schizophrenia.

Keywords: Schizophrenia, Intracortical Myelin, Speed of Processing

Disclosure: Janssen: Honoraria, Grant (Self); Alkermes:, Grant (Self); Precision Health Economics: Honoraria (Self)

W119. Auditory N100 Amplitude Deficits Predict Conversion to Psychosis in the North American Prodrome Longitudinal Study (NAPLS-2) Cohort

Erica Duncan*, Brian J. Roach, Nicholas Massa, Holly K. Hamilton, Peter M. Bachman, Aysenil Belger, Ricardo E. Carrion, Jason K. Johannesen, Gregory A. Light, Margaret A. Niznikiewicz, Jean Addington, Carrie E. Bearden, Kristin S. Cadenhead, Tyrone D. Cannon, Barbara A. Cornblatt, Thomas H. McGlashan, Diana O. Perkins, Ming T. Tsuang, Elaine F. Walker, Scott W. Woods, Nima Nasiri, Daniel H. Mathalon

Atlanta Veterans Affairs Health Care System; Emory University, Decatur, Georgia, United States

Background: Schizophrenia typically emerges in late adolescence with prodromal symptoms indicative of a dysfunction in thinking, mood and cognition. A greater understanding of brain changes in the prodrome could lead to improved early treatment and potentially to improved course later in life. The auditory N100 is an event related potential (ERP) component evoked by acoustic stimuli. Auditory N100 generators have been localized to the auditory cortex, with additional contributions from frontal and parietal sources. While N100 amplitude reduction has been repeatedly observed in schizophrenia, its status in individuals at clinical high risk for psychosis (CHR) remains unclear. Accordingly, using data from the 8-site North American Prodrome Longitudinal Study (NAPLS-2), we examined whether auditory N100 amplitude is reduced in CHR individuals relative to healthy controls (HC), and further whether it predicts conversion to psychosis in CHR individuals during a 24-month follow-up period.

Methods: Subjects included CHR individuals (n=552; 42.8% female; age (mean±sd)=19.21±4.38 years) who met criteria for the psychosis risk syndrome based on a SIPS interview, and a demographically similar group of HC (n=236; 47.0% female; age=20.44±4.73 years). Based on clinical follow-up assessments, CHR individuals were further classified as converters to full psychosis (CHR-C; n=73) and non-converters who completed 24 months of follow-up without converting to psychosis (CHR-NC; n=225). Electroencephalography data were collected during an auditory oddball task consisting of frequent (80%) standard tones (500 Hz, 50 ms duration), infrequent (10%) target tones (1000 Hz, 50 ms duration) requiring a button press, and infrequent (10%) novel distractor sounds (variety of natural and man-made sounds, mean duration 250 ms) presented in a pseudorandom sequence with a stimulus-onset asynchrony of 1250 ms. After pre-processing and data cleaning, EEG epochs were averaged to form ERPs time-locked to each of the oddball stimulus types. N100 peak amplitudes in response to each stimulus type were measured at midline scalp electrodes Cz and Fz where N100 is largest, and were subsequently transformed to age- and site-corrected z-scores, reflecting deviations in standard units from the age-specific norms provided by HC at each site. N100 z-scores were compared across groups using Group x Stimulus Type x Electrode repeated measures ANOVAs. Prediction of time to conversion to psychosis in the CHR group was assessed using Cox Proportional Hazard regression models.

Results: CHR individuals as a group showed significantly smaller (i.e., less negative) N100 amplitudes than HC ($F(1,786)=3.92$, $p=0.048$), an effect that did not significantly interact with Stimulus Type or Electrode. In the model comparing three groups (CHR-C, CHR-NC, and HC), a significant Group x Stimulus Type x Electrode interaction emerged ($F(4,966)=4.64$, $p=0.002$). To parse this interaction, the Group x Trial Type interaction was tested at each electrode, reaching trend level significance at Cz ($F(4,1021)=2.15$, $p=0.076$) but not at Fz ($F(4,1030)=0.94$, $p=0.441$) where the Group effect was also not significant ($p=0.079$). At Cz, Group effects were tested for each trial type and found to be significant for Standard ($F(2,531)=3.77$, $p=0.024$) and Novel ($F(2,531)=5.16$, $p=0.006$) stimuli, but not Targets ($F(2,531)=0.83$, $p=0.437$). For Standards, post-hoc tests showed N100 to be significantly reduced in CHR-C relative to HC ($p=0.018$) but not relative to CHR-NC ($p=0.139$), with no difference between CHR-NC and HC ($p=0.467$). For Novels, post-hoc tests showed N100 to be significantly

reduced in CHR-C relative to both HC (p=0.014) and CHR-NC (p=0.005), who in turn did not significantly differ from each other (p=0.871). Finally, a Cox regression predicting time to conversion to psychosis in the entire CHR sample from N100 amplitude z-scores (electrode Cz) was run separately for each stimulus type. An earlier conversion to psychosis was predicted by smaller N100 responses to novel (Wald = 10.72, p=0.001, Exp(B)=1.57) and standard (Wald = 6.16, p=0.013, Exp(B)=1.38) stimuli, but not target stimuli (p=0.584).

Conclusions: Reduced auditory N100 amplitude, an established biomarker and candidate genetic endophenotype for schizophrenia, is reduced in CHR individuals, indicating that its reduction precedes the onset of the schizophrenia. Further, greater N100 amplitude deficits in CHR individuals, particularly to task irrelevant but attention-grabbing novel sounds, predicts both a greater likelihood of subsequent conversion to psychosis and the imminence of psychosis onset. Our results suggest that pathophysiological alterations in auditory cortical circuitry subserving processing of sounds, particularly variable and unexpected sounds, contributes to the risk for psychosis in CHR individuals.

Keywords: Prodromal Schizophrenia, Event Related Potentials, N100

Disclosure: Teva Pharmaceuticals, Inc: Grant (Self)

W120. Linking Language Features to Clinical Symptoms and Multimodal Imaging in Individuals at Clinical High Risk for Psychosis

Abstract not included.

W121. Characterizing Resilience in Schizophrenia With Data-Driven and Machine Learning Approaches

Sunny Tang*, Katrin Hänsel, Lindsay Oliver, Colin Hawco, Erin Dickie, Majnu John, Miklos Argyelan, Pamela DeRosse, Robert Buchanan, Aristotle Voineskos, Anil Malhotra

Zucker Hillside Hospital, Northwell Health, Glen Oaks, New York, United States

Background: Schizophrenia spectrum disorders (SSD) are associated with significant functional impairment. However, individuals are not affected uniformly. A proportion of individuals with SSD are functionally resilient and remain engaged and active despite their diagnosis. An understanding of the demographic, cognitive, clinical and neurocircuitry correlates of functional resilience is critical for promoting recovery in all individuals affected by SSD.

Methods: Participants were 282 people with SSD, aged 18–55 years, 34% female. Multidimensional functioning was assessed with the Birchwood Social Functioning Scale (BSFS) and Quality of Life Scale (QLS). Functionally relevant sub-groups were identified using k-means and hierarchical Ward's methods based on BSFS and QLS subscales. Scree plot analysis suggested a three-cluster solution. Principal component analysis reduced functional items into three components: social and individual engagement, occupational and role functioning, and independent functioning. Detailed phenotyping included task-based fMRI (a social-emotional task where participants observed or imitated emotional faces), as well as assessment of social and nonsocial neurocognition, personal and interpersonal traits, psychosis symptoms, and negative symptoms in particular (with the Scale for the Assessment of Negative Symptoms). To extract characteristics most related to functional resilience from among the 47 demographic, cognitive, clinical and neurocircuitry variables, a machine-learning algorithm was generated to optimize accuracy of predicting the k-means functional grouping using all combinations of three-, four- and five-variable Latent Discriminant Analysis (LDA). Analyses were performed in R.

Results: K-means and Ward clustering consistently produced a three-cluster solution with a low-functioning group, a high-functioning/resilient group, and an intermediate group with preferentially preserved social and interpersonal engagement, but impaired occupational and role functioning. The optimized three-variable LDA included avolition, anhedonia and neural activity pattern during an emotional imitate/observe task. The four-variable LDA additionally included alogia, and the five-variable LDA added family history of SSD. Membership in the functional groups was predicted by the three-variable LDA solution with 69% accuracy, by the four-variable LDA solution with 74% accuracy, and by the five-variable LDA solution with 77% accuracy. Post hoc comparisons among functional groups revealed that symptoms of avolition, anhedonia, and alogia were lowest in the functionally resilient group and highest in the low-functioning group. A pattern of increased neural activation during the imitate/observe task was most associated with membership in the functionally resilient group. And, family history of SSD was more common in the intermediate group (35%) compared to the resilient (27%) and low-functioning groups (23%).

Conclusions: Absence of negative symptoms and increased neural activation in response to social-emotional tasks are key correlates of functional resilience in SSD. Family history of SSD was associated with impaired role functioning but relatively preserved social and individual engagement. Identification of modifiable contributors to functional resilience may provide new avenues to promoting recovery in individuals with SSD.

Keywords: Schizophrenia (SCZ), Risk and Resilience, Negative Symptoms, Multimodal Neuroimaging, Functioning

Disclosure: North Shore Therapeutics: Stock / Equity (Self), Winterlight Labs: Consultant (Self)

W122. Distinct Hierarchical Alterations of Intrinsic Neural Timescales Account for Different Manifestations of Psychosis

Kenneth Wengler*, Andrew Goldberg, George Chahine, Guillermo Horga

Columbia University/New York State Psychiatric Institute, New York, New York, United States

Background: Hierarchical perceptual-inference models of psychosis may provide a holistic framework for understanding psychosis in schizophrenia and may explain why positive symptoms such as hallucinations and delusions tend to cluster together yet sometimes manifest in isolation. To test this class of models, we used a recently developed resting-state functional MRI measure of intrinsic neural timescale (INT), which reflects the time window of neural integration and has been shown to capture hierarchical brain gradients. We specifically applied INT to test whether hallucinations and delusions are associated with distinct alterations at low and high levels of neural hierarchies, respectively, manifesting as symptom-specific differences in hierarchical gradients. We further explored global INT differences in schizophrenia and assessed the robustness and reliability of this fMRI measure.

Methods: Resting-state fMRI data were collected from two publicly available datasets: 1) HCP (N = 100 healthy and unrelated); 2) SchizConnect (N = 127 schizophrenia; N = 158 matched healthy controls). INT maps were estimated as previously described (Watanabe et al., eLife, 2019). Briefly, the autocorrelation function of the fMRI signal at each voxel (or vertex) was estimated and the sum of the autocorrelation coefficients during the initial positive period was calculated. T1w/T2w (myelin) and cortical-thickness maps were obtained from high-resolution structural

scans in the HCP dataset. The HCP-multimodal parcellation was used to facilitate further analysis. The robustness of INT to potential confounds (e.g., head motion) and the test-retest reliability were evaluated. Permutation tests were used to determine statistical significance and correct for multiple comparisons. All analyses controlled for head motion, age, and gender. A large-scale biophysical model (Chaudhuri et al., Neuron, 2015) was used to recapitulate in vivo findings through local changes in excitation-inhibition (E/I) ratios.

Results: In a first hierarchy validation step, several anatomically-informed hierarchies of parcels in the auditory, visual, and somatosensory systems were tested and the ordering for each sensory system that explained the most variance in structural measures of hierarchy (T1w/T2w and cortical thickness) was selected. All selected models had 9 hierarchical levels and explained more variance than random orderings (all: Ppermutation ≤ 0.003). Validation of the winning hierarchies was then performed both in-sample (HCP) and out-of-sample (SchizConnect controls) by correlating hierarchical position with INT, which generally yielded significant and consistent effects in the expected direction (higher INT at higher levels) across samples. Our hypothesis was then tested in these hierarchies.

We evaluated the relationships between symptom severity and INT values using a regression model predicting INT with scores for each of 7 symptoms (hallucinations, delusions, conceptual disorganization, emotional withdrawal, social withdrawal, blunted affect, and alogia) as regressors. Coefficients for hallucination and delusion severity were then included in a regression model with main effects and interactions of symptoms on hierarchical INT gradients. Critically, within this model we found hierarchical-gradient effects that differed significantly between hallucinations and delusions in the expected directions for 2/3 systems (auditory system, symptom-by-hierarchical-level interaction: t42 = 4.59, Ppermutation = 0.001; visual: t42 = -2.06, Ppermutation = 0.083; somatosensory: t42 = 3.50, Ppermutation = 0.011; set-level test for symptom interactions in 2/3 systems: Ppermutation = 0.014). In the auditory and somatosensory systems, patients with higher hallucination severity had a compressed hierarchical gradient consistent with increased INT at lower levels of the hierarchy. In contrast, patients with higher delusion severity had an expanded hierarchical gradient consistent with increased INT at higher levels. Post-hoc analyses showed specificity to positive symptoms and consistency across alternative definitions of the hierarchies.

Using a large-scale biophysical model, the differential patterns of hierarchical effects for hallucinations and delusions were recapitulated by locally increased E/I at low and high levels, respectively. Under this model, locally increased E/I causes increased recurrent excitation that leads to increased INT, yielding changes in the hierarchical gradients consistent with those observed in vivo.

In an exploratory analysis, relative to controls, patients exhibited a small-to-moderate, but widespread, reduction of INT (Ppermutation = 0.013) of comparable magnitude in low and high hierarchical levels.

Robustness analyses uncovered a small but systematic effect of head motion associated with decreased INT (Ppermutation = 0.01). No effects were observed for gender or age (all Ppermutation > 0.174). INT maps showed excellent reliability [median ICC (2,1) ± interquartile range: 0.94 ± 0.03].

Conclusions: We have presented evidence for distinct hierarchical alterations in neural timescales as a function of hallucination and delusion severity, lending initial neural support for hierarchical models of psychosis. Furthermore, we demonstrated that INT is a reliable method that, in combination with biophysical modeling, may provide a new, promising approach to investigate E/I imbalance across neuropsychiatric disorders.

Keywords: Psychosis, Intrinsic Neural Timescale, Excitation-Inhibition Balance, Hierarchical Perceptual Inference, Schizophrenia

Disclosure: Nothing to disclose.

W123. Differential Genetic Architecture of Cognition, Education and Schizophrenia via Local Genetic Correlations

Max Lam*, Todd Lencz

The Feinstein Institute for Medical Research, New York, New York, United States

Background: Pleiotropic association between cognitive ability, educational attainment, and schizophrenia have been reported by our group previously (PMID: 31374203), demonstrating a dissociation between adult synaptic pruning and early neurodevelopmental pathways. More recently, Genomic SEM methods have allowed more precise deconvolution of genetic effects in cognition and education, allowing a non-cognitive factor underlying educational attainment to be extracted. Here, we employ complementary methods to further investigate the differential genetic architecture underlying cognitive and non-cognitive factors derived from educational attainment GWAS.

Methods: First, we performed MTAG on GWAS summary statistics (i) cognition and schizophrenia, and (ii) the non-cognitive factor and schizophrenia. MAGMA pathway analysis was conducted on the results using the Molecular Signature DB v 5.3 as annotation. Second, we carried out rho-HESS on the same GWAS summary statistics. Local genetic correlations we then compared with global genetic correlations computed via LDSC.

Results: MAGMA pathway revealed that neurodevelopmental pathways were associated with cognition and schizophrenia, including regulation_of_cell_development (Pbonf = 1.92E-07), central_nervous_system_neuron_differentiation (Pbonf = 4.28E-06), and positive_regulation_of_nervous_system_development (Pbonf = 2.51E-05). By contrast, synaptic pathways were associated with the conjunction of non-cognitive factors and schizophrenia, including synapse_organization (Pbonf = 4.11E-05), postsynapse (Pbonf = 0.00328), and regulation_of_synaptic_-vesicle_clustering (Pbonf = 0.00341). These results are consistent with our earlier findings. Global genetic correlations were significant and negative for cognition and schizophrenia (rg = -0.245), and were of similar magnitude, but in the positive direction, for the non-cognitive factor and schizophrenia (rg = 0.222), consistent with earlier reports. While local genetic correlation demonstrated that a majority (~60%) of genomic segments followed these global patterns, we also identified 95 nominally significant genomic segments (~4% of the genome) demonstrating a positive correlation between cognition and schizophrenia, as well as 63 nominally significant genomic segments (~3% of the genome) demonstrating a negative correlation between the non-cognitive factor and schizophrenia. Notably, 8 regions were common to both of these sets, demonstrating unusual patterns of correlation between schizophrenia and both cognitive and non-cognitive factors; these regions included several lipid metabolism genes such as LRP1B, SPTLC3, and ACACA.

Conclusions: The results that we show here demonstrate that pleiotropic analyses are important for dissociating biological mechanisms in large-scale genomic analyses of correlated traits. Moreover, the examination of global genetic correlations, while increasingly popular, may obscure local regions that follow anomalous patterns and can reveal additional biology.

Keywords: GWAS, Schizophrenia (SCZ), Cognition, Pleiotropy Analysis, Genomics

Disclosure: Nothing to disclose.

W124. Proteogenomics Identifies Novel Biological Effects of Schizophrenia Risk Loci

Matthew MacDonald*, Lora McClain, Lambertus Klei, Megan Garver, Nathan Yates, David Lewis, Robert Sweet, Bernie Devlin

University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, United States

Background: Genome wide association studies have identified common variation associated with schizophrenia. Studies integrating this "risk" and transcriptomic variation have identified dozens of genes whose transcriptomic variation rises and falls with risk genotypes. However, for many loci containing risk variation, no effect on transcription is observed. We hypothesized that a portion of these "missing links" are due to our limited knowledge of how proteomic variation relates to genetic variation in the brain human, as the genome can regulate the proteome mechanisms that "skip" transcript levels.

Methods: Grey matter homogenates were prepared from right hemisphere auditory cortex grey matter of 48 schizophrenia and 48 matched control subjects. Protein expression was measured by targeted proteomics (N = 400 proteins). Genotyping was conducted on Illumina Global Screening Arrays. Protein Quantitative Trait Loci (pQTLs) were detected by linear regression of subjects' protein level using MatrixQTL, with the model adjusting for ancestry (significant eigenvectors), sex, diagnosis and technical confounds. FDR < 0.05 was used for significance, following common practice.

Results: We identified cis pQTLs for 9 proteins, 4 of which did not have GTEx-reported eQTLs. Interestingly, for one risk locus for schizophrenia, a leading risk SNP was a pQTLs for one protein and an eQTL for an unrelated gene.

Conclusions: This study confirms the capacity of proteogenomics to identify biological effects of common genetic variation that would be missed by transcriptomic approaches. Studies to expand the number of proteins assayed in these subjects and identify the biological mechanism by which the risk SNP regulates protein levels are underway.

Keywords: Schizophrenia (SCZ), Postmortem Brain Tissue, Proteomics, Genomics

Disclosure: Nothing to disclose.

W125. Role of Molecular Rhythms in the Human Striatum in Subjects With Schizophrenia or Bipolar Disorder

Kyle Ketchesin*, Wei Zong, Mariah Hildebrand, Kelly Cahill, Madeline Scott, Vaishnavi Shankar, Jill Glausier, David Lewis, George Tseng, Colleen McClung

University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Background: Schizophrenia and bipolar disorder are debilitating psychiatric disorders associated with significant disturbances in sleep and circadian rhythms, including altered peripheral gene expression rhythms. A recent study from our group used a time-of-death analysis of RNA-sequencing (RNA-seq) data and found diurnal rhythms in gene expression in the human postmortem dorsolateral prefrontal cortex in schizophrenia and control subjects. Notably, subjects with schizophrenia had a distinct set of rhythmic transcripts that were enriched for mitochondrial-related functions. However, the role of molecular rhythms in other regions associated with core symptoms of schizophrenia and bipolar disorder remains unknown.

Methods: In the current study, we aimed to determine whether molecular rhythms are altered across the human dorsal and ventral striatum in subjects with schizophrenia or bipolar disorder. Nucleus accumbens (NAc), caudate, and putamen tissue samples from male and female schizophrenia, bipolar disorder, and comparison subjects were obtained through the University of Pittsburgh Brain Tissue Donation Program and the NIH NeuroBioBank. We generated a psychosis cohort consisting of subjects with schizophrenia and bipolar disorder with psychosis. The bipolar disorder cohort consisted of subjects without psychosis. RNA-seq was performed on the NAc, caudate, and putamen tissue samples. Nonlinear regression was used to detect circadian patterns of transcript expression based on individual subjects' time of death. Sinusoidal curves were fitted to the expressed data using the nonlinear least-squares method and coefficient of determination (R^2) was used as a measure of goodness-of-fit. The empirical p-value was estimated by comparing the observed R^2 to the null distribution of R^2 generated from 1000 randomly shuffled time-of-death data. Transcripts with a gain or loss in rhythmicity between the psychosis (caudate/putamen: n = 35; NAc: n = 34) or bipolar disorder (n = 21) subjects and age- and sex-matched comparison subjects (n = 34) was determined using the difference in R^2 between the cohorts.

Results: We observed significant rhythms in transcript expression in the NAc, caudate, and putamen in comparison subjects without psychiatric or neurological disorders. Subjects with psychosis or bipolar disorder had distinct sets of rhythmic transcripts in each striatal region that showed low overlap with comparison subjects. In each striatal region, psychosis and bipolar disorder subjects exhibited subsets of transcripts that gain or lose rhythmicity relative to comparison subjects, with many more transcripts showing a loss of rhythmicity. In the NAc of comparison subjects, many of the top rhythmic transcripts were non-coding RNAs, particularly small nucleolar RNAs (snoRNAs). Interestingly, many of these snoRNAs in the NAc were not rhythmic or had lower amplitude rhythms in psychosis and bipolar disorder subjects. Transcripts in the NAc that gain rhythmicity in psychosis subjects were enriched for dopamine receptor signaling. In the caudate, transcripts that lose rhythmicity in psychosis and bipolar disorder subjects were highly enriched for pathways related to translation, including eIF2 signaling. In the putamen, transcripts that lose rhythmicity in psychosis and bipolar disorder subjects were enriched for pathways related to cellular stress, including glucocorticoid receptor signaling and unfolded protein response.

Conclusions: These data suggest that rhythms in transcript expression in the dorsal and ventral striatum of psychosis and bipolar disorder subjects are largely distinct from comparison subjects. Interestingly, many of the top rhythmic transcripts in the NAc of comparison subjects were snoRNAs, known for their role in guiding chemical modifications of other RNAs such as ribosomal and transfer RNAs. These snoRNAs appear to lose rhythmicity in schizophrenia and bipolar disorder subjects, suggesting potential alterations in circadian regulation of processes such as ribosomal biogenesis and protein synthesis. Transcripts that gain rhythmicity in the NAc of psychosis subjects are enriched for pathways related to dopamine function that are known to be altered in psychosis. This increase in dopamine-related rhythmicity could be due to a hyperdopaminergic state in subjects with psychosis, which will be the focus of future animal studies. In both psychosis and bipolar disorder subjects, there is a loss in rhythmicity in pathways related to translation and cellular stress in the caudate and putamen, respectively. We are currently performing additional analyses to explore differential expression in transcripts between groups in each striatal region. Future studies in animals will also be necessary to determine the molecular mechanisms underlying these rhythmic changes across the human dorsal and ventral striatum.

Keywords: Postmortem, Circadian Rhythms, Striatum, Schizophrenia, Bipolar Disorder

Disclosure: Nothing to disclose.

W126. Association of Mitochondrial Genetic Variants in the Risk for Schizophrenia

Wei Deng, Marquis Vawter, Michael Cronquist Christensen, Antonio F. Pardiñas, Schizophrenia Working Group, James Kennedy, Lei Sun, Vanessa Gonçalves*

Centre for Addiction and Mental Health, University of Toronto, Toronto, Canada

Background: Schizophrenia is a complex disorder with both clinical and biological heterogeneity, and to-date, effective diagnosis, treatment and prevention strategies are largely absent, in-spite of the recent success in identifying contributing genetic variants from the nuclear genome. Mitochondrial DNA (mtDNA) plays a crucial role in the brain suggesting that genetic variants in this system may contribute to the etiology of mental illnesses. Previous studies of primary mitochondrial diseases have observed impairment in brain function and high prevalence of psychosis among cases. Here, we aim to identify novel mitochondrial variants associated with schizophrenia using a subset of the Psychiatric Genomics Consortium (PGC) schizophrenia samples, which had mtDNA available for analysis.

Methods: Our analysis included 7,182 schizophrenia cases and 4,817 healthy controls, all European-Caucasians. After standard quality control and imputation, we recovered 107 mtDNAs that were shared across the samples, including 51 common variants and 56 rare variants (MAF < 0.01). Haplogroups were assigned using HaploGrep2 and tested for association using generalized linear regression models. For common variants, we performed a logistic regression while adjusting for sex. We then grouped rare variants by mtDNA genes, which were tested for association using SKAT.

Results: Haplogroups J-T ($\chi2 = 13.82$, $p = 0.0002$) and U ($\chi2 = 8.36$, $p = 0.003$) are associated with the risk of schizophrenia. For common SNPs analysis, two SNPs reached significance (0.05/51; p=0.0009). The most significant SNP is MT-15452 (MT-CYB; $p = 0.0001$), which is also one of the defining mutations of haplogroup J. The other one is MT-10463 (MT-TR, p= 0.0003), which is one of the mutations that define haplogroup T. Rare variants analyses were conducted on the 56 rare SNPs inside five mtDNA protein coding genes. Rare variants from complex I genes (NADH encoded by MT-ND1, 2, 3, 4, 5 and 6) are collectively associated with SCZ ($p = 0.0002$) while adjusting for the effect of sex.

Conclusions: This preliminary study found evidence that common and rare mitochondrial genetic variants might influence schizophrenia risk. Analysis in a larger sample is warranted and is currently ongoing by our team through the PGC. Our study is the largest thus far in major psychosis and brings new perspective for the schizophrenia genetic architecture by including the overlooked mitochondrial variants.

Keywords: Mitochondrial DNA, Schizophrenia (SCZ), Biomarkers for Risk Assessment

Disclosure: Nothing to disclose.

W127. Measurement of fALFF From Resting-State fMRI at Baseline Predicts Acute Treatment Response in First Episode Psychosis

Todd Lencz*, Miklos Argyelan, Ashley Moyett, Michael Birnbaum, Juan Gallego, Majnu John, Anita Barber, Philip Szeszko, Delbert Robinson, Anil Malhotra

Zucker Hillside Hospital, Glen Oaks, New York, United States

Background: Clinical response to antipsychotic (AP) drug treatment is highly variable, yet prognostic biomarkers are lacking. The goal of the present study was to test whether fractional amplitude of low frequency fluctuations (fALFF), as measured from resting state fMRI data, can serve as a potential biomarker of treatment response to APs.

Methods: Subjects included 130 patients with first episode psychotic disorders (mean age = 22 years) and minimal exposure to APs (median exposure = 5 days; all patients <2 years). All subjects underwent scanning while entering 12 weeks of prospective treatment with second-generation APs (risperidone or aripiprazole). Consistent with our prior studies, stringent treatment response criteria were applied for ratings obtained on weeks 1, 2, 3, 4, 6, 8, 10, and 12: response required 2 consecutive ratings of much or very much improved on the CGI, as well as a rating of ≤3 on psychosis-related items of the BPRS-A. By these criteria, 84 patients were classified as responders; these subjects did not differ from 46 non-responders on age, sex, medication, or scan movement (FD and DVARS).

All fMRI exams were conducted on a 3T scanner (GE Signa HDx, n=77; Siemens PRISMA, n=53). On the Signa, the resting-state scan lasted 5 minutes, during which 150 EPI volumes were obtained (TR = 2000 ms, TE = 30 ms, matrix = 64*64, FOV = 240 mm; 40 contiguous 3mm oblique axial slices). On the PRISMA, two 7-minute 17-second resting-state runs were obtained, one each with AP and PA phase encoding directions. Resting scans contained 594 whole-brain volumes, each with 72 contiguous axial/oblique slices in the AC-PC orientation (TR=720ms, TE=33.1ms, matrix = 104x90, FOV = 208mm, voxel = 2x2x2mm, multi-band acceleration factor=8).

Raw resting state data were preprocessed with despiking, linear trend removal, spatial smoothing, and grand mean scaling. Utilizing Fourier Transformation at every voxel, we calculated the power of BOLD signal in the low frequency range of 0.01–0.10 Hz and divided it by the power of BOLD signal across the entire frequency range (0–0.25 Hz) to calculate fALFF. Voxelwise fALFF was compared between responders and non-responders using t-tests implemented in SPM with age, sex, scanner, and movement (FD) as nuisance covariates, and applying a height threshold of p<0.005 and FDR-corrected cluster size p<.05.

Results: Compared to non-responders, patients who would later meet strict criteria for clinical response demonstrated significantly greater baseline fALFF in five brain regions: bilateral orbitofrontal cortex, bilateral superior frontal cortex, and right pars opercularis. Additionally, one large (k=203) region in the left posterior temporal cortex demonstrated greater baseline fALFF in non-responders compared to responders, although this region did not meet FDR-corrected significance.

Conclusions: Baseline resting state measures, obtained relatively easily even in impaired, early-phase patients with psychosis show promise as prognostic biomarkers. Combined with prior literature, results suggest that prefrontal deficits may be a critical target for antipsychotic treatment, whereas temporal abnormalities may be associated with lingering positive symptoms.

Keywords: Resting-State fMRI, Antipsychotic Response, First Episode Psychosis

Disclosure: Nothing to disclose.

W128. A Proton Magnetic Resonance Spectroscopy Study in Patients With Remitted Psychotic Depression Compared to Controls With an Examination of Effects of Antipsychotics vs. Placebo on Brain Metabolites

Hideaki Tani*, Iska Moxon-Emre, Natalie Forde, Nicholas Neufeld, Kathleen Bingham, Ellen Whyte, Barnett Meyers, George Alexopoulos, Matthew Hoptman, Anthony Rothschild, Hiroyuki Uchida, Alastair Flint, Benoit Mulsant, Aristotle Voineskos

Centre for Addiction and Mental Health, Toronto, Canada

Background: While psychotic depression is associated with poorer outcomes, the neurobiology of disease is not well-

understood, and to our knowledge there is no published magnetic resonance spectroscopy (MRS) study in this illness to date. MRS can non-invasively measure brain metabolite concentrations. A further consideration in neuroimaging studies, particularly in MRS studies, in where the effects of medication may be more notable on neurochemical measures is that participants are almost always medicated. In the treatment of psychotic depression, antipsychotics are often used in combination with antidepressants. Historically, dopamine has been the focus of antipsychotics, although recent MRS studies also suggest that antipsychotics decrease glutamate in the frontal cortex and increase myo-inositol (mI) in the thalamus. However, such studies have not been placebo-controlled, limiting interpretation related to causality.

In the present study, we compared the brain metabolite concentration between patients with remitted psychotic depression and healthy individuals. Moreover, we examined if the use of olanzapine was related to metabolite level change in comparison with placebo.

Methods: MRS data was obtained from a nested, multi-site multimodal neuroimaging study examining patients with psychotic depression (Study of the Pharmacotherapy of Psychotic Depression (STOP-PD) II). All patients in the STOP-PD II randomized controlled trial were treated with sertraline (150-200 mg/day) and olanzapine (15-20 mg/day) for 12 to 20 weeks and were then randomly assigned to either continue sertraline plus olanzapine or switch to sertraline plus placebo. Baseline MRS scans were obtained at the time of randomization. Follow-up scans were collected either at the time of relapse or once sustained remission was achieved 36 weeks after their baseline scan. Controls completed one MRS scan. For the MRS scans, a Point-RESolved Spectroscopy (PRESS) sequence was acquired at 3 Tesla. We used data from two sites (CAMH and U.Mass) which acquired water-scaled metabolite concentrations. MRS acquisition parameters were as follows: At CAMH: repetition time (TR) = 2000 ms, echo time (TE) = 35 ms, spectra width = 5000 Hz, 4096 datapoints, 128 water-suppressed and 16 water-unsuppressed averages, 8 number of excitations. At U.Mass: TR = 2000 ms, TE = 30 ms, spectra width = 2500 Hz, 1024 datapoints, 128 water-suppressed and 128 water-unsuppressed averages, 16 number of excitations. The voxels were placed in the left dorsolateral prefrontal cortex (L-DLPFC) (30 x 30 x 15 mm (CAMH) or 25 x 25 x 15 mm (U.Mass)) and bilateral supragenual anterior cingulate cortex (SACC) (30 x 20 x 15 mm). Water-scaled glutamate + glutamine (Glx), glycerophosphocholine + phosphocholine (Cho), mI, N-acetylaspartate + N-acetylaspartylglutamate (NAA), and creatine + phosphocreatine (Cr) concentrations were estimated with a corresponding basis set provided by LCModel and were corrected for water concentrations of the cerebrospinal fluid, gray matter, and white matter compartments. Linear regression models were used to compare metabolite levels between baseline for patients and the single time point acquisition for controls using analysis of covariance including age and sex. Moreover, we examined longitudinal changes in brain metabolites between olanzapine and placebo groups. Given that different TEs were applied across sites, we analyzed data from each site separately then meta-analyzed the effect sizes as a standardized mean difference (SMD). Furthermore, given that the interval between scans (in days) varied among subjects, we estimated the main effects of group and time by using a linear mixed-model regression. Time was measured and a treatment-group by time interaction was modeled, with age and sex as covariates along with a random intercept to account for within-subject variability and one to account for site variability.

Results: MRS data for 40 remitted patients (53.3 ± 13.9 years, male = 14) with psychotic depression and 46 controls (42.2 ± 16.8 years, male = 26) were included after quality control. Patients were older ($p = 0.002$) and had a lower proportion of males ($p = 0.046$). When the data were meta-analyzed, remitted patients with psychotic depression demonstrated higher Cho (SMD = 0.77; 95% CI, 0.26–1.28; $p = 0.003$) and mI (SMD = 0.66; 95% CI, 0.20–1.11; $p = 0.005$) in the L-DLPFC and higher mI (SMD = 0.56; 95% CI = 0.12–1.00; $p = 0.01$) in the SACC after adjusting for age and sex. Among the patients, longitudinal data were available for 15 in the olanzapine and 18 in the placebo groups. Those randomized to placebo showed a decrease in mI (SMD = 0.96; 95% CI = 0.21–1.71; $p = 0.01$) and Cr (SMD = 1.08; 95% CI, 0.33–1.84; $p = 0.005$) in the SACC compared to those randomized to continue on olanzapine; no differences between groups were present in L-DLPFC. When the time between scans was modeled and the data were harmonized across sites, placebo group showed a decrease in mI (estimate ± standard error, 0.00378 ± 0.00165, $t(32.9) = 2.295$, $p = 0.03$) and Cr (0.00461 ± 0.00102, $t(29.7) = 4.501$, $p < 0.001$) compared to olanzapine in the SACC.

Conclusions: Cho and mI in remitted patients with psychotic depression are higher than controls. Moreover, olanzapine may maintain mI and Cr levels. Future placebo-controlled studies with a large sample size are needed to confirm the finding of this study and to reveal the role of metabolite level changes as a result of antipsychotic treatment in relation to the functional outcome.

Keywords: 1H-MRS, Psychotic Depression, Antipsychotics, Placebo-Controlled Trial

Disclosure: Nothing to disclose.

W129. High Inflammation and Metabolic Dysfunction are Associated With Increased Negative Symptoms in Patients With Schizophrenia

David Goldsmith*, Nicholas Massa, Brian Miller, Brad Pearce, Andrew Miller, Erica Duncan

Emory University School of Medicine, Atlanta, Georgia, United States

Background: Bidirectional relationships between inflammation and metabolic dysfunction may contribute to the pathophysiology of psychiatric illnesses like schizophrenia. Inflammation has been implicated in the development of schizophrenia and recent data supports an association between increased inflammation and negative symptoms of schizophrenia. This is significant given the lack of current effective treatments for negative symptoms which are closely associated with the poor functional outcomes seen in schizophrenia. Many individuals with schizophrenia have metabolic abnormalities at illness onset which may be further exacerbated with antipsychotic treatment. Previous data in patients with depression have demonstrated that altered glucose metabolism defined a subset of depressed patients with high inflammation and anhedonia. Similarly, we have shown that inflammation and metabolic dysfunction are associated with disruptions in circuits that are relevant for reward and motivation. Motivational deficits are a core negative symptom of schizophrenia and we thus hypothesized that individuals with schizophrenia who had high inflammation and metabolic dysfunction would have increased negative symptoms compared to those with low inflammation and metabolic dysfunction.

Methods: 88 patients (87.5% male; mean age 48.27 years, sd10.45) with schizophrenia recruited from the Atlanta VA for two different studies were combined for this analysis. Inflammatory markers for the two studies were run in two separate labs and thus concentrations of TNF, IL-6, IL-1beta, IL-10, and MCP-1 (all previously shown to be altered in patients with schizophrenia) were Z-scored and added to create an inflammatory composite score. Lipid panels for each subject were obtained from the VA electronic medical record that were most recent to their study visit. Total negative symptom from the PANSS were significantly correlated with total cholesterol ($r=0.401$, $p=0.002$) and the LDL: cholesterol ratio ($r=0.427$, $p=0.002$) and were used in subsequent analyses. Median splits of the inflammatory composite score, total

cholesterol, and LDL:cholesterol ratios were used to define high versus low inflammation and high versus low metabolic dysfunction for each subject. Independent t-tests were used to test differences in negative symptoms (total negative symptoms subscale score and the individual negative symptom items from the PANSS) between individuals with: a) high inflammation and high metabolic dysfunction vs low inflammation and low metabolic dysfunction; b) high inflammation and high metabolic dysfunction vs high inflammation and low metabolic dysfunction; and c) high inflammation and high metabolic dysfunction vs low inflammation and high metabolic dysfunction. Logistic regression models were then used to test these relationships while accounting for covariates that were significantly different between the groups.

Results: There were significantly higher negative symptoms in the high inflammation and high metabolic dysfunction subjects vs the low inflammation and low metabolic dysfunction subjects as defined by both total cholesterol (PANSS blunted affect: t=-2.08, p = 0.049; PANSS emotional withdrawal: t=-2.76, p=0.011; total negative symptoms (N-total) t=-2.561, p=0.017) and the LDL: cholesterol ratio (PANSS blunted affect: t=-2.66, p=0.014; PANSS emotional withdrawal: t=-2.73, p=0.012; N-total t=-2.51, p=0.019). There were significantly higher negative symptoms in subjects with high inflammation and high metabolic dysfunction vs subjects with high inflammation and low metabolic dysfunction as defined by both total cholesterol (PANSS emotional withdrawal: t=-3.51, p=0.01; N-Total t=-2.89, p=0.008) and the LDL:cholesterol ratio (PANSS emotional withdrawal: t=-3.47, p=0.002; N-Total t=-3.12, p=0.005). There were no significant differences between subjects with high inflammation and high metabolic dysfunction vs subjects with low inflammation and high metabolic dysfunction. Because age and smoking status were significantly different between groups, we ran logistic regression models using these as covariates and found similar results for total cholesterol, but significance was reduced to a trend level for the LDL: cholesterol ratio.

Conclusions: These results suggest that individuals with high inflammation and metabolic dysfunction may represent a subset of individuals with schizophrenia who have worse negative symptoms. Increased inflammation appears to be necessary to drive these relationships as high metabolic dysfunction alone did not appear to support differences in negative symptoms between individuals with high vs low inflammation. Future work should seek to replicate these findings in larger samples. High inflammation and metabolic dysfunction may represent a transdiagnostic signature for symptoms related to positive valence systems (i.e., anhedonia and motivational deficits) and could represent novel therapeutic targets for these difficult to treat symptoms.

Keywords: Schizophrenia (SCZ), Negative Symptoms, Inflammation, Cytokines, Metabolic Defect

Disclosure: Nothing to disclose.

W130. Gut Microbiome in Schizophrenia Shows Altered Functional Pathways Related to Aging, Immune Modulation, and Cardiovascular Risk: Implications for Therapeutic Discovery

Tanya Nguyen*, Tomasz Kosciolek, Rebecca Daly, Yoshiki Vázquez-Baeza, Austin Swafford, Rob Knight, Dilip Jeste

University of California San Diego, School of Medicine, San Diego, California, United States

Background: Individuals with schizophrenia are more prone to diseases associated with aging, namely cardiovascular diseases (CVD) and exhibit age-associated physiological changes, such as inflammation, at earlier ages. A growing body of literature strongly supports the hypothesis of accelerated biological aging in persons with schizophrenia. Understanding the mechanisms of potential accelerated aging is imperative to improving the quality and quantity of life in schizophrenia. Emerging evidence has linked the gut microbiome changes to schizophrenia. However, the mechanisms underlying the connection between the gut microbiota and the phenotype of persons with schizophrenia are unclear. The objectives of this study were to: 1) Utilize a newly-developed compositionally-aware method incorporating reference frames to identify differentially abundant microbes and pathways; 2) Characterize the functional potential of the gut microbiome; and 3) Evaluate the microbiome as a diagnostic predictive tool by constructing taxonomy-based and function-based classifiers using supervised learning models.

Methods: This study included 48 persons with chronic schizophrenia and 48 matched non-psychiatric comparison subjects (NCs). To account for potential methodological differences that might bias results and to control for clinical factors and known major drivers of microbiome changes, NCs were matched to schizophrenia subjects on sequencing plate, age, sex, BMI, and antibiotic use. Stool samples were collected and assayed using 16S rRNA sequencing. PICRUSt was performed to predict functional potential of microbial communities, based on metagenomes inferred from 16S data.

Results: Patients with schizophrenia demonstrated significant beta-diversity differences in microbial composition (unweighted UniFrac pseudo-F = 2.41, p = 0.002) and predicted genetic functional potential (KEGG orthologs, Jaccard pseudo-F = 2.41, p = 0.01; EC numbers, Jaccard pseudo-F = 2.49, p = 0.008) compared to NCs. Alpha-diversity of taxa and functional pathways were not different between the groups. Differential abundance testing revealed that the family Lachnospiraceae was associated with schizophrenia; the log-ratio of Lachnospiraceae, relative to the top-20 ranked taxa (i.e., taxa most associated with NCs), was significantly higher in schizophrenia compared to NCs (Z = -2.81, p = 0.005). Functional pathways related to trimethylamine-N-oxide (TMAO) reductase and Kdo2-lipid A biosynthesis were altered in schizophrenia. Log-ratios of TMAO reductase (Z = -3.99, p < 0.001) and Kdo2-lipid A biosynthesis (Z = -3.85, p < 0.001), relative to bottom-20 pathways, were significantly lower in the schizophrenia group than in NC group. Spearman's correlations revealed that log-ratios of TMAO reductase were positively correlated with age (ρ = 0.373, p = 0.013) and Framingham Risk Score (ρ = 0.393, p = 0.026), whereas the log-ratio of Kdo2-lipid A was negatively associated with TNFα (ρ = -0.632, p = 0.006) and IL-10 (-0.761, p = 0.001) in schizophrenia but not in NCs. Random forests analyses revealed that microbial taxa (75% accuracy, 0.83 AUC) and functional profiles (KEGG: 65% accuracy, 0.65 AUC; MetaCyc: 74% accuracy, 0.73 AUC) predicted differentiation of patients with schizophrenia from NCs with good accuracy.

Conclusions: To our knowledge, this is the first study to show that persons with schizophrenia have altered metabolic pathways related to TMAO reductase and Kdo2-lipid A biosynthesis. TMAO reductase is an enzyme that catalyzes the reduction of TMAO to trimethylamine. TMAO has been implicated in the pathogenesis of atherosclerotic coronary artery disease, glucose intolerance, and insulin resistance. Findings suggest that persons with schizophrenia may have less ability to clear TMAO due to lower levels of TMAO reductase, leading to increased levels of TMAO. Correlations with age and Framingham Risk Scores suggest that this enzyme may be relevant in aging and CVD in schizophrenia. Kdo2-lipidA is the active component of lipopolysaccharide (LPS) on most Gram-negative bacteria, which stimulates host immune responses. Results showing alterations in the Kdo2-lipid A biosynthesis pathway and its positive relationship to pro-inflammatory cytokine TNFα in schizophrenia is consistent with evidence that schizophrenia is associated with a chronic pro-inflammatory state. Together, our findings suggest that the microbiota may impact the pathophysiology of the disease through modulation of

functional pathways related to immune signaling/response and lipid and glucose regulation, which might have implications for accelerated biological aging in schizophrenia. Although data are cross-sectional and we cannot infer causal relationships, these findings lay possible groundwork for future investigations to elucidate the role of the gut microbiome in the development, presentation, and progression of schizophrenia. This research also has important clinical implications for developing novel therapeutics for schizophrenia. Therapies that target enzymes and receptors involved in TMAO and Kdo2-lipid A biosynthesis pathways are emerging targets for immunopharmacological exploitation.

Keywords: Gut Microbiome, Schizophrenia- Novel Treatment, Accelerated Aging, Cardiovascular Physiology, Inflammation

Disclosure: Nothing to disclose.

W131. Novel Behavioral Approaches for Analyzing Temporal-Tracking, Context-Time Integration, and Time Cell Activity in Mice

Abstract not included

W132. Capturing Clinical Symptoms With Ecological Momentary Assessment: Convergence of Momentary Reports of Psychotic and Mood Symptoms With Diagnoses and Standard Clinical Assessments

Michelle Miller, Philip Harvey*, Colin Depp, Raeanne Moore, Amy Pinkham

Miller School of Medicine, University of Miami, Miami, Florida, United States

Background: Real time assessment of clinical symptoms and functional behaviors via mobile devices is becoming more widely used in both research and clinical settings. Data collected by momentary assessment is not affected by recall bias and may be considerably more valid than retrospective reports of everyday functioning and mood. Given the current interest in remote clinical trials and enhancement of between-visit assessments, examining whether EMA can be validly performed and used as an adjunct to other clinical assessments has intrinsic value. This study used Ecological Momentary Assessment (EMA) to examine clinical symptoms and related these sampling results to structured clinical ratings in individuals with schizophrenia or bipolar disorder. We hypothesized that EMA-assessed psychotic symptoms would correlate with clinically assessed psychotic symptoms via equivalent items on the PANSS at post-EMA assessments. We also hypothesized that EMA-reported psychotic symptoms would be specifically convergent with clinical ratings of psychotic symptom severity at this assessment, as EMA-reported psychotic symptoms are associated with clinical ratings of symptom severity across different symptoms, including delusions and hallucinations.

Methods: Three times a day for 30 days, participants with bipolar disorder (n=71; BPI) or schizophrenia (n=102; SCZ) completed smartphone-based surveys assessing five psychosis-related and five mood symptoms, in addition to reporting their location and who they were with at the time of survey completion. Participants also completed Positive and Negative Syndrome Scale (PANSS) and MADRS interviews with trained raters at baseline and at the end of the sampling period. Mixed-model repeated-measures (MMRM) analyses examined diagnostic effects and the convergence between clinical ratings and EMA sampling.

Results: 12,406 EMA samples were collected from 173 participants, with 80% adherence to prompts. EMA-reported psychotic symptoms manifested substantial convergence with equivalent endpoint PANSS items. Patients with SCZ had more severe PANSS and EMA psychotic symptoms. Patients with BPI reported more momentary depression and more severe depression based on MADRS ratings. Being home and alone correlated with the occurrence of both hallucinations and delusions. Ratings of reduced emotional experience on the PANSS were associated with greater numbers of surveys answered while home alone. There were no changes in symptom severity scores as a function of the number of previous assessments, reflecting no exposure effects..

Conclusions: EMA surveyed clinical symptoms converged substantially with commonly used clinical rating scales in a large sample, with high adherence. This suggests that remote assessment of clinical symptoms is valid and practical and was not associated with alterations in symptoms as a function of reassessment, with additional benefits of "in the moment" sampling such as eliminating recall bias and the need for informant reports. EMA should be a valuable adjunct for treatment studies, including psychosocial and pharmacological approaches. Further, as there was no evidence of changes in symptoms as a function of repeated reassessments, these data suggest that conditions with high risk of placebo effects, such as negative symptoms in schizophrenia, PTSD, and major depression, could be validly sampled with EMA with reduced risk of placebo responses compared to in person ratings.

Keywords: Bipolar I & II Disorder, Schizophrenia; Functional Capacity; Technology, Ecological Momentary Assessment.

Disclosure: Acadia: Advisory Board (Self), Bioexcel: Consultant (Self), Boehringer-Ingelheim: Consultant (Self), Alkermes: Advisory Board (Self), Minerva Pharma: Consultant (Self), Otsuka Pharma: Consultant (Self), Regeneron Pharma: Consultant (Self), Roche Pharma: Advisory Board (Self), Sunovion Pharma: Advisory Board (Self), Teva Pharma,: Consultant (Self), Takeda Pharma: Grant (Self), iFunction, inc,: Stock / Equity (Self).

W133. Incomplete Hippocampal Inversion in Schizophrenia

Maxwell Roeske*, Maureen McHugo, Ilwoo Lyu, Stephan Heckers

Vanderbilt University Medical Center, Nashville, Tennessee, United States

Background: Incomplete hippocampal inversion (IHI) is an anatomical variant of the human brain resulting from an arrest in brain development, especially prevalent in the left hemisphere. We hypothesized that IHI is more common in schizophrenia and contributes to these hippocampal structural differences.

Methods: We studied 199 schizophrenia patients and 161 healthy control participants with 3-Tesla MRI to establish IHI prevalence and the relationship of IHI with hippocampal volume, asymmetry, and shape in both males and females. We measured IHI with rigorous, quantitative criteria to assess prevalence and severity. We employed linear mixed models to investigate the effect of IHI on hippocampal volume and asymmetry. Shape analysis was completed using a modified SPHARM-PDM toolkit.

Results: Schizophrenia patients had more frequent IHI in both the left ($p < 0.01$) and right ($p = 0.04$) hemisphere. IHI severity was significantly greater in the left ($p < 0.01$) hemisphere in schizophrenia patients. Severe IHI cases were associated with a higher rate of automated segmentation failure. IHI contributed to smaller hippocampal volume ($p < 0.001$) and increased volume asymmetry ($p < 0.001$) in schizophrenia. In contrast to significant IHI differences, we did not find significant group differences in our vertex-wise analysis of hippocampal shape.

Conclusions: The increased prevalence and severity of IHI supports the neurodevelopmental model of schizophrenia. The impact of this developmental variant deserves further exploration in studies of the hippocampus in schizophrenia.

Keywords: Schizophrenia (SCZ), Hippocampus, Human Neuroimaging, Brain Development, Hippocampal Shape

Disclosure: Nothing to disclose.

W134. Orexin Receptor Antagonists Reverse Aberrant Dopamine Neuron Activity and Related Behaviors in Rodent Models of Psychosis

Stephanie Perez*, Hannah Elam, Daniel Lodge

UT Health Science Center At San Antonio, San Antonio, Texas, United States

Background: Psychosis is a serious mental disorder that is commonly observed in multiple disease states, such as, schizophrenia and post-traumatic stress disorder (PTSD). Symptoms of psychosis have been repeatedly demonstrated to stem from hyperactivity in the dopamine system; however, targeting this system directly produces undesirable side-effects. Further, because there is no overt histopathology present within dopamine neurons, it is suggested that upstream brain regions that regulate the activity of these neurons are dysfunctional. We have recently reported that the paraventricular nucleus of the thalamus (PVT) works in concert with the ventral hippocampus (vHipp) to regulate dopamine system function; however, the PVT has yet to be investigated as a target for the treatment of psychosis. Because of the dense expression of orexin receptors in the thalamus, we posit that this region is a possible target for the pharmacological regulation of dopamine neuron activity.

Methods: Here we used two different rodent models (males rats only; n= 5-15 rats per group), the methylazoxymethanol acetate (MAM) model and a stress-induced (using a two-day inescapable foot-shock paradigm) model, which display pathological alterations consistent with schizophrenia and post-traumatic stress disorder (PTSD), to determine whether orexin receptor blockade can restore ventral tegmental area (VTA) dopamine system function and related behaviors. We used in vivo electrophysiology, in anesthetized rats, to measure dopamine neuron population activity following the administration of TCS 1102 (a dual orexin receptor antagonist (DORA); both intraperitoneal and intracranial into the PVT in MAM- and saline -treated rats), orexin A & B peptides (intracranial in the PVT in naïve rats), and Suvorexant (a DORA), SB334867 (orexin 1 receptor antagonist) or EMPA (orexin 2 receptor antagonist; intraperitoneal in stress-induced rats). Additionally, stress-induced rats were evaluated for sensorimotor gating deficits and sensitivity to psychomotor stimulants (MK-801). Administration of Suvorexant, SB334867 or EMPA (systemically in stress-induced rats) were found to restore normal dopamine system function, while only Suvorexant or SB334867 were able to reverse deficits in behaviors related to psychosis.

Results: Aberrant dopamine system function in MAM-treated rats was normalized by the systemic administration of TCS 1102. The potential site of action was determined by direct administration of the orexin peptides A & B (into the PVT), where they significantly increase VTA dopamine neuron population activity in naïve rats. Further, the direct administration of TCS 1102 (into the PVT) produced the same beneficial effects seen with systemic administration in MAM-treated rats. Stress-induced rats also displayed aberrant dopamine system function, as well as deficits in sensorimotor gating and an increased sensitivity to psychomotor stimulants. Moreover, targeting the orexin 1 receptor was more effective than the orexin 2 receptor in reversing stress-induced deficits.

Conclusions: Taken together these data suggests that the orexin system represents a novel site of therapeutic intervention for psychosis across multiple disease states, via an action in the PVT.

Keywords: Dual Orexin Receptor Antagonist, Paraventricular Nucleus of the Thalamus, Schizophrenia (SCZ), Post-Traumatic Stress Disorder, Orexin System

Disclosure: Nothing to disclose.

W135. Impact of Vapourized Cannabis Flower on Schizophrenia-Relevant Neural Circuitry and Behaviour in a Neurodevelopmental Rat Model of Schizophrenia

Bryan Jenkins, Shoshana Buckhalter, Melissa Perreault, Jibran Khokhar*

University of Guelph, Guelph, Canada

Background: Cannabis use is common in patients with schizophrenia and is associated with a significant worsening of the overall course of the disease. There is also evidence suggesting that cannabis may act as a risk factor for the development of schizophrenia. This study was designed to assess the causal schizophrenia-like changes in brain circuitry and behavior that arise from vaporized cannabis (of varying constituent profiles) exposure.

Methods: Male Sprague-Dawley rats (n=30) were injected with either ibotenic acid (neonatal ventral hippocampal lesion [NVHL]) or artificial cerebrospinal fluid (sham) on postnatal day 7. A separate cohort of unmanipulated animals was also tested. In adulthood, animals (n=10) were implanted with depth electrodes across multiple brain regions (i.e., nucleus accumbens [NAc], cingulate cortex [Cg], prefrontal cortex [PFC], hippocampus [Hip]) to collect local field potentials.

These animals were also tested on a behavioural battery including assays of somatosensory gating such as prepulse inhibition. Animals were exposed once with either vaporized THC-only cannabis (11% THC) or balanced THC-CBD (11% THC and CBD) cannabis flower in a within-animal randomized cross-over design with a 2-week washout, and the effects on schizophrenia-like behaviour and circuitry were assessed.

Results: NVHL rats displayed significant sensorimotor gating deficits ($p<0.005$), as well as altered power and coherence features across multiple brain regions (including decreased spectral power was observed within the NAc, Cg and Hip, especially in the gamma range (>32–100 Hz; $P<0.05$), as well as decreased coherence between the Hip-PFC and Hip-Cg ($p<0.05$)). As we have previously shown with pure THC, the THC-only cannabis flower produced decreased gamma power in sham animals (Cg theta and gamma; PFC theta and gamma; Hip theta; NAc theta, beta and gamma; $p<0.05$) and worsened the dampened power observed in the NVHL rats across multiple brain regions (Cg and PFC theta, beta and gamma (compared to NVHL baseline); Hip theta; NAc theta and gamma; $p<0.05$). The presence of CBD in the balanced flower reduced the gamma suppression in sham animals (Cg, PFC and NAc gamma; $p<0.01$), and in some cases, even recovered the baseline and THC-flower induced deficits observed in the NVHL rats (Cg gamma; NAc beta and gamma; $p<0.05$ compared to baseline and/or THC-flower). Similar findings were also observed on coherence in NVHL rats between Hip-NAc with significant worsening observed in the THC-flower ($p<0.05$) but not after balanced flower vapour exposure. For Cg-PFC, both types of cannabis significantly increased coherence in NVHL rats, possibly related to the acute worsening effects of cannabis on cognitive function in schizophrenia. Sensorimotor gating deficits were also significantly worsened by exposure to both types of cannabis (main effect of drug; $p<0.05$).

Conclusions: These studies suggest that acute exposures to cannabis can worsen schizophrenia-related circuit and behavioural changes in a rat model of schizophrenia. Furthermore, in schizophrenia model animals, the presence of equal amounts of cannabidiol may ameliorate this worsening. Future studies will

assess the effects of chronic exposures, and attempt to reverse the circuit dysfunctions observed in these studies via pharmacological and neuromodulatory approaches.

Keywords: Cannabis, Schizophrenia-Like Behavior, Vapor, Electrophysiology

Disclosure: Nothing to disclose.

W136. Aberrant Nonlinear Dynamical Interactions Between Default Mode Network Hemodynamics and Alpha EEG Power in Schizophrenia

Michael Jacob*, Parham Pourdavood, Terrence Deacon, Brian Roach, Daniel Mathalon, Judith Ford

University of California, San Francisco, School of Medicine, San Francisco, California, United States

Background: Patients with schizophrenia (SZ) exhibit neural processing deficits in addition to impairments in metabolism, leading some to propose that SZ represents a deficit in neuroenergetic coupling. The investigation of neurometabolic coupling is complicated by the dynamical complexity of neural signals, hemodynamic signals and their interactions, with growing evidence that standard linear modeling assumptions may be less accurate in certain tasks or disease states. Nonlinear dynamical systems analysis has advanced our understanding of the complexity of neural signals and a loss of neural complexity has been observed in psychosis (towards either greater signal randomness or excessive regularity). The impact of nonlinear dynamics on neurometabolic coupling in SZ is largely unknown.

Methods: We recruited SZ participants (n=55) and healthy controls (HC, n=43) for simultaneous recording of electroencephalography and fMRI (EEG-fMRI) to capture the dynamics of neural and hemodynamic signals. During a 6-minute scan, subjects were instructed to rest with their eyes open. EEG power was segregated into a broadband, scale-free component as well as residual rhythmic power in five legacy frequency bands (delta, theta, alpha, beta, gamma). We extracted the blood oxygen level dependent (BOLD) time-series from the anterior and posterior hubs of the default mode network (DMN) and focused on its relationship to posterior alpha EEG, given the task-free protocol and correlation between DMN and alpha in the prior literature. DMN BOLD and EEG alpha signals were subject to convergent cross mapping (CCM, also known as Sugihara causality), a dynamical systems analytic approach to infer the directionality of interactions, complexity and causality between nonlinear time series. CCM yields a causal directionality value for each subject (0 to 1, where "1" is perfect causation) and for each causal direction (EEG "causing" BOLD and BOLD "causing" EEG), indicating the magnitude of the impact of each time series on the other. This approach allows inferences regarding unidirectional (eg. EEG "causing" BOLD without evidence of BOLD "causing" EEG) as well as bidirectional interactions (EEG and BOLD both have causal influence on each other). CCM values for each subject and causal direction were entered into a repeated measures ANOVA to assess the impact of illness on EEG-BOLD causal directionality. Symptomatology was evaluated using the positive and negative syndrome scale (PANSS).

Results: CCM analyses in the posterior hub of the DMN (posterior cingulate) identify bidirectional coupling in SZ. Alpha EEG power drives BOLD hemodynamics (causal direction: EEG to BOLD) in HC (mean=0.050, s.e.m.=0.018) and SZ (mean=0.0311, s.e.m.=0.014), whereas BOLD hemodynamics drive alpha power (causal direction: BOLD to EEG) primarily in SZ (mean=0.0525, s.e.m.=0.020) and less so in HC (mean=0.0091, s.e.m.=0.025). These findings yield a group X causal direction interaction effect ($F(1,96) = 5.95$, $p=0.017$ and no main effect of group or causal direction; p's > 0.45). In the anterior hub (medial prefrontal cortex) we find only a main effect of causal direction ($F(1,96)=6.7338$, $p=0.011$ where causal direction: EEG to BOLD is greater than causal direction: BOLD to EEG) with no effect of group and no group x causal direction interaction effect (p's > 0.46). CCM directionality: alpha EEG to posterior cingulate BOLD was inversely correlated with total negative symptoms ($r=-0.3949$, $p=0.0028$) but not total positive symptoms ($r=-0.1472$, $p=0.2837$).

Conclusions: In a reversal of classical neural-hemodynamic coupling, patients with SZ show a reduced impact of alpha EEG on the BOLD signal in the posterior hub of the DMN and an elevated impact of posterior cingulate BOLD on alpha power. These findings suggest an aberrant hemodynamic modulation of resting alpha rhythms; a potential compensation for poor hemodynamic recruitment, leading to a pathologic feedback cycle. Our findings are consistent with the identification of metabolic abnormalities in SZ, including changes in genes impacting energy balance, mitochondrial impairments and vascular alterations which may all contribute to impaired neuroenergetic coupling. We further hypothesize that these aberrant metabolic signals in SZ impair neural information processing and contribute to pathophysiology; as is suggested by finding greater impairment in EEG to BOLD causality associated with more negative symptoms. Our approach highlights the benefit of applying a dynamical systems framework to relate nonlinear interactions between neural and metabolic signaling in elucidating pathophysiology.

Keywords: EEG-fMRI, Neurometabolism, Dynamical Systems, Schizophrenia

Disclosure: Nothing to disclose.

W137. Matrix Metalloproteinase 9 (MMP9) and its Association With Hippocampal Volume in Schizophrenia

Johanna Seitz*, Magdalena Seethaler, Nikos Makris, Jarrett Rushmore, Kang-Ik K. Cho, Elizabeth Rizzoni, Mark Vangel, Olcay Senay Sahin, Carina Heller, Ofer Pasternak, Filip A. Szczepankiewicz, Carl-Frederik Westin, Jan Losak, Libor Ustohal, Josef Tomandl, Lubomir Vojtisek, Petr Kudlicka Ing, Martin Jani, T. Wilson Woo, Tomas Kasparek, Zora Kikinis, Marek Kubicki

Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States

Background: Perineuronal nets (PNNs) are extracellular matrix structures that envelop parvalbumin (PV)-containing inhibitory interneurons. PNNs play critical roles in dendritic and synaptic plasticity in part by regulating the development and integrity of PV neurons. Among the key molecular regulators of PNN remodeling are matrix metalloproteinases (MMPs), a large family of extracellularly acting zinc-dependent proteases. Matrix metalloproteinase 9 (MMP9) is the most prevalent MMP in the central nervous system, and it has been implicated in the pathophysiology of schizophrenia.

Several studies have demonstrated MMP9 upregulation in the blood of patients with schizophrenia and it has been suggested that minocycline, an antibiotic and MMP9 inhibitor, ameliorates symptom's severity in patients with schizophrenia. Notably, MMP9 regulates long-term potentiation and plays an essential role in the control of neuroplasticity in the hippocampus. Excessive dendritic pruning in the hippocampus, hippocampal volume loss, and hippocampal functional impairments are among the most consistent symptoms in patients with schizophrenia.

These findings have prompted our current investigation into the relationship between MMP-9 and hippocampal volumes in healthy individuals and subjects with schizophrenia.

Methods: 34 healthy individuals (mean age = 32.50, male = 21, female = 13) and 30 age and sex-matched subjects with schizophrenia (mean age = 33.07, male = 19, female = 11)

underwent a blood draw and T1-weighted magnetic resonance imaging. The hippocampus was automatically segmented utilizing the FreeSurfer 6.0 hippocampal sub-field parcellation module. ANCOVAs (corrected for age and sex) were conducted to compare MMP9 plasma levels and hippocampal subfield volumes between subjects with schizophrenia and healthy controls. Spearman's correlations were utilized to investigate the association between symptoms, medication, and MMP9 plasma levels in patients. Partial correlations (controlled for age, sex, group) were used to test for the relation between MMP9 plasma levels and hippocampal volumes in patients and HC.

Results: Patients displayed MMP9 plasma levels almost twice as high as healthy individuals ($F(1, 60) = 21.19$, $p<.0001$). MMP9 levels correlated with negative symptoms in patients ($R = .39$, $p < .035$). Further, patients had smaller overall hippocampal volumes ($F(1,60) = 10.34$, $p < .002$) and smaller subfield volumes (including the presubiculum, subiculum, CA1, CA3, CA4, HATA, and tail). Across both groups the total hippocampal ($R = -.25$, $p < .049$), CA1 ($R = -.28$, $p < .032$), and subiculum ($R = -.28$, $p < .030$) volume correlated negatively with MMP9 plasma levels.

Conclusions: The present study establishes an association between MMP9 and the hippocampus volume in SCZ.

We observed lower hippocampal volumes and higher MMP9 plasma levels in patients with SCZ when compared to HC.

Our finding of higher MMP9 levels, that were associated with negative symptoms, is in line with recently published work reporting that MMP9 mRNA expression is more pronounced in deficit SCZ.

Given the crucial role of the hippocampus for memory, emotion processing, and higher-level cognition, which are all affected in SCZ, it is one of the most studied brain structures in psychosis and indeed the hippocampus is the most affected subcortical brain region in SCZ

Our study adds to the literature by demonstrating a negative association between hippocampal volume and MMP9 plasma levels, which might be specific for the subiculum and CA1.

Our findings might pave a new avenue towards neuroprotective treatments. For example, MMP9 inhibitors such as minocycline can regulate MMP9 and could reverse neurodegeneration and improve negative symptoms in SCZ [77]. More extensive studies are needed to replicate our findings and to investigate the association between MMP9 and brain pathology along the SCZ spectrum.

Keywords: Matrix Metalloproteinase-9 (MMP-9), Magnetic Resonance Imaging, Hippocampus, Psychosis

Disclosure: Nothing to disclose.

W138. Evidence for Schizophrenia-Specific Pathophysiology of Nicotine Dependence

Heather Ward*, Adam Beerman, Uzma Nawaz, Mark Halko, Amy Janes, Lauren Moran, Roscoe Brady

Brigham and Women's Hospital & Harvard Medical School, Boston, Massachusetts, United States

Background: The prevalence of smoking in schizophrenia is three-times higher than the general population and tobacco use is a factor in 53% of deaths in schizophrenia. Prior imaging studies of candidate brain circuits have not converged on a biological basis for schizophrenia's link to nicotine addiction. We therefore employed an entirely data-driven analysis of the connectome to identify both shared and schizophrenia-specific circuits of nicotine addiction.

Methods: We reanalyzed existing data from two neuroimaging studies using a data-driven approach. In the first cohort, thirty-five smokers (18 schizophrenia, 17 controls) underwent resting-state fMRI imaging and clinical characterization. We conducted a transdiagnostic assessment to identify shared and diagnosis-specific circuits of nicotine use. A multi-variate pattern analysis of whole-connectome data was used to identify the strongest links between daily cigarette consumption and functional connectivity. In the second cohort, twelve participants with schizophrenia and 12 controls were enrolled in a randomized, controlled crossover study of transdermal nicotine patch with resting-state fMRI. We calculated mean change in DMN connectivity (FCnicotine – FCplacebo) and correlated change in whole-network functional connectivity with nicotine dose.

Results: In cohort 1, the strongest ($p<.001$) correlate between functional connectivity and daily cigarette consumption was driven by individual variation in the topography of the Default Mode Network (DMN). This effect was entirely driven by participants with schizophrenia despite the fact that groups were matched for severity of nicotine dependence. In cohort 2, we observed a linear relationship between nicotine dose and reduction in DMN connectivity ($R=-0.50$; 95% CI -0.75 to -0.12, $p<.05$). There was a significant effect of diagnosis on DMN connectivity. Schizophrenia subjects had hyperconnectivity compared to controls in the placebo condition ($p<.05$), which was no longer significant during nicotine administration.

Conclusions: It has been hypothesized that the biological basis of nicotine dependence is different in schizophrenia and in non-schizophrenia populations. We here provide direct evidence in support of this hypothesis by demonstrating that tobacco use is strongly linked to brain network organization only in participants with schizophrenia. Our results suggest that the high prevalence of nicotine use in schizophrenia may be a product of both a hyperconnected DMN that interferes with cognitive performance and is more sensitive to nicotine in schizophrenia compared to controls. Future experiments will directly test the acute effect of nicotine on this network in schizophrenia and control populations.

Keywords: Cortical Circuit Function, Schizophrenia, Nicotine Dependence, Resting State Functional Connectivity

Disclosure: Nothing to disclose.

W139. Repeated Ethanol Exposures Alter the Reward-Dependent Striatopallidal Neuronal Activities in the Dorsomedial Striatum

Seungwoo Kang, Doo-Sup Choi*

Mayo Clinic College of Medicine and Science, Rochester, Minnesota, United States

Background: The dorsomedial striatum (DMS) is an important brain region for shaping reward-seeking and coordinating movement. A growing body of evidence indicates the adaptation of the neuronal and astrocytic activities in the DMS produces distinctive reward-seeking behaviors. Recently, we demonstrated that optogenetic and pharmacological activation of adenosine receptor A2AR-expressing striatopallidal (SP) neurons in the DMS could reduce alcohol-seeking behavior in mice. However, little is known about the spatiotemporal activities of the neurons and astrocytes in the DMS that represent reward-dependent signatures in a cell-type specific manner.

Methods: We examined the cellular activities in the DMS during a three-arm choice task using fiber-photometry and microendoscopy calcium imaging. GCaMP6s, a genetically encoded calcium-dependent fluorescent indicator, was selectively expressed in the A2AR-expressing SP or ALDH1L1-expressing astrocytes by Cre-LoxP recombination system. Mice were given access to water, sucrose (15% w/v), and ethanol (15% v/v) solution on the three-arm choice task in a Y-maze arena for 10 min. In addition, we sought to investigate whether the chronic intermittent ethanol (CIE) exposures alter the synchronization of the cellular activities in the DMS with specific behavioral patterns. Briefly, mice were

exposed to air or vapored ethanol in vapor inhalation chamber for four weeks. Each daily cycle consisted of ethanol vapor for 16 h followed by 8 h of abstinence in their home cage. This was repeated each day for 4 consecutive days, followed by 3 days of abstinence.

Results: We observed that, at 72 h withdrawal from CIE exposure, mice showed the increased time spent in ethanol zone without changes in the time spent in sucrose zone compared to those of ethanol-naïve counterparts. In parallel, in the SP neurons of ethanol-naïve mice, Ca2+ transients were robustly increased sequentially as the mice were approaching and tasting ethanol, while those Ca2+ transients were significantly reduced by the ethanol withdrawal. No significant changes in Ca2+ transient patterns were observed in those neurons as the mice were approaching and tasting sucrose. The increases in Ca2+ transients of the SP neurons and astrocytes during other exploratory movements including turning and rearing were observed, but not changed by the CIE exposure.

Conclusions: Our results indicate that the DMS SP neuronal activities are responsive to ethanol, which could be progressively dampened upon repeated ethanol exposures, suggesting that SP neuronal activities represent reward-dependent behavioral flexibility.

Keywords: Dorsal Striatum, Ethanol, Reward-Seeking Behavior, Astrocyte-Neuron Interaction, Calcium Imaging

Disclosure: Peptron Inc: Advisory Board (Self)

W140. Plasticity Changes in Prelimbic Neurons Projecting to Nucleus Accumbens After Heroin Self Administration and Abstinence: Role of DRD1 and DRD2 Dopamine Receptors

Robert Cole, Claudia Trotter, Daniela Cossio, James Otis, Jacqueline McGinty*

Medical University of South Carolina, Charleston, South Carolina, United States

Background: Dysregulation of a subset of neurons in the prelimbic (PL) cortex that project to the nucleus accumbens (NAc) core is implicated in relapse to drug-seeking after both cocaine and heroin self-administration (SA); however, the underlying cellular mechanisms have not been thoroughly investigated. Specifically, a critical gap in our knowledge exists about the type of changes in PL cortex that drive relapse to drug-seeking after forced abstinence without extinction training. Further, there is no information about changes in intrinsic and synaptic plasticity in PL-NAc core neurons that express Drd1 vs. Drd2 dopamine receptors in abstinence/relapse models.

Methods: Male and female Long Evans wildtype (WT), Drd1-Cre + (D1), and Drd2-Cre+ (D2) rats were implanted with a jugular catheter and their nucleus accumbens core (NAc core) was infused with retrograde virus AAVrg-hSyn-eGFP (WT) or AAVrg-hSyn-DIO-eGFP (Cre+) to isolate PL-NAc projecting neurons. After recovery, rats were trained to self-administer heroin on a FR1 descending dose heroin schedule (2 mg/ml days 1-2, 1 mg/mL days 3-4, 0.5 mg/mL days 5-14) or received yoked saline. Following 7 days of homecage abstinence, rats were decapitated and acute brain slices of the PFC (300 um) were prepared for whole-cell patch clamp recordings of PL->NAc neurons. Layer V PL-NAc neurons were identified by the presence of GFP and all recordings occurred in oxygenated aCSF that contained 100 uM picrotoxin to isolate glutamatergic transmission. Current-clamp recording were employed to examine intrinsic cellular activity such as rheobase and action potential (AP) frequency. Voltage-clamp recordings were used to characterize synaptic plasticity following heroin SA. Measurements of synaptic plasticity included baseline spontaneous excitatory postsynaptic currents (sEPSCs) and AMPA to NMDA ratios.

Results: Following 7 days of heroin abstinence, overall AP firing was significantly higher in WT animals (17.0±2.8) compared to saline-treated WT animals (11.55±0.6; p=0.04). This increase of AP firing was not associated with changes in rheobase measurements between heroin (135.5±11.5pA) and saline (146.7±11.9pA; p=0.61) groups. Moreover, heroin abstinent WT rats had increased sEPSC amplitudes (15.2±1.2pA vs 11.6±1.2pA; p=0.049), sEPSC frequency (2.9±0.2 vs 1.7±0.3; p<0.01) compared to WT saline rats, but sEPSC decay did not differ (7.2±0.6ms vs 7.2±0.8ms; p=0.95). Because sEPSC amplitude and frequency are associated with post- and pre-synaptic mechanism respectively, we further examined changes in the AMPA/NMDA ratios following heroin abstinence. We found AMPA/NMDA ratios were significantly higher in the heroin group (1.6±0.2) than in the saline group (0.9±0.1; p<0.01).

Because prefrontal dopamine plays a role in controlling aspects of addiction-like behavior, we investigated changes in specific Drd1+ or Drd2+ PL->NAc projecting neurons. Preliminary data show that following 7d of heroin abstinence, Drd1+ PL->NAc neurons also exhibit a significant increase in overall AP firing (19.6 ±0.7) compared to saline Drd1+ rats (13.8±4.2; p=0.02). Interestingly, heroin treated Drd2+ PL->NAc neurons exhibited a trend toward a decrease in AP firing (10.7±0.6) compared to PL->NAc neurons in saline rats (13.9±2.2; p=0.06). Further analysis of synaptic plasticity measures is currently ongoing.

Conclusions: This study demonstrates that heroin-induced neuroadaptations in PL->NAc neurons following 7 days of homecage abstinence in WT rats are due to increases in intrinsic neuronal excitability in addition to changes in synaptic plasticity. Furthermore, preliminary evidence suggests that these heroin-induced changes in PL->NAc projection neurons appear to be mediated by Drd1+ neurons after abstinence. Our preliminary findings also suggest that the role of Drd2+ PL->NAc cells may be differentiated from Drd1+ PL->NAc cells following heroin abstinence. Future experiments will explore the role of these neuroadaptations after relapse as well as targeting downstream signaling related to Drd1 and Drd2 as possible pharmacotherapies for substance use disorders.

Keywords: Opioid Addiction, Medial Prefrontal Cortex, D1 Dopamine Receptors, D2 Dopamine Receptor, Nucleus Accumbens Core

Disclosure: Nothing to disclose.

W141. Chemogenetic Inhibition of Orbitofrontal Cortex Reveals Distinct Role of Subtypes of Cortical Projection Neurons in Regulating Decision-Making in a Probability Discounting in Rats Task

Zackari Murphy*

University of Washington School of Medicine, Seattle, Washington, United States

Background: A prominent feature of many neuropsychiatric disorders, including drug addiction, is maladaptive decision-making. This often presents as impulsivity, which can be defined as an inability to appropriately consider the consequences of a behavioral action. The orbitofrontal (OFC) is essential for encoding and updating reward evaluations, and changes in OFC function are thought to underlie the impulsivity that develops in addiction. However, cortical pyramidal projection neurons can be subdivided into two major types with distinct inputs and projection targets, molecular and receptor profiles, morphologies and electrophysiological characteristics. Intratelencephalically (IT) cortical neurons have sparse apical tufts, minimal h-currents, are regular spiking and project bilaterally to striatum and contralateral cortex. In contrast, pyramidal tract (PT) cortical neurons have thick apical tufts, prominent h-currents and are burst firing send their main axon into the pyramidal tract with collateral projections to

ipsilateral striatum and subcortical structures. Given the distinct cellular properties and connectivity patterns of these two populations, they are poised to regulate decision-making processes in unique ways. However, studies have not specifically examined the role of IT and PT neurons in impulsive behavior.

Methods: We used an intersectional viral vector approach in male Long Evans rats to express inhibitory Gi/o-coupled DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) selectively in IT or PT neurons (or both) of the ventro-lateral OFC. Activation of DREADDs by clozapine-n-oxide (CNO) produces transient decreases in neuronal activity and allowed us to examine the effect of these manipulations on behavior in a probability-discounting task. To target both IT and PT neurons, a retrograde FLP (AAVrg-EF1a-FLP) was injected into the left hemisphere pons and the right hemisphere nucleus accumbens (Nac), while a retrograde CAV2-CRE was injected into the right hemisphere pons and left hemisphere NAc. Flp-dependent (Left hemisphere: AAV1-DIOFRT-hm4Di-EYFP) and CRE-dependent (Right hemisphere: AAV8-hSyn-DIO-hm4DiHm4Di-mCherry) DREADDS were injected into the OFC. To target IT neurons, CAV2-CRE was injected into one hemisphere of the NAc and a CRE-dependent DREADD was injected into the contralateral OFC. AAVrg-FLP was then injected into the other NAc hemisphere and a FLP-dependent DREADD was injected into the contralateral OFC. To target PT neurons, CAV2-CRE was bilaterally injected into the pons, while a CRE-dependent DREADD was injected bilaterally into the OFC. As an additional control, a nonselective DREADD (AAV8-hSyn-hm4Di) was injected bilaterally into the OFC. Rats then underwent training in a probability discounting paradigm. During this task, one lever would always yield one food pellet following each lever press ("safe" option), while the other lever would yield three food pellets with a decreasing chance of reward depending on time block from 100% to 50% to 25% to 12.5% ("risky" option). After ~30 days to establish a stable probability-discounting curve, rats were tested both CNO (3 mg/kg, ip) or vehicle in a counter-balanced fashion with re-baselining between tests. Rats then underwent a reversal task, where the "safe" and "risky" levers were switched; following 7 sessions of training rats received counterbalanced CNO and vehicle tests with additional training in-between. Brains were then processed for immunohistology verification of DREADD expression.

Results: Nonselective neuronal inhibition of OFC led to a significant overall reduction in choice of the risky reward option (two-way ANOVA, Main effect of treatment). However, neither selective inhibition of IT nor PT neurons, nor dual inhibition of both IT and PT neurons, altered the discounting curve. When examining patterns of choice based on the outcome (win or lose) and subsequent pattern (stay on option or shift to alternative), nonselective OFC inhibition impacts choice based on outcome (three-way ANOVA Treatment x Outcome), while dual IT and PT inhibition alters rates based on both outcome and whether a choice was on the same lever or shifted to the alternative option from the previous action (three-way ANOVA, Treatment x Outcome x Choice). However, choice patterns were not affected by selective IT or PT inhibition. In the reversal task, only IT neuron inhibition significantly increased the number of reversals (paired t-test). In contrast, only PT neuron inhibition significantly increased the number of trials needed to make a subsequent reversal when the start of a reversal resulted in a reward (three-way ANOVA, Treatment x Outcome x Choice).

Conclusions: These data confirm and extend previous results that the OFC regulates impulsive choice, as assessed in a probability discounting task. Unexpectedly, manipulations specifically targeting IT and PT neurons had no effect on discounting curves, suggesting that the effects of the nonselective manipulation may be due to alterations in interneuron activity. Nonetheless, these data suggest that IT and PT cortical neurons are important for and play distinct roles in the regulation of reversal learning. Future studies using c-Fos mapping and fiber photometry are planned to determine whether IT and PT neurons in other regions of the prefrontal cortex may play a different role in decision-making in the probability discounting task.

Keywords: Risk Taking Behaviors, DREADDs, Orbitofrontal Cortex (OFC)

Disclosure: Nothing to disclose.

W142. Epigenetic Effects of Sex and Early-Life Stress on Cocaine Addiction Vulnerability

Devin Rocks, Ivana Jaric, John Greally, Masako Suzuki, Marija Kundakovic*

Fordham University, Bronx, New York, United States

Background: Early-life traumas, such as neglect and abuse, significantly increase the risk for drug abuse and addiction, including cocaine use disorder, in later life. In addition, there are sex differences in cocaine addiction, with females progressing more rapidly into substance use disorder than males, which is at least in part due to sex hormone effects. However, the interaction between sex and stress and, in particular, the biological mechanisms underlying stress- and sex hormone-induced addiction vulnerability remain poorly understood. We hypothesized that early life stress and sex hormones interact to bring about sex-specific cocaine vulnerability, acting via epigenetic mechanisms.

Methods: To study this, we first performed a well-established early-life stress (maternal separation, MS) paradigm in C57BL/6J mice, which included 3-hour daily separations of pups from their mothers in the first two weeks of life, from postnatal day (PD) 1-14. We then examined the risk for cocaine addiction in late adolescence/early adulthood (PD54-60) using the cocaine-induced conditioned place preference (CPP) with the low (2.5 mg/kg) or high (10 mg/kg) cocaine dose (N=15-25 mice per sex/separation group/dose). For the duration of the CPP test, the estrous cycle stage of each female animal was determined daily, using vaginal cytology. To analyze the CPP data, we conducted three-way ANOVA with sex (male, female), maternal separation (no separation, separation), and cocaine dosage (2.5 mg/kg, 10 mg/kg) as factors. For females, we also analyzed the data separately, accounting for the estrous cycle stage. To address whether epigenetic mechanisms may be involved in sex-specific and estrous cycle-dependent cocaine responses, we ran a separate cohort of control C57BL/6J mice. At P54, we performed a genome-wide chromatin accessibility analysis (ATAC-seq) on FACS-purified neuronal nuclei from the nucleus accumbens (NAc) of proestrus (high-estrogenic) females, diestrus (low-estrogenic) females, and males, following 1-hour or 4-hour acute (10 mg/kg) cocaine treatments (N=9 animals per group/time point). The bioinformatics analysis of the ATAC-seq data included differential chromatin accessibility, gene ontology, and KEGG pathway analyses among the examined groups.

Results: Our study shows that early-life stress increases cocaine preference in a dose- and sex-dependent manner. Male mice exposed to early-life stress show increased cocaine CPP after the low (2.5 mg/kg) cocaine dose that is not sufficient to induce cocaine preference in control males ($p<0.001$); the high (10 mg/kg) cocaine dose induces CPP in both MS and control male groups. On the contrary, both low (2.5 mg/kg) and high (10 mg/kg) cocaine doses have a more profound effect in stress-exposed female mice compared to control females ($p<0.001$). Interestingly, we found that the CPP results in control females vary with the estrous cycle ($p<0.01$), with early-life stress skewing this behavior toward increased cocaine preference. Furthermore, we found sex-specific, estrous cycle-dependent chromatin organizational changes in the NAc, a key brain reward area, in response to acute cocaine treatment. Close to 1,000 gene regions in NAc

neurons changed chromatin accessibility following cocaine exposure in both male and proestrus female mice, with a very little overlap between sexes. Chromatin differences were found nearby genes involved in neuronal function, synaptic plasticity, and the brain reward pathway, implicating different genes and pathways in cocaine-induced plasticity in males and females.

Conclusions: We show that early-life stress increases cocaine preference in later life, but the results are different in males and females and depend on the cocaine dose. Acute cocaine exposure exerts sex-specific, estrous cycle-dependent effects on neuronal chromatin organization in the nucleus accumbens, the key reward area, and nearby neuronal genes implicated in addiction. Overall, this study has implications for improving our understanding of the role of stress and sex hormones in sex-specific addiction vulnerability, and reveals candidate epigenetic mechanisms underlying sex differences in drug abuse and addiction.

Keywords: Cocaine Addiction, Sex Difference, Sex Hormones, Epigenetic, Early Life Stress

Disclosure: Nothing to disclose.

W143. Cocaine and Sucrose Rewards Recruit Different Seeking Ensembles in the Nucleus Accumbens Core

Ana-Clara Bobadilla*, Eric Dereschewitz, Lucio Vaccaro, Jasper Heinsbroek, Michael Scofield, Peter Kalivas

University of Wyoming, Laramie, Wyoming, United States

Background: Poorly regulated reward seeking is a central feature of substance use disorder. Convergent findings using different biomarkers reveal that only ~2-5% of cells encode a putative cocaine ensemble, including deltaFosB immune-labeling, Daun02-inactivation of cFos expressing neurons, and the number of nucleus accumbens Medium Spiny Neurons (MSNs) exhibiting phasic activity. When animals are exposed to two rewards, in vivo measurements of neuronal firing in the nucleus accumbens reveal ~20% overlap between neurons responding to self-administration of different types of reward such as cocaine, water, regular chow or sucrose, while a study using FISH reports that 50% of activated neurons in the infralimbic prefrontal cortex respond to both ethanol and saccharin. These results suggest a finely tuned specificity of ensembles. Here we comprehensively characterize the specific ensembles of neurons built through experience that are linked to two antagonistic behavioral strategies: seeking and extinction learning. We additionally address the question of whether or not addictive drugs usurp the neuronal networks recruited by natural rewards by evaluating cocaine- and sucrose-associated ensembles within the same animal.

Methods: We use targeted recombination in active populations (TRAP) strategy, specifically FosCreERT2/+/Ai14 (cFos-TRAP) transgenic mice to deposit a cFos-driven Cre recombinase-tdTomato reporter into neurons activated during cue-induced reward seeking and extinction in order to tag cells as potentially encoding these behaviors. The construct fuses Cre-recombinase to the estrogen receptor (ER) under the cFos promoter and allows nuclear Cre expression in the presence of ER agonist 4-hydroxytamoxifen (4-OHT), specifically in the cells expressing cFos. To characterize the seeking and extinction ensembles, these mice underwent the well-described rodent behavioral model of cocaine self-administration (SA), extinction training and cue-induced reinstatement of seeking. To define and compare different reward-specific ensembles within the same animal, we developed a poly-reward cocaine and sucrose self-administration paradigm in mice, where each reward is associated to a different discrete cue. After undergoing extinction training in absence of cues, mice are first re-exposed to one cue in presence of 4-OHT, allowing the tagging of the reward-specific ensemble with tdTomato, and a few days later exposed to the second cue, followed by immediate Fos tagging. Using this paradigm, we were able to assess the neurons included in the cocaine or sucrose ensembles, and to quantify the overlap between the two populations within the same animal exposed to both types of reward.

Results: We tagged ~1% of neurons in the core subregion of the accumbens activated during cue-induced seeking for cocaine or sucrose. The majority of tagged cells in the cocaine- or sucrose-seeking ensembles were D1-MSNs, and specifically activated during seeking, not during extinction or when mice remained in the home cage. Using the cocaine and sucrose poly-reward model, we found ~70% distinction between the cells constituting the cocaine- compared to the sucrose-seeking ensemble. Establishing that cocaine recruits an ensemble of nucleus accumbens core neurons largely distinct from neurons recruited into an ensemble coding extinction learning from drug use or for sucrose seeking.

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

Keywords: Neuronal Ensemble, Reward Circuitry, Cocaine Seeking

Disclosure: Nothing to disclose.

W144. Anatomical and Behavioral Characterization of Noradrenergic Galanin and its Receptors Following Chronic Morphine and Precipitated Withdrawal

Stephanie Foster*, Saumya Karne, David Weinshenker

Emory University School of Medicine, Atlanta, Georgia, United States

Background: The neuropeptide galanin and its receptors are dynamically regulated in the noradrenergic locus coeruleus (LC) in response to various conditions including exercise and opioid exposure. Understanding the modulators and effects of noradrenergic galanin is important, as it is implicated in regulating stress responses and attenuating opioid reward and withdrawal. The galanin receptor subtype GalR1 may be particularly relevant, since it is reported to be upregulated in the LC following morphine withdrawal. Here, we evaluated the effects of chronic morphine and precipitated withdrawal on GalR1 expression in the LC using an in situ hybridization approach that offers enhanced spatial and cell-type specificity, which was lacking in previous studies. We also assessed the consequences of increasing or decreasing noradrenergic galanin on withdrawal behaviors using genetically altered mice. We predicted that withdrawal would increase GalR1 expression in the LC, and that depleting galanin in the LC would exacerbate withdrawal symptoms, while overexpression would reduce symptoms.

Methods: Eighteen C57BL/6J mice (males and females) were used for in situ hybridization studies. Mice were assigned to one of three groups: saline control, chronic morphine, or precipitated withdrawal ($n = 6$ per group). Mice received five consecutive days of twice daily intraperitoneal injections of saline or escalating doses of morphine (20, 40, 60, 80, 100 mg/kg). On day 6, mice were administered a final dose of saline or 100 mg/kg morphine, and 2 h later, all mice received subcutaneous naloxone (1 mg/kg). Three hours after naloxone, all mice were sacrificed and brains were processed for in situ hybridization. RNAscope (Multiplex Fluorescent Assay) was performed on LC tissue using probes for tyrosine hydroxylase (TH), galanin, and GalR1, and images were analyzed using Imaris software. The average percent of TH-positive cells co-expressing GalR1 and the average number of GalR1 puncta per TH-positive cell were compared across groups using one-way ANOVA. For behavioral studies, morphine (same dosing regimen as above) was administered to wild-type (WT) mice and those either lacking or overexpressing galanin in

noradrenergic neurons (NE-GAL KO and NE-GAL OX, respectively (n = 7-10 per group). Following naloxone administration, mice were video recorded for 30 min, and withdrawal symptoms (weight loss, diarrhea, jumping, rearing, paw tremor, wet dog shakes, sniffing, and backwards steps) were quantified. Behavioral data were analyzed via two-way ANOVA.

Results: Preliminary image analyses suggested that only 35-40% of TH-positive LC neurons co-expressed GalR1, and expression levels overall were very low (1-2 puncta/cell). In contrast, abundant GalR1 expression was observed in brainstem TH-negative cells outside the LC. Importantly, neither chronic morphine nor precipitated withdrawal significantly altered the percent of TH-positive LC cells that co-expressed GalR1 or the number of GalR1 puncta per cell. Analyses of withdrawal behaviors showed that neither depletion nor overexpression of noradrenergic galanin strongly affected withdrawal behaviors compared to WT, as there was no main effect of genotype for either study.

Conclusions: Our findings differ from previous findings in mice and suggest that (1) GalR1 expression attributed to the LC may mostly reflect non-noradrenergic cells in nearby brain regions, and (2) LC-derived galanin may not be necessary for a typical withdrawal response. Together, our findings suggest that noradrenergic galanin does not strongly mediate opioid withdrawal effects.

Keywords: Opioid Addiction, Galanin, Noradrenergic, Opioid Withdrawal

Disclosure: Nothing to disclose.

W145. Npas4 Regulates Cocaine-Conditioned Behaviors and Synaptic Transmission in a Cell Type-Specific Manner

Brandon Hughes*, Makoto Taniguchi, Christopher Cowan

Medical University of South Carolina, Charleston, South Carolina, United States

Background: Hallmark features of substance use disorders, as defined by DSM-V criteria, include patterns of drug use, dependence, and compulsive seeking despite negative and/or harmful outcomes. During the course of drug use, persistent neuroadaptations develop in the nucleus accumbens (NAc), a brain region predominately composed of dopamine D1 receptor- and D2 receptor-expressing medium spiny neurons (MSNs). The progression from casual drug use to abuse is potentially mediated, at least in part, by the strong associations formed between the euphoric effects of the drug and the environmental contexts and cues linked to drug use experiences. As such, these external cues can become powerful triggers for relapse. However, the molecular and cellular mechanisms underlying these powerful and enduring drug-context memories are not well understood. One possible regulator of neuroadaptations in the NAc responsible for drug reward-related learning is the activity-dependent transcription factor, neuronal PAS Domain Protein 4 (Npas4). We previously showed that constitutive knockout of Npas4 in the NAc reduces cocaine-conditioned behaviors, and multiple studies in the literature report that Npas4 can regulate excitatory and inhibitory synapse balance and synaptic transmission in a cell type-dependent manner. As such, we sought to examine the possible cell type-specific regulation and role of Npas4 in the adult rodent NAc during and following repeated cocaine experiences.

Methods: Since NPAS4 is induced in D1- and D2-MSNs following a cocaine reward experience, we designed a Cre-dependent AAV2-Npas4 shRNA virus to reduce Npas4 in only these cell types. The virus was bilaterally infused into the NAc of young adult D1-Cre or D2-Cre BAC transgenic mice (male and female) then, three weeks later, they underwent cocaine conditioned place preference and time spent in the cocaine-paired chamber on the test day was measured (n = 11-14/group; unpaired t-test). To determine the role of Npas4 in D2-MSN synaptic transmission, we performed patch-clamp recordings of D2-MSNs after Npas4 knockdown, as well as a circuit-based approach using channelrhodopsin (ChR)-evoked currents from PFC-NAc afferents. Ongoing studies are 1) investigating the cell type-specific role of Npas4 during cocaine self-administration and 2) using single-cell RNA sequencing (scRNA-seq) to further characterize the Npas4-inducing cell types in the NAc after cocaine conditioning.

Results: Following exposure to a novel drug-paired environment, a small population of NAc neurons induce NPAS4 and, of those, ~50% are D1R- or D2R-expressing MSNs. Npas4 knockdown in D2-, but not D1-, MSNs of the NAc caused a significant reduction in cocaine CPP ($p < 0.01$). Preliminary data suggests this D2-MSN-specific effect of Npas4 knockdown is associated with hyperactivation of D2-MSNs. In addition, ongoing studies suggest a possible role for Npas4 in D2-MSNs for drug seeking behavior following cocaine self-administration. Moreover, preliminary scRNA-seq analysis reveals interesting cell type-specific populations, including D2-MSNs, that show enrichment and induction of Npas4 following cocaine conditioning.

Conclusions: Together, our findings suggest that NAc Npas4 plays a critical cell type-specific role to shape the circuit adaptations required to support the development of drug reward-context memories. Npas4 appears to regulate cocaine conditioned behaviors by modulating E/I balance of D2-MSNs, and possibly other cell types, within the NAc.

Keywords: Cocaine Addiction, Nucleus Accumbens, Conditioned Place Preference, Homeostatic Synaptic Transmission, Npas4

Disclosure: Nothing to disclose.

W146. Phosphoproteomic Identification of Differential Neural Signaling Induced by Low to High Dose Alcohol in the Nucleus Accumbens

Mary Torregrossa*, Megan Bertholomey, Angus Nairn, TuKiet Lam

University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Background: Alcohol use disorders (AUDs) cause significant health and financial burdens to society; however, the neural mechanisms underlying the development of AUDs are not well understood. One of the challenges for understanding AUDs is that alcohol itself is a very ubiquitous drug, affecting numerous aspects of cellular function. Historically, work on understanding the targets of alcohol in the brain have focused on high concentrations of alcohol that correspond to either binge-like intoxication or even loss of consciousness. The neural targets of concentrations that correspond to 1-2 drinks (social drinking) that mediate the initial reinforcing effects have been less well characterized. However, the individual response to low doses of alcohol is known to be quite variable with some experiencing extreme negative effects, while others experience strong positive effects. The different ways individuals respond to low doses can be predictive of risk for developing an AUD. Thus, we used phosphoproteomics as an unbiased screen for proteins and signaling systems regulated by low versus medium versus high doses of alcohol, and compared effects across males and females, as a first step at comparing groups known to have biologically different responses to alcohol.

Methods: In adult male and female Sprague-Dawley rats, we gave intraperitoneal (i.p.) injections of ethanol (100% diluted to desired concentration in saline) at three doses and compared to control rats that were either uninjected or that received saline injections. We administered doses to target blood alcohol concentrations (BACs) of 13 mg/dL (supports operant self-

administration), 80 mg/dL (binge-like), or 230 mg/dL (excessive intake). About 10 min after injection, corresponding to peak BACs, rats were killed by focused microwave irradiation, blood was collected to measure BACs, and the nucleus accumbens was dissected and processed for analysis of phosphorylated proteins by quantitative label-free mass spectrometry in collaboration with the Yale/NIDA Neuroproteomics Center. Normalized phosphopeptide abundance was analyzed by ANOVA across doses and between sexes for each phosphopeptide. Only phosphopeptides meeting set confidence thresholds were analyzed. Changes in phosphopeptide abundance across doses relative to the combination of the two control groups was analyzed by Ingenuity Pathway Analysis (IPA) to identify pathways significantly regulated by each dose of ethanol.

Results: We identified 250 unique phosphopeptides regulated by ethanol relative to no injection and saline control groups. Of the 250 ethanol regulated phosphopeptides, only two were significantly altered by all three doses. Indeed, each dose produced largely non-overlapping changes in protein phosphorylation, with the medium dose producing almost 3X the number of alterations as the high dose and 5X the number of alterations as the low dose. We also identified 168 phosphopeptides showing sex differences in response to low dose ethanol that were independent of baseline sex differences. Finally, IPA indicated that while all doses altered synaptogenesis signaling, there were distinct pathways regulated by each dose. For example, the low dose regulated GABA receptor signaling, the medium dose regulated opioid and glutamate signaling, and the high dose regulated calcium and immune signaling.

Conclusions: In conclusion, we identified phosphopeptides and signaling pathways uniquely regulated across low, medium, and high doses of alcohol, with the amount of differentially phosphorylated proteins showing an inverted-U dose effect pattern. In addition, we found that low dose alcohol results in distinct protein phosphorylation events in males and females that may explain known sex differences in the propensity to consume alcohol.

Keywords: Alcohol, Proteomics, Sex Differences, Phosphorylation, Dose Response

Disclosure: Nothing to disclose.

W147. Missplicing of Long Non-Coding RNAs in Brain in Patients With Alcohol Use Disorder

Ilya Blokhin*, Derek Van Booven, Dayne Mayfield, Claes Wahlestedt

University of Miami Jackson Memorial Hospital, Miami, Florida, United States

Background: Alcohol use disorder (AUD) is a problematic pattern of alcohol use leading to clinically significant impairment. In the United States, 14% of adults currently meet criteria for AUD, 29% – met AUD criteria once during their lifetime. Annual cost of AUD and alcohol-related disorders is ~$250 billion, and prevalence of AUD is increasing. Even though multiple studies have focused on AUD, etiopathogenesis of this disease remains unclear. As a result, current medications for AUD are not highly efficacious, and majority of patients with AUD eventually relapse. The lack of clinical translation may stem from a traditional focus on protein-coding genes (PCGs). PCGs constitute only ~2% of genome, however, while ~70-80% of genome are transcribed. The most understudied class of non-coding features is long non-coding RNAs (lncRNAs) which are non-coding RNAs >200 nucleotides in length with no coding potential. lncRNAs are difficult to study since they function in a splice-specific manner. Splicing is a part of messenger RNA (mRNA) processing which facilitates the removal of introns from a synthesized mRNAs to produce mature mRNAs consisting only of exons. To date, the splicing landscape of lncRNAs in brain of healthy individuals and patients with AUD has not been interrogated. We tested the hypothesis that lncRNAs are aberrantly spliced in patients with AUD.

Methods: Postmortem human brain samples (30 patients with AUD and 30 controls) were obtained from the New South Wales Tissue Resource Centre at the University of Sydney. Fresh frozen samples of the superior frontal gyrus (SFC), nucleus accumbens (NA), basolateral amygdala (BLA), and central nucleus of amygdala (CNA) were collected from each sample. For RNA sequencing, samples were processed using the TruSeq RNA Library Prep Kit v.2 and sequenced on the Illumina HiSeq 2000. To evaluate the transcriptome conventionally (in non-splice-specific manner), we employed edgeR in Bioconductor. Evaluation of the transcriptome in a splice-specific fashion was performed using STAR alignment with StringTie/BallGown pipeline. To evaluate missplicing events, resulting bam files from the STAR alignment were indexed with samtools for use by rMATS software package.

Results: We found that lncRNAs were ~11-fold more spliced in SFC, NA, BLA, and CNA compared to transcripts from PCGs. Higher rates of splicing were observed across all brain regions in a highly consistent manner, suggesting that the rate of lncRNAs splicing in the brain is tightly regulated. Given that each PCG gives rise to ~5 mature mRNA transcripts, these data indicate that individual lncRNA gene may be capable of generating a diverse transcriptomic pool. We also observed that more lncRNAs were misspliced (than differentially expressed) in brain of patients with AUD. Specifically, we found that expression of 29 lncRNAs was altered in AUD: 4 – in SFC, 2 – in NA, 15 – in BLA, and 8 – in CNA. Analysis of misspliced lncRNAs identified 54 missplicing events: 26 events were observed in SFC, 2 – in NA, 24 – BLA, and 2 – in CNA.

Conclusions: In summary, our data suggest that lncRNAs are extensively spliced in the brain and that AUD is accompanied by missplicing of multiple lncRNAs. As lncRNAs and spliceosome can be targeted more specifically than proteins via oligonucleotide-based technologies, and these therapies can be delivered to the central nervous system, identification of affected components of the spliceosome and functionally important misspliced lncRNAs may reveal new mechanisms of etiopathogenesis of AUD and suggest novel therapeutic targets.

Keywords: Alcohol Use Disorder, RNA Splicing, Non-Coding RNA

Disclosure: Nothing to disclose.

W148. Hedgehog-Interacting Protein Acts in the Habenula to Regulate Nicotine Seeking

William Howe*, Stephanie Caligiuri, Karim Elayouby, Purva Bali, Zuxin Chen, Xin-An Liu, Vanessa Lehmann, Alexander Smith, Jessica Ables, Maya Williams, Paul Johnson, Alexandra DiFeliceantonio, Kristin Beaumont, Robert Sebra, Ines Ibanez-Tallon, Paul Kenny

Virginia Tech, Blacksburg, Virginia, United States

Background: Major breakthroughs in our understanding of the mechanisms of tobacco dependence were spurred by large-scale human studies seeking to identify genetic loci influencing vulnerability to lung cancer. Allelic variation in the α3/α5/β4 nicotinic acetylcholine receptor (nAChR) subunit gene cluster was shown to increase lung cancer risk, and subsequent studies demonstrated that these same alleles also increased vulnerability to tobacco dependence in humans. Mechanistic studies in rodents showed that α5 nAChRs control the sensitivity of the medial habenula (mHb)-interpeduncular nucleus (IPN) circuit to nicotine, which signals the aversive properties of the drug, constituting a neurophysiologic "brake" on nicotine intake. As such, nicotine-induced alterations in the function of this circuit are likely to play

key roles in promoting the development of tobacco addiction and thereby influence risk of smoking-related diseases. However, little is currently known about mechanisms downstream of nAChRs in the mHb-IPn circuit that regulate the motivational properties of nicotine.

Methods: We used single cell RNA sequencing (scRNASeq) to identify genes and cell types in the mHb-IPN modified by nicotine exposure, a combination of translating ribosome affinity purification (TRAP) and in situ hybridization (RNAscope) to confirm scRNAseq results, and CRISPR-CAS9 gene editing in mice self-administering nicotine to assess the role of genes identified by our scRNASeq results in regulating the motivation to consume nicotine. All experimental protocols in animal studies were approved by the Mt. Sinai Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Sex was included as a variable in all analyses (n=~8-15 per experiment, males and females).

Results: We collected the mHb from mice exposed to a behaviorally relevant dose of nicotine (1.2 mg kg-1) or saline and performed scRNAseq. Cell cluster classification and identification of differentially expressed genes identified 14 distinct cell clusters in habenula from saline- and nicotine-treated mice. One cluster of cells that showed robust nicotine-induced changes in gene expression contained markers characteristic of cholinergic neurons, consistent with the large population of cholinergic neurons contained in the ventral portion of the medial habenula. The majority of these cells also expressed Slc17a6 transcripts, consistent the fact that habenular cholinergic neurons co-release glutamate. The gene encoding Hedgehog-interacting protein (HHIP), which regulates hedgehog as well as nitric oxide signaling was upregulated by nicotine in these cells. Interestingly, allelic variation in HHIP is a major genetic risk factor for COPD, a disease that leads to poor lung function and like lung cancer is associated with tobacco smoking. We followed up this intriguing finding by first collecting habenula tissue from Chat-Cre mice and, using TRAP, found that Hhip transcripts were expressed at ~7-fold higher levels in cholinergic neurons compared with other cell types in the habenula. Co-localization of Chat and HHIP was further confirmed with RNAscope. Thus, HHIP is robustly expressed in habenula cholinergic neurons that are transcriptionally responsive to nicotine.

To test the functional role of HHIP-mediated signaling in nicotine self-administration, we used CRISPR/Cas9 to cleave Hhip in the habenula of mice. AAVs expressing sgRNA-Hhip or sgRNA-control and Cre recombinase were injected into the habenula of Rosa26LSL-spCas9-eGFP mice. We confirmed that Hhip was efficiently cleaved and protein levels of HHIP were reduced in the habenula of mice that received AAV-sgRNA-Hhip compared to controls. Behaviorally, the locomotor-suppressing actions of nicotine (1.2 mg kg-1) were attenuated in mice treated with AAV-sgRNA-Hhip, and numbers of c-Fos immunopositive cells were also lower in the IPn compared with the control AAV-sgRNA-eGFP mice ($p<0.05$). As habenula cholinergic neurons provide the major source of excitatory input to the IPn, these findings suggest that HHIP regulates habenula-IPn circuit function. Finally, sgRNA-Hhip and sgRNA-control mice were trained to respond for intravenous nicotine infusions (0.03-0.6 mg kg-1 per infusion). Responding for food rewards was similar in both groups ($p>0.10$), however mice treated with AAV-sgRNA-Hhip in the mHb consumed greater quantities of nicotine than those treated with AAV-sgRNA-eGFP, an effect was apparent when high unit doses of nicotine were available for self-administration ($p<0.05$). Habenula AAV-sgRNA-Hhip mice also showed robust craving-like increases in nicotine-seeking responding compared with AAV-sgRNA-eGFP mice following a period of forced abstinence ($p<0.05$).

Conclusions: HHIP risk alleles are associated with poor lung function and increased vulnerability to COPD. It is generally assumed that HHIP acts exclusively in the lungs. However, our data suggest that HHIP is densely expressed by habenula cholinergic neurons, which regulate aversive behavioral responses to nicotine that protect against developing tobacco addiction. Our findings suggest that, in addition to its actions in the lungs, HHIP may also influence COPD risk by acting in the habenula to regulate the motivational properties of nicotine contained in tobacco smoke.

Keywords: Nicotine Addiction, Single-cell RNA Sequencing, CRISPR/Cas9, Self-Administration, Systems Neuroscience

Disclosure: Takeda, Inc: Consultant (Self)

W149. Central Amygdala Projections to the Nucleus Accumbens Core Regulates Binge Drinking

Angela Ozburn*

Oregon Health & Science University, VA Portland Health Care System, Portland, Oregon, United States

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 subjects. Our previous studies have found that increasing neuronal activity in the NAc core reduced binge drinking in female C57BL/6J mice, and decreasing activity reduced drinking in males. Here, we hypothesized that specific projections to the NAc core could be important for initiation and maintenance of binge drinking, and these circuits may be different in males and females. The central amygdala (CeA) projects to the NAc core, is an important regulator of alcohol drinking, and exhibits sex-dependent physiological responses to alcohol (Logrip et al., 2018). We asked whether 1) neuronal projections from the CeA to the NAc core, or 2) corticotropin releasing factor (CRF) in the NAc core could regulate binge drinking in male and/or female C57BL/6J.

Methods: For experiment 1, mice were injected with AAV2 Cre-GFP into the NAc core and a Cre-inducible DREADD [AAV2 DIO-hM3Dq, -hM4Di, or -mCherry] into the CeA. We tested the effects of altering CeA to NAc activity on binge-like ethanol intake ("Drinking in the Dark", DID) and measured intake of other fluids/tastants using a serial drinking in the dark design (n=11-19/sex/virus group; as described in Purohit et al., 2018). During the first week of ethanol DID, mice were treated with vehicle to establish baseline 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. We subsequently measured the effects of vehicle or CNO on intake of water and saccharin in the dark. For experiment 2, we bilaterally implanted guide cannula into the NAc core of male and female mice, allowed them to recover, and performed a 4d DID. We measured baseline ethanol intake on d1-3, and on d4, we microinfused 0, 0.05, or 0.5ug CRF into the NAc core prior to DID (n=7-10/sex/dose).

Results: We found that stimulation of excitatory, but not inhibitory, DREADDs in neurons projecting from the CeA->NAc core significantly decreased binge-like ethanol drinking ($p<0.001$). For serial tastant testing, we found that increasing CeA->NAc core activity modestly reduced water intake ($p<0.01$), but had no effect on saccharin intake. CNO did not alter intake of ethanol, water, or saccharin in mice expressing hM4Di. We found that CRF in the NAc core could mimic the effects of CNO stimulation of hM3Dq in CeA->NAc core projections and was sufficient to reduce ethanol intake in a dose dependent manner ($p<0.05$). Surprisingly, we did not observe any significant sex differences.

Statistics: Experiment 1: In the absence of any sex x treatment interactions, male and female data were collapsed and one-way ANOVAs were performed for ethanol intake data. Significant results observed for the hM3Dq group: one way ANOVA carried out for ethanol intake [$F(1.6,34.5) = 11.1$, $p<0.001$, Tukey's post-hoc VEH1 vs CNO $p<0.01$**, CNO vs VEH2 $p<0.001$***] and Student's t-tests were carried out for water ($t=3.3$, $df=21$, $p<0.01$) and saccharin (n/s).

Experiment 2: Two-way ANOVA. Main effect of dose $F(2,43)=4.5$, $p<0.05$. No significant effect of sex and no dose x sex interaction observed.

Conclusions: We found that CeA->NAc core projection neurons and intra-NAc CRF regulate binge drinking in a similar manner for both females and males. Because the CeA is sexually dimorphic, and CRF actions in the NAc core are sexually dimorphic, we are currently studying whether intra-NAc CRF antagonism blocks the ability of CeA->NAc stimulation to reduce binge drinking similarly in male and female mice.

Keywords: Binge Drinking, Neurocircuitry, DREADDs, CRF

Disclosure: Nothing to disclose.

W150. Assessment of the Abuse Potential of Daridorexant, a New Dual Orexin Receptor Antagonist for the Treatment of Insomnia Disorder: Data From Preclinical and Clinical Studies

Mike Ufer*, Steiner Steiner, Anke Post, Jasper Dingemanse, Michael Toeroek, Maria Elena Giusepponi, David Mayleben, Ingo Fietze, Kerri Schoedel, Michaela Tessari, Dalma Seboek Kinter

Idorsia Pharmaceuticals Ltd, Allschwil, Switzerland

Background: Dual orexin receptor antagonists (DORAs) selectively inhibit wake-promoting effects of orexin neuropeptides, differentiating them from commonly used allosteric GABA-A receptor modulators that non-specifically inhibit neuronal excitation.

A comprehensive analysis of abuse potential is required for compounds with central nervous system (CNS) activity, based on structural similarity to other drugs of abuse, pharmacological sites of action, behavioral animal studies, and data from a human abuse potential (HAP) as well as large Phase 3 studies.

We present comprehensive preclinical and clinical data on the abuse potential of daridorexant following recent completion of two phase 3 studies.

Methods: Daridorexant pharmacological sites of action were determined in an off-target screen of >120 common CNS targets. Reinforcing properties were tested in an intravenous self-administration study in rats (n=9 per group: three daridorexant doses, positive and negative controls) and interoceptive similarity to zolpidem in a rat drug-discrimination study (n=11; cross-over design). Physical dependence was assessed by monitoring possible withdrawal signs after 4 weeks chronic treatment in rats (n=10 per group).

The HAP study, designed in compliance with applicable FDA guidance, was a randomized, double-blind, double-dummy, placebo- and active-controlled six-period crossover study in healthy recreational sedative users. In each study period, a single, oral, morning dose of either daridorexant (50, 100, or 150 mg), placebo, or active control, i.e., suvorexant (150 mg) or zolpidem (30 mg), was administered. Each subject had to demonstrate greater drug-liking for both control drugs than for placebo in a qualification phase prior to the core treatment phase. Primary pharmacodynamic (PD) endpoint was Emax of the bipolar drug-liking visual analog scale (VAS) ranging from 0 (maximum disliking) to 100 points (maximum liking) assessed over 24 h. Several secondary subjective and objective pharmacodynamic endpoints, safety/tolerability, and the pharmacokinetics (PK) of daridorexant were also assessed.

Two large complementary double-blind, placebo-controlled, phase 3 registration trials in patients with insomnia disorder assessed the efficacy and safety of nightly daridorexant doses of 10 mg, 25 mg, and 50 mg for 3 months. Safety endpoints included the assessment of adverse events (AEs), which also comprised reporting of AEs associated with abuse potential. Withdrawal after 3 months' exposure was evaluated during a week placebo run-out based on benzodiazepine withdrawal symptoms questionnaire (BWSQ) scores and AEs.

Results: Daridorexant and its precursors displayed no structural similarity to other drugs of abuse. Daridorexant did not bind to any known abuse-associated CNS targets based on molecular profiling. In rats, daridorexant was not self-administrated, did not generalize to zolpidem, and did not produce withdrawal symptoms ($p>0.05$; post-hoc tests following ANOVA).

The core treatment phase of the HAP study included 72 subjects of which 63 completed all six periods. The study was valid per pre-specified criteria, i.e., drug-liking of suvorexant and zolpidem was greater than placebo based on a 15-point validity margin ($p<0.0001$).

At the highest phase 3 dose (50 mg), drug-liking VAS Emax (mean; 95% CI) of daridorexant (73.2; 69.0-77.5) was significantly lower ($p<0.001$) compared to supratherapeutic doses of suvorexant (80.7; 77.0-84.5) and zolpidem (79.9; 76.2-83.5). No significant differences were determined at daridorexant 100 mg (79.1; 75.0-83.3) and 150 mg (81.3; 77.7, 84.8). Such dose-related patterns were also observed for most secondary endpoints. At each daridorexant dose, drug-liking VAS scores were greater than placebo.

Both control drugs and daridorexant were safe. AEs of euphoric mood were less frequent at all doses of daridorexant (3.0-5.8%) compared to suvorexant (9.0%) and zolpidem (20.3%). Daridorexant PK was consistent with earlier trials indicating quick absorption (tmax 1 h) and elimination (t1/2 7-9 h).

In the phase 3 studies in 1854 patients with insomnia disorder, the incidence of all pre-defined abuse-associated events was balanced across all study groups; accidental overdose was the most frequently reported event, without indication of dose dependency. Furthermore, there was no indication of withdrawal based on AEs, BWSQ scores or ECG readings during run-out.

Conclusions: Daridorexant did not bind to abuse-related brain targets and did not show any abuse or dependence potential in the key rat studies (drug discrimination, self-administration, and dependence potential). Among recreational drug users pre-selected based on liking of sedatives, daridorexant exhibited similar drug-liking effects as suvorexant and zolpidem at supratherapeutic doses. At the highest phase-3 dose of 50 mg, daridorexant showed lower effects than both control drugs with respect to the primary and most secondary endpoints. Phase-3 data indicated no relevant abuse or dependence potential of daridorexant in patients with insomnia.

Keywords: Dual Orexin Receptor Antagonist, Abuse Potential, Dependence

Disclosure: Idorsia Pharmaceuticals Ltd: Employee (Self)

W151. Translational Evidence for Ketogenic Diet-Induced Reductions of Alcohol Dependence Symptoms

Corinde Wiers*, Leandro Vendruscolo, Jan-Willem Van der Veen, Peter Manza, Juan Gomez, Michael Michaelides, Sophie Elvig, Janaina Vendruscolo, Ehsan Shokri-Kojori, Danielle Kroll, Dana Feldman, Katherine McPherson, Catherine Biesecker, Rui Zhang, Kimberly Herman, Sara Turner, Shanna Yang, Melanie Schwandt, Dardo Tomasi, Anders Fink-Jensen, Helene Benveniste, Nancy Diazgranados, Gene-Jack Wang, George Koob, Nora D. Volkow

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Individuals with alcohol use disorder (AUD) show high plasma levels of the alcohol metabolite acetate and elevated

brain metabolism of acetate at the expense of reduced brain glucose metabolism. We hypothesized that a deficit of energy substrates during withdrawal when acetate and ketone levels in plasma decline may contribute to withdrawal severity and neurotoxicity in AUD, and that a ketogenic diet (KD) may reduce these effects.

Methods: We studied the effects of KD on alcohol withdrawal symptoms, craving, and brain ketone concentrations in AUD inpatients (n=33, 10 female) during alcohol detoxification, who were randomized into a KD (n=19; 4:1 ratio of grams of fat: grams of carbohydrate and protein) or Standard American (SA) diet (n=14; 50% carbs; 15% protein, 35% fat) intervention for 3 weeks. By providing a mostly liquid diet, study procedures were double blind and there were no group differences in diet expectation at study completion, indicating successful blinding. In parallel, we studied the effects of KD on alcohol consumption and brain glucose uptake in rats. Repeated Measure ANOVAs with group (KD/SA) and time were used for analyses.

Results: AUD inpatients who received KD versus SA required significantly lower use of benzodiazepines during the first week of detoxification, when withdrawal symptoms were the strongest (group x time interaction: $F_{3,93}=4.0$, $p=0.01$). Over a 3-week treatment, patients on a KD versus SA showed significantly lower "wanting" (group x time: $F_{2,56}=4.9$, $p=0.048$) and increased dorsal anterior cingulate cortex (dACC) reactivity to alcohol cues (interaction $p<0.05$ family-wise error corrected), consistent with enhanced control over craving as assessed with functional magnetic resonance imaging. Spectroscopic imaging of the dACC suggested alternative brain energy sources to glucose (i.e., elevated acetoacetate, acetone, glutamate and glutamine; KD>SA all $p<0.05$) and lower neuroinflammation (i.e, lower myo-inositol and choline; KD<SA all $p<0.05$) in the KD versus SA group. In rats, a KD induced higher ketone bodies and lower glucose levels in the blood (group x time interaction, ketones: $F_{2,68}=107.30$, $p<0.0001$; glucose: $F_{2,68}=43.09$, $p<0.0001$), and positron emission tomography imaging indicated decreased use of glucose in the brain compared with rats on regular chow ($t_{48}=1.68$; $p=0.05$). A history of a KD blocked the escalation of alcohol drinking in a rat model of alcohol dependence ($F_{3,32}=5.71$, $p=0.003$).

Conclusions: Here, we provide translational evidence for beneficial effects of a KD on alcohol-related behavior including withdrawal, craving and alcohol intake in AUD.

Keywords: Alcohol Use Disorder - Treatment, Ketogenic Diet, Multimodal Neuroimaging

Disclosure: Nothing to disclose.

W152. Vigilance Impedes Habitual Information Processing by Activation of Astrocytes in the External Globus Pallidus

Sa-Ik Hong*, Matthew Baker, Doo-Sup Choi

Mayo Clinic College of Medicine, Rochester, Minnesota, United States

Background: Vigilance, a state of readiness to respond to certain changes, is closely related to motivated behavior. The external globus pallidus (GPe) which converges the output of indirect medium spiny neurons (iMSNs) in the dorsomedial striatum (DMS) and dorsolateral striatum (DLS), is the main node involved in habitual information processing and vigilance. However, it remains unclear how GPe astrocytes, the orchestrator of neuronal processing, contribute to the relationship between vigilance and habitual behavior.

Methods: Mice were trained for shaping habitual reward-seeking behavior in the nose-poke operant chambers with 20% sucrose reward. During this operant conditioning for habit learning, we employed the in vivo calcium imaging to measure the cellular activity of GPe astrocytes. To examine whether vigilance alters habitual behavior, we provided mice with attentional stimulation before reward evaluation. In addition, we recorded changes in GPe astrocytic activity by attentional stimulation. Then, we assessed whether GPe astrocyte regulates habitual behavior by using GFAP promoter-driven expression of hM3Dq, the excitatory designer receptors exclusively activated by designer drugs (DREADDs).

Results: Repeated habit learning developed homogeneous and stable habitual behavior. Genetically encoded calcium indicator in GPe GFAP-expressing astrocytes displayed intracellular calcium dynamics when mice approached the magazine where mice receive the reward. However, repeated habit learning reduced GPe astrocyte dynamics. Interestingly, attentional stimulations increased intracellular calcium ion level in the GPe astrocytes. Moreover, these stimulations yielded flexible reward evaluation and dampened habitual behavior. Similarly, chemogenetic activation of the astrocytes in the GPe reduced habitual behavior. On the other hand, the GPe astrocyte activation did not change the reward-seeking behavioral patterns when mice were trained to have flexible reward evaluation.

Conclusions: Together, our results indicate that vigilance impedes habitual behavior and GPe astrocyte may be a key factor of this relationship between vigilance and habitual behavior. Thus, GPe astrocyte could be a potential therapeutic target for maladaptive habitual behaviors, such as addiction, obsessive-compulsive disorders, and other decision-making disorders.

Keywords: Attention, Habit, Globus Pallidus, Astrocyte

Disclosure: Nothing to disclose.

W153. Interindividual Variability in Foraging Behavior Relates to Drug Addiction and a Marker of Midbrain Dopamine Function

Candace Raio*, Sara Constantino, Kathryn Biernacki, Darla Bonagura, Joany Xue, Kenneth Wengler, Guillermo Horga, Anna Konova

New York University, New York, New York, United States

Background: A prominent feature of addiction is the tendency to exploit a previously rewarding resource despite its diminishing returns. Such behavior is aptly captured in animal foraging models that have recently been extended to humans. Catecholaminergic systems are thought to underlie such behavior, but a precise empirical account of this is lacking in humans.

Methods: We recruited 21 individuals with opioid use disorder (OUD) and 21 socio-demographically matched control participants (25-70 y of age, 43% women). OUD participants were drawn from a larger longitudinal study on heroin relapse in treatment-seeking individuals. Participants completed a foraging task, during which they made sequential decisions between "harvesting" a depleting resource for monetary rewards or incurring a cost to "travel" to a replenished resource. To more directly assess catecholaminergic contributions to foraging behavior, we further acquired high-resolution (<0.7 mm2 in-plane) neuromelanin-sensitive MRI scans, a non-invasive technique used to reliably probe long-term dopamine and norepinephrine function, in a subset (n=27) of OUD and control participants. Our imaging protocol was optimized to separately localize dopaminergic nuclei (substantia nigra, ventral tegmental area, SN/VTA) and the noradrenergic locus coeruleus (LC-NE), both of which have been theoretically linked to foraging behavior and are thought to underlie changes in neural circuits implicated in addiction.

Results: Our foraging task results replicate previous reports that healthy control participants over-harvest relative to the optimal policy, especially as travel times to new patches increase (travel time effect: $p=0.009$). Interestingly, OUD participants tended to over-harvest even more than matched controls. This tendency to over-harvest in OUD was coupled with a marked insensitivity to

travel times (travel time effect: p=0.79; travel time X diagnosis interaction: F=3.10, p=0.08). These group differences held when controlling for age, sex and cognitive variables, and over-harvesting scaled with increased years of opioid use (OUD: r=0.47, p=0.048). Our imaging analysis revealed a dissociation whereby, across participants, over-harvesting was associated with lower neuromelanin signal contrast in dopaminergic nuclei (SN/VTA, r=0.41, p=0.03, and separately within VTA, r=0.38), but not in LC (p=0.55).

Conclusions: Our findings suggest that individual differences in foraging behavior are related to interindividual variability in dopaminergic—but not noradrenergic—circuit function that informs reward rates in dynamic decision environments and may serve as a marker for maladaptive reward-seeking behavior.

Keywords: Reward-Based Decision-Making, Opioid Use Disorder, Neuromelanin-Sensitive MRI, Patch Foraging

Disclosure: Nothing to disclose.

W154. Exposure to Smoking Context Potentiates Habitual Motor Response

Jason Oliver*, Brett Froeliger, Matthew Engelhard, Cynthia Conklin, Francis McClernon

Duke University, Durham, North Carolina, United States

Background: Contexts associated with past drug use can evoke powerful cravings and potentially drive relapse among individuals trying to abstain. Research on this topic has traditionally emphasized the effects that proximal drug-related stimuli (e.g. images of lit cigarettes) have on self-reported urge or physiological changes consistent with increased motivation. Across a series of studies, our team has shown that these effects on urge/motivation extend to the broader contexts in which smoking occurs, even in the absence of proximal smoking-related stimuli. However, it is also plausible that smoking contexts influence other neurocognitive processes relevant to smoking behavior. For example, contexts that are highly stimulating might reduce cognitive control by overloading cognitive resources. Contexts rich in reinforcers might increase action tendency by recruiting appetitive drive systems. Importantly, such effects could stem from either the conditioned associations that are typically emphasized or the physical properties of the contexts themselves. Though theoretical at present, if such effects were confirmed it would have broad implications for the etiology of addictive disorders and other neuropsychiatric conditions, as well as directly inform the development and tailoring of novel interventions. For instance, if research reveals reduced cognitive control in smoking-related contexts, it might suggest the need for cognitive-training or neurotherapeutics aimed at increasing cognitive control in addition to traditional interventions aimed at reducing craving. This project served as an initial attempt to begin examining the effects of smoking contexts on specific neurocognitive processes. We hypothesized that exposure to smoking-related contexts would reduce inhibitory control in smokers.

Methods: Adult cigarette smokers (N = 45) were drawn from one of two parent studies examining the effects of context on smoking behavior. Participants completed a novel Go-NoGo task designed to assess habitual motor responses and response inhibition while viewing smoking and non-smoking contexts. Using data from our prior work, we identified five contexts smokers most frequently report strongly associating with smoking (Porch, Yard, Bus Stop, Outside Work, Outside Bar/Restaurant) and five contexts most frequently report associating with abstinence (Store, Inside Work, Inside Restaurant, Inside Religious Venue, Inside Other Business). Two images of each context category were selected for inclusion and further validated using a machine learning algorithm we have developed to categorize images as smoking or non-smoking contexts. Images were then modified to include the letter "P" or "F" in one of four corners of the image. Participants were instructed to respond with a button press when one letter was presented ("Go" stimulus; 85% of trials) and withhold response during the other ("NoGo" stimulus; 15% of trials). Letter assignments were counterbalanced across participants. Participants completed 400 trials in a pseudorandom order that were divided evenly across image categories (smoking context, non-smoking context). Each image was presented for 750 ms, with a 250 ms inter-trial interval.

Results: Accuracy on "Go" trials was significantly higher for smoking context trials than non-smoking context trials ($F = 54.86$, $p < .000001$; Nonsmoking: $M = 0.84$, $SD = 0.10$; Smoking: $M = 0.89$, $SD = 0.08$). No differences in accuracy were found for "NoGo" trials ($F = 0.06$, $p = .814$; Nonsmoking: $M = 0.73$, $SD = 0.11$; Smoking: $M = 0.72$, $SD = 0.12$). Findings for response time on correct "Go" trials paralleled findings for accuracy, with smokers responding significantly faster on smoking context trials than non-smoking context trials ($F = 28.18$, $p = .000003$; Nonsmoking: $M = 533.1$, $SD = 28.1$; Smoking $M = 521.3$, $SD = 36.3$). Analyses using a signal detection approach indicated accuracy effects were driven by both enhanced stimulus detection while viewing smoking images ($F = 12.89$, $p < .001$) and the adoption of a more liberal response bias ($F = 13.23$, $p < .001$). Effects did not differ as a function of sex, smoking rate, or nicotine dependence (all p's > .05).

Conclusions: Smokers do not experience deficits in response inhibition when viewing smoking-related contexts but do experience increased habitual motor response tendencies as evinced by increased accuracy and reduced response time for "Go" trials. Signal detection results indicate the cause of this effect is multifaceted, stemming from both improved detection of action-promoting stimuli and the adoption of a more liberal response bias when viewing smoking contexts. Together, these findings provide the first support for the notion that smoking-related contexts can influence basic neurocognitive processes that are not specific to smoking motivation and could conceivably influence a range of different behaviors. Future research is needed to determine if effects are driven by conditioned associations with smoking or specific physical features common to each image category (e.g. indoor versus outdoor setting, basic perceptual characteristics). The primary limitations of this project include the modest sample size, the small number of image categories included, and the absence of a non-smoker comparison group. Nonetheless, these preliminary findings are promising and support the need for further research examining contextual influences on neurocognitive processing in addiction.

Keywords: Smoking, Impulsivity, Cognition

Disclosure: Nothing to disclose.

W155. Resting-State Nucleus Accumbens Connectivity Predicts Adherence to Extended-Release Naltrexone Treatment in Opioid Use Disorder

Zhenhao Shi*, Kanchana Jagannathan, Corinde E. Wiers, Charles P. O'Brien, Anna Rose Childress, Daniel D. Langleben

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Monthly injectable extended-release naltrexone (XR-NTX) is an effective relapse prevention treatment for opioid use disorder (OUD). However, XR-NTX's effectiveness is limited by non-adherence that often leads to relapse and increases risk of fatal overdose. Studies show substantial individual variability in non-adherence. Identifying risk factors for non-adherence is crucial for improving the effectiveness of XR-NTX. Neuroimaging techniques have shown great promise in elucidating the neural underpinnings of response to addiction treatments. Specifically, resting-

state functional magnetic resonance imaging (rs-fMRI) is among the most common neuroimaging protocols and one of the easiest to implement with minimal task demand on patients. Using rs-fMRI, prior research has shown alterations in the functional connectivity between the nucleus accumbens (NAcc) and cortical regions (e.g. prefrontal cortex) in OUD patients. The current study aimed to use rs-fMRI to investigate the role of NAcc connectivity in adherence to XR-NTX in OUD patients during the first three months of treatment, a period associated with the highest rate of premature dropout.

Methods: Forty-three detoxified OUD patients (30 male, 13 female; 19-56 years-old) were offered up to three months of XR-NTX treatment (i.e. three injections). Adherence was defined as completing all three injections, and non-adherence otherwise. Prior to the first injection, patients completed a 5-minute rs-fMRI session, during which they were required to relax, stay still and awake, and keep their eyes open. rs-fMRI data underwent standard preprocessing steps including slice time correction, realignment, segmentation, normalization to the Montreal Neurological Institute (MNI) space, smoothing, detrending, band-pass filtering (0.01–0.1 Hz), and removal of motion artifacts and signals from the white matter and cerebrospinal fluid. The bilateral NAcc was anatomically defined as the seed region using the Harvard-Oxford Atlas. NAcc connectivity was computed by correlating the time course of the NAcc and that of every voxel of the rest of the brain, which was then Fisher Z-transformed to yield a connectivity map for each patient. Using a leave-one-out cross-validation framework (LOOCV, 43 iterations), feature selection was performed within the training set by conducting a two-sample t-test on the connectivity map between the adherent and non-adherent patients. After applying a threshold of voxel-level $p<0.001$ to the t-map, connectivity strengths of the peak voxels of the surviving clusters were selected as features and entered in a LASSO logistic regression model with adherence as the outcome. Model coefficients and the regularization hyperparameter (λ) were optimized using an internal, nested 10-fold cross-validation. Classification accuracy was indexed by the cross-validated area under the receiver operating characteristic curve (ROC AUC) and its 95% bootstrap confidence interval (bootCI, 100 iterations). A Monte-Carlo permutation test (1000 iterations) was used to determine the p-value of the ROC AUC.

Results: Twenty-three of the 43 patients (53.49%) completed all three injections (i.e. "adherent"), and 20 (46.51%) missed at least one injection (i.e. "non-adherent"). Adherence was predicted by pre-treatment NAcc connectivity with the ventromedial prefrontal cortex (vmPFC; MNI coordinates x/y/z=0/41/–19 in 38 out of 43 LOOCV iterations) and left posterior superior temporal gyrus (pSTG; x/y/z=–66/–22/11 in 42 LOOCV iterations, –63/–22/11 in 1 LOOCV iteration). Specifically, the adherent patients showed greater NAcc-vmPFC and NAcc-pSTG connectivity than the non-adherent patients. Classification of the adherent vs. non-adherent patients achieved a cross-validated ROC AUC of 0.80 (95% bootCI, [0.62, 0.91], p=0.019), with a maximum overall classification accuracy of 76.74% at positive predictive value=84.21% and negative predictive value=70.83%.

Conclusions: Our data suggest that pre-treatment connectivity of the NAcc with the vmPFC and pSTG may serve as a predictive biomarker of treatment adherence in OUD patients receiving XR-NTX. Greater NAcc connectivity with the vmPFC and pSTG may reflect greater integrity of the corticostriatal system, which may in turn contribute to better adherence and lower risk of relapse. Our preliminary findings suggest that adjunctive interventions that improve corticostriatal functioning such as non-invasive neuromodulation, cognitive training, and psychosocial treatments may enhance adherence to XR-NTX in OUD.

Keywords: Opioid Use Disorder, Treatment Adherence, Brain Connectivity, Machine Learning, Treatment Outcome Prediction

Disclosure: Nothing to disclose.

W156. Accelerated Aging of the Amygdala in Alcohol Use Disorder: Implications for the "Dark-Side" of Addiction

Dardo Tomasi*, Corinde E. Wiers, Peter Manza, Ehsan Shokri Kojori, Michele-Vera Yonga, Rui Zhang, Danielle Kroll, Dana Feldman, Katherine McPherson, Catherine Biesecker, Melanie Schwandt, Nancy Diazgranados, George Koob, Gene-Jack Wang, Nora Volkow

National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland, United States

Background: Chronic alcohol intake hijacks brain stress pathways involving the amygdala giving rise to a dysphoric state that promotes relapse. Despite the prominent role of the amygdala in preclinical studies of neuroadaptations associated with the negative emotional states during alcohol withdrawal, there are few clinical studies on the neurobiological underpinnings of negative emotional states in alcohol use disorder (AUD). Here we studied the role of the amygdala and other subcortical volumes during alcohol withdrawal in patients with AUD, in association with aging and detoxification. We hypothesized that the amygdala would emerge as a prominent feature of machine learning classifiers for distinguishing AUD from healthy controls (HC), would show accelerated aging in AUD, and would recover during detoxification. We hypothesized that amygdala volume would be negatively associated with negative emotions while controlling for age and detoxification in AUD.

Methods: AUD participants were admitted for detoxification and had at least 5 years' history of heavy drinking. All participants had a negative breath test result for alcohol consumption and a negative urine drug screen on days of testing (except for benzodiazepines in AUD patients) and were free of psychoactive medications within 24 hours of study procedures. Fifty-two AUD patients and 53 healthy controls (HC) underwent high-resolution structural MRI. The two groups did not differ in age or gender proportion. Thirty-two AUD patients were scanned twice, 2 weeks apart during early and later withdrawal, to assess the effect of abstinence on MC-features. The structural preprocessing pipelines of the Human Connectome Project based on FreeSurfer were used to quantify 45 subcortical volumes, defined in the Automatic Subcortical Segmentation atlas. We developed a new and simple morphometry-based classifier (MC) based on subcortical volume features to predict group membership and study the association of the features with age and negative emotions. ANCOVA with main effects of group and age and age-by-group interactions was used to assess if subcortical volumes predicting group membership are prone to accelerated aging in AUD while controlling for confounding effects from differences in intracranial volume and gender. Paired t-tests were used to assess the effect of withdrawal on the MC-features that differentiated AUD from HC, by comparing subcortical volumes at baseline and at the end of detoxification. A false discovery rate corrected of 0.05 was used to report significant effects.

Results: We found significant alterations of volumes in AUD patients, including 31±6% enlargement of ventricles and cerebrospinal fluid (CSF) volume and 11±2% volume reductions of the amygdala, hippocampus, caudate, accumbens, putamen, thalamus, ventral diencephalon (DC), cerebellum, brain stem, and corpus callosum studied during the first week of alcohol detoxification. Using these volumetric features, MC achieved 80% accuracy in the classification of AUD and HC in the Discovery Cohort and 67% accuracy in the Validation Cohort. Several MC-features demonstrated significant effects of age. Particularly the volumes of the 3rd and lateral ventricles, choroid plexus, CSF, as well as white matter (WM) and non-WM hypointensities, showed increases with age, whereas the volumes of the cerebellar cortex, accumbens, putamen, and ventral DC decreased with age across

all participants. Higher amygdala volume was associated with higher negative urgency and anxiety in AUD but not in HC. The volumes of right-amygdala, right-hippocampus, and left cerebellum and thalamus, the 3rd and left-inferior-lateral ventricle, and both lateral ventricles recovered significantly with abstinence (0.9-24.7%). Larger amygdala volumes at baseline were associated with more severe anxiety and impulsivity in AUD.

Conclusions: Reduced subcortical volumes in stress and reward nuclei and enlargement of CSF partitions are consistent with alcohol-induced accelerated aging, and their partial recovery indicates that some of these changes might revert with protracted abstinence in AUD. As we hypothesized and our reproducible findings suggest, subcortical structures were among core brain regions negatively affected in AUD. Our findings are consistent with the hypothesis that alcohol exacerbates aging effects in the brain and provide novel insights into such effects in subcortical regions, including the amygdala. In summary, we document significant volumetric changes in subcortical regions including the amygdala that showed exacerbated atrophy with aging, that partially recovered with detoxification in AUD patients. We also document an association between amygdala volume and anxiety and negative urgency. The effects corroborate in AUD the involvement of the amygdala in the withdrawal/negative stage, described as the 'dark side of addiction'.

Keywords: Alcoholism, Reward, Cortisol Response to Stress

Disclosure: Nothing to disclose.

W157. Sex-Related Differences in Corticolimbic Kappa Opioid Receptor Availability Among Individuals With Alcohol Use Disorder Compared to Healthy Controls

Yasmin Zakiniaeiz*, Aishwarya Vijay, Bart de Laat, Nabeel Nabulsi, Kelly Cosgrove, Yiyun Huang, Suchitra Krishnan-Sarin, Evan Morris

Yale University School of Medicine, New Haven, Connecticut, United States

Background: Alcohol abuse is one of the leading causes of disability in the United States and female drinkers are more vulnerable than male drinkers to many of the consequences of alcohol use [NIAAA 2017 Women & Alcohol]. Alcohol has been shown to interact with numerous neurotransmitter systems to exert its pharmacological effects. The endogenous kappa opioid receptor (KOR) system has been strongly implicated in positive and negative reinforcing effects of alcohol, negative affect, and stress [Bruchas et al. 2010 Brain Res]. Dysregulation of the KOR system by chronic alcohol use contributes to individual differences in alcohol use behaviors [Kosterlitz et al. 1989 NIDA Res Monogr] and blockade of KORs decreases alcohol intake in dependent but not in nondependent rats [Walker & Koob 2008 NPP]. A large body of work shows that women are more likely to drink to regulate negative affect/stress, while men are more likely to drink for positive reinforcement [Logrip et al. 2018 Alcohol]. These differences may be attributed to neuroadaptations in the KOR system, particularly in the coupling of amygdalo-frontal cortex region projections, which are critical for negative affect/stress regulation [Tej et al. 2015 NPP]. Currently approved alcohol use disorder (AUD) medications have a relatively low efficacy, were initially developed with male samples and, do not consider sex-specific targets. We previously observed that individuals with AUD had lower corticolimbic levels of available KOR than healthy control (HC) subjects (Vijay et al. 2018 NPP]. We also observed that healthy males had higher levels of available KOR than healthy females [Vijay et al. 2016 AJNMMI], suggesting that men and women with AUD should be compared to their sex-matched HC counterparts. The goal of this study is to examine the KOR system in women and men with AUD compared to their HC counterparts. Based on preclinical and human behavioral studies, and our prior work mentioned above, we hypothesized that men with AUD will have lower KOR than HC men in striatum, amygdala, and frontal cortex, and that women with AUD vs. HC women will have lower KOR in amygdala, but will show no differences in frontal cortex and striatum.

Methods: Fifty-two individuals with AUD (19 females) and 25 HC subjects (9 females) underwent positron emission tomography (PET) scans with [11C]LY2795050, a selective, high-affinity, KOR antagonist radiotracer with favorable kinetics for imaging KOR in vivo [Kim et al. 2013 JNM]. Partial-volume correction was applied to all AUD and HC subject PET data to correct for potential atrophy. Volume of distribution (VT) of the tracer was estimated regionally as a measure of KOR availability. VT values of AUD versus HC were compared for 3 a priori regions of interest based on their behavioral involvement–striatum (reinforcement), amygdala (negative affect) and frontal cortex (negative affect/stress regulation). Independent-samples t-tests were used to compare VT values for men with AUD vs. HC men and, women with AUD vs. HC women.

Results: We found preliminary evidence of AUD-related differences in KOR availability between sexes. KOR availability was significantly lower in men with AUD compared to HC men in striatum ($p=0.038$, Cohen's $d=0.374$) and amygdala ($p=0.013$, Cohen's $d=0.597$), and trending in the same direction in frontal cortex ($p=0.069$, Cohen's $d=0.420$). Women with AUD had lower KOR availability than HC women in amygdala ($p=0.008$, Cohen's $d=0.319$), but did not show differences in KOR availability in striatum ($p=0.973$, Cohen's $d=0.302$), and frontal cortex ($p=0.787$, Cohen's $d=0.370$).

Conclusions: Partially consistent with our hypotheses, KOR availability was lower in men with AUD than HC men in striatum and amygdala, and lower, but not statistically significant, in frontal cortex. Women with AUD vs. HC showed lower KOR availability in amygdala, but no differences in frontal cortex and striatum. These data suggest that men and women with AUD have different patterns of KOR neuroadaptations compared to their HC counterparts. In women, the pattern of amygdalo-frontal decoupling of KOR may explain why women drink to regulate negative affect. These findings point towards a possible neurobiological basis for sex differences in alcohol use behaviors and potential differences in clinical effectiveness of opioid receptor-targeted medications. Future work should relate KOR availability to behavioral measures, and to treatment efficacy.

Keywords: Alcohol Use Disorder, Sex Differences, Kappa Opioid Receptor, Positron Emission Tomography (PET), Corticolimbic

Disclosure: Nothing to disclose.

W158. Maternal Brain Responses During Infant-Oriented Face Mirroring in Postpartum Women With Opioid Use Disorder

James Swain*, S. Shaun Ho

Renaissance School of Medicine, Stony Brook University, South Setauket, New York, United States

Background: Maternal reflective functioning, which is critical to sensitive behaviors and child outcomes, is threatened by the opioid use disorder (OUD) epidemic. From 1999 to 2014, the incidence of pregnant women with OUD quadruped and currently about 2.5% of pregnant women use opioids chronically. Despite current "gold standard" buprenorphine treatment (BT) to prevent withdrawal, mothers with BT/OUD are at high risk for relapse and associated problems of stress, depression, polysubstance use and maladaptive parenting behaviors that can lead to child maltreatment and costly foster care utilization. Opioid-induced deficits in maternal behaviors are well-characterized in preclinical rodent models, but little is known of how BT/OUD may affect human parenting and maternal brain

function, critical to sensitive behavior and child outcome. We know that human maternal behaviors are governed by an evolutionary conserved Maternal Behavior Neurocircuit (MBN) that regulates maternal caring and aggressive behaviors. We have also recently established abnormalities for human BT/OUD mothers brain responses to own-baby cry and resting state functional connectivity between reciprocally inhibiting care and aggression behavior circuits. Here, we propose to extend our functional magnetic resonance imaging (fMRI) studies to a face mirroring task that examines effects of BT/OUD on own-child-oriented maternal empathy responses, relating to reflective function, sensitive behavior and child outcome.

Methods: We studied 22 mothers, including 6 mothers receiving BT for OUD and 16 non-OUD mothers as a comparison group (CG), aged 18-40. They underwent an fMRI Child Face Mirroring Task (CFMT), at 4 months postpartum. In CFMT, the participants viewed their own child's pictures that were repeatedly presented in three different task conditions, OBSERVE, REACT, and JOIN, that were interleaved in a random order. In OBSERVE, the participants were instructed to coldly observe the child' picture without imitating the child's facial expressions or emotions. In REACT, the participants were instructed to respond to the child as they normally would. In JOIN, the participants were instructed to empathically imitate (mirroring) the child's facial expressions and emotions. Each task condition for their own child was presented in four blocks (16s/block). In each block, four pictures of the child were consecutively presented for 4 seconds per picture. Each of these four pictures exhibited one of four different emotional expressions, Joyful, Distressed, Ambiguous, and Neutral expressions, presented in a random order. fMRI data were analyzed using statistical parametric mapping software (SPM8).

Results: We examined the group differences in each task condition related to the own child. In Observe condition, BT/OUD mothers, as compared to CG, showed greater neural responses in the extended amygdala and hypothalamus. In React condition, BT/OUD, as compared to CG, showed lesser neural responses in the dorsal striatum. In Join condition, BT/OUD, as compared to CG, showed lesser neural responses in the anterior cingulate cortex, supplemental motor area, right precentral area, bilateral anterior insula/frontal inferior operculum, and thalamus.

Conclusions: We show preliminary effects of BT/OUD on neural responses during child-oriented mirroring. As compared to the comparison group, BT/OUD mothers showed hypo-activated mirroring-dependent neural system function when performing child-oriented face mirroring. This included hypo-activated dorsal striatum when they responded to their child as they normally would, and hyper-activated extended amygdala and hypothalamus when they coldly observe their child. Further work is needed to connect these MBN mechanisms with parenting behavior, child outcome. However, these preliminary results suggest potential mechanisms for mothers with BT/OUD that may be modifiable with treatments that focus on augmenting reflective function to reduce transgenerational mental health risks.

Keywords: Opioid Use Disorder, Maternal Brain, Maternal Behavior, Functional MRI (fMRI), Empathy

Disclosure: Nothing to disclose.

W159. Prenatal Exposure to Opioids Disrupts the Development of the Dopamine System During Adolescence: A Role for Microglia?

Caroline Smith*, Tania Lintz, Madeline Clark, David Potter, Karen Malacon, Veronica Kim, Young Jo, Staci Bilbo, Elena Chartoff

Duke University, Durham, North Carolina, United States

Background: In the past decade, rates of opioid use disorder have increased to epidemic proportions. Devastatingly, this includes a dramatic increase in disorders among pregnant mothers and, therefore, the incidence of prenatal exposure in their children. While the symptoms of the resulting neonatal abstinence syndrome (NAS) can be acutely managed with opioid replacement therapies, very little is known about the long-term consequences of prenatal opioid exposure for brain development and adult behavior. The dopamine system is critical to motivated behaviors such as social behavior and addictive behavior, among others. We recently showed that microglia, the resident immune cells of the brain, engulf dopamine D1 receptors (D1R) during adolescence, leading to a decrease in D1R within the nucleus accumbens (NAc) in adulthood in males but not females. Here, we hypothesize that prenatal opioid exposure disrupts the appropriate microglial pruning of D1R in the adolescent NAc of males, thereby increasing vulnerability to addictive behaviors in adulthood.

Methods: Female rats were trained to self-administer oxycodone (0.1mg/kg/infusion; 4hrs/day) prior to mating. Subsequently, the treatment group (IVSA) continued to self-administer the drug until the day before parturition, while the control group (CON) did not. A number of outcomes were measured in offspring during the perinatal period including body weight, behavior (righting-reflex and incline plane) and ultrasonic vocalizations. Both male and female offspring were sacrificed at 3 timepoints: postnatal days (PND) 20, 30, and 55. Immunohistochemistry was used to co-label tissue for both D1R and microglia (Iba1). In a separate series, sections were stained for dopamine D2 receptors (D2R) and tyrosine hydroxylase (Th). 2-way ANOVAs (treatment x age) were used to evaluate the data in each sex separately.

Results: In both males and females, we observed higher expression of D1R, as well as D2R and Th, in the NAc at P30 as compared to both younger and older timepoints, consistent with a developmental peak in the dopamine system during adolescence. Interestingly, prenatal opioid exposure increased D1R density in the NAc of both sexes at P55, suggesting a failure of developmental pruning. In males, IVSA increased D2R at P30, but this increase did not persist into adulthood. No effects of prenatal opioid exposure were observed on Th immunoreactivity. All significance was set at $p<0.05$.

Conclusions: Together, these findings suggest that prenatal opioid exposure disrupts the developmental trajectory of dopamine receptor expression within the NAc, without changing dopamine input per se. We are currently using IMARIS image reconstruction software to assess microglial phagocytosis of D1R and investigating the impact of these changes on adult addictive behaviors.

Keywords: Opioid Addiction, D1 Dopamine Receptors, Developmental Trajectory, Microglia Engulfment, Prenatal Drug Exposure

Disclosure: Nothing to disclose.

W160. Patterns and Reasons for Kratom Use Assessed by a Consumer Survey and Social Media Monitoring

Jack Henningfield*, Michael Barr, Daniel Wang, Marilyn Huestis

Pinney Assoc & Johns Hopkins University School of Medicine, Bethesda, Maryland, United States

Background: Kratom products are prepared from leaves of a tree in the coffee family (Mitragyna speciosa) native to Southeast Asia (SEA). Leaves contain several dozen alkaloids but mitragynine is most abundant and appears to account for most of the effects associated with kratom consumption. Mitragynine is a partial mu-opioid receptor agonist with low reinforcing and respiratory depressant effects as compared to morphine-like opioids, and diverse other effects that can produce caffeine-like alertness, and relaxation depending on the dose, as well as naloxone reversible

relief of pain in animal models. Use is almost exclusively by the oral route in the form of tea-like decoctions, or as leaf powder added to food or beverages or encapsulated to mask its bitter flavor. Although kratom is not approved by the United States Food and Drug Administration (US FDA) for any therapeutic use, surveys indicate that it is used to enhance work performance; relieve pain, stress, and depressed mood; and increasingly for self-management of opioid withdrawal to achieve opioid abstinence. There are an estimated 10-16 million kratom consumers in the US.

The purpose of this study is to better understand kratom use by employment of a new kratom consumer survey and a newly created social media monitoring platform. Social media enables capture of community-driven discussion that may provide information complementary to structured surveys.

Methods: Internet survey: An Internet survey was conducted using the Survey Monkey platform. Participants were recruited through Facebook and Google, and through the American Kratom Association's email list. Data were collected July through September 2019. The survey exclusively focused on kratom use for health and well-being, There were 3 main arms of the survey – 1) users taking kratom to support/manage withdrawal symptoms and 2) users taking kratom to support/ manage a "disease or symptom " or 3) Both – Treating withdrawal and a disease/ symptom. The survey was reviewed and approved by the independent ethics review board Canadian SHIELD Ethics Review Board.

Social media monitoring: A tool was created to extract data from online forum posts that primarily involve people who use kratom. Searches focused on discussions that provide insights about such topics as: the advantages and disadvantages of various kratom preparations (e.g. beverages, powders, capsules), dosing to minimize undesired effects, and opinions about regulation. Searching and data extraction are obtained from the three major kratom discussion websites: www.reddit.com/r/kratom (October 2015 – present), https://drugs-forum.com/forums/kratom.76/ (January 2003 – January 2020), and https://www.thekratomforum.com/index.php (September 2008 – present). A Microsoft PowerBI dashboard was created to visualize the Azure data. These authors and other experts also performed online monitoring of the websites.

Results: Survey: Among survey participants, 68.5% (n=7,064) reported that they use kratom to "treat a disease or symptom (including pain, fatigue, for energy, etc.)". 28.0% (n=2,891) reported using kratom for both "treating withdrawal AND a disease/symptom". Among consumers using kratom exclusively to treat "a disease or symptom", 44% (n=3,105) reported using to treat pain; 11% (n=776) reported use to treat anxiety; and 10.7% (n=757) reported use to treat fatigue. Of kratom consumers who reported use solely to treat drug withdrawal/addiction/dependency, drugs that were the most commonly reported as the cause of such symptoms included prescription pain pills (45.6%), heroin (17.0%), alcohol (14.0%).

Social media monitoring: Data collection is ongoing, and the poster will include results obtained through October 2020. However, several lines of discussion provide real world insights about how people prepare kratom leaf for consumption; kratom use patterns and effects; advice to minimize undesired effects such as nausea; motivations for use; concerns about kratom bans in some states; and support of states that have initiated their own kratom regulatory frameworks to ensure product purity, regulate maximum allowable alkaloid levels, and require that packaging, labeling, marketing meet state standards. The discussions also indicate a high degree of awareness and reliance upon published and presented scientific studies with respect to kratom safety and plausible kratom benefits.

Conclusions: The two studies reported in this poster replicate and extend the results of earlier surveys in the United States, and field studies in South East Asia that also evaluated patterns of use, reasons for use, and the effects of kratom. The data indicate that many people turn to kratom because approved pharmaceutical products were not effective, accessible, tolerable or acceptable, and/or due to preferences for "natural" products. Both surveillance approaches support earlier conclusions that for many people kratom is useful to self-manage substance use disorders and other disorders, as well as for general health and well-being.

Whereas the Internet survey, like others, provides invaluable quantitative data on patterns and reasons for use, the social media monitoring provides a rich source of complimentary real world data about factors that influence kratom use, patterns of use, and consequences. This would appear to be a particularly rich source of ideas to guide future research as well as real world considerations to inform kratom policy and regulation.

Keywords: Internet Survey, Social Media Monitoring, Opioid Use Disorder, Treatment, Natural Dietary Product

Disclosure: Pinney Associates: Employee (Self)

W161. Automated Facial Grimace Tracking to Assess Spontaneous Withdrawal Following Neonatal Morphine (P1-14) in CFW Mice and Altered Affective and Psychostimulant-Induced Phenotypes in Adolescence

Kristyn Borrelli*, Emily J. Yao, William W. Yen, Qiu T. Ruan, Julia C. Kelliher, Melanie M. Chen, Richard K. Babbs, Jacob A. Beierle, Elisha M. Wachman, Alberto Cruz-Martin, Camron D. Bryant

Boston University School of Medicine, Boston, Massachusetts, United States

Background: The Opioid Use Disorder epidemic has led to higher incidence of Neonatal Opioid Withdrawal Syndrome (NOWS). NOWS-affected infants born to opioid-dependent mothers display body weight deficits, inconsolability, insomnia, and increased pain sensitivity. The neurobiological basis of NOWS is largely unknown, but mouse models will help facilitate mechanistic discovery and treatment development. In order to understand both the short- and long-term behavioral and transcriptional adaptations induced by perinatal opioid exposure, we induced repeated cycles of spontaneous opioid withdrawal during a sensitive period of neurodevelopment. Our prior studies demonstrated increased thermal nociceptive sensitivity as well as sex- and time-dependent increases in ultrasonic vocalizations and withdrawal induced locomotor activity in outbred mice (ACNP 2020). Here, we sought to extend the repertoire of withdrawal behaviors and examined changes in emotional pain processing. We have also conducted new transcriptome and exon usage analyses. Additionally, we assessed affective, cognitive, and drug-related phenotypes during adolescence to gauge potential long-term effects of perinatal opioid exposure.

Methods: We treated male and female neonatal outbred Cartworth Farms White (CFW) mice (a.k.a. Swiss Webster) with morphine sulfate (MOR; 15.0 mg/kg, subcutaneous) or saline (SAL) 1x or 2x/day from P1 to P14, the approximate third trimester-equivalent of human gestation. On P15, pups (n=20 SAL/20 MOR) were injected with 4.0 mg/kg naloxone (NAL; intraperitoneal) or saline (10 SAL/10 NAL per MOR treatment group) and videos of facial expressions were collected for 20 minutes. Automated scoring of facial grimace was performed with Rodent Face Finder and Automated Mouse Grimace Scale (aMGS) software to gauge spontaneous withdrawal severity. In a separate cohort, brainstem (containing pons and medulla) was collected on P14 (n=6 SAL/6 MOR, 3 M/3F per treatment) and processed for transcriptomic analysis via mRNA sequencing (RNA-seq) using Nova-seq (100 bp paired-end sequencing). Pathway analysis was performed in Cytoscape using KEGG enrichment terms and network plots were generated using gene-gene interactions imported from the STRING database. In a separate cohort (n=16 SAL/20 MOR), following neonatal MOR treatment, adolescent behaviors were

assayed from ~P25-36. Assays included Light/Dark (~P25), Elevated Plus Maze (~P26), Barnes Maze (~P27-31), and assessment of methamphetamine (2.0 mg/kg; intraperitoneal)-induced locomotor activity (~P32-36).

Results: Surprisingly, MOR-treated pups showed overall reduced facial grimace pain scores compared to SAL-treated pups, as well as an interaction between treatment and sex. In addition, analysis across time revealed a distinct divergence pattern unique to each treatment group. The female MOR-NAL showed significantly decreased average pain scores 5-10 min post-injection (when systemic NAL reaches peak availability in the brain). Sex-combined brainstem transcriptome pathway analysis indicated disruptions within gene networks relevant to developmental processes, food consumption, and muscle contraction. Sex-specific analysis identified opposing effects on ribosomal and mitochondrial gene expression, as there was downregulation of genes within these networks in males and upregulation in females. During adolescence, MOR treatment was linked to decreased spatial memory performance (Barnes Maze; 1-way ANOVA with Treatment as between-subjects factor; $p<0.05$), reduced anxiety-like behaviors (Light/Dark and Elevated Plus Maze; 1-way ANOVA, effect of Treatment: $p<0.05$), and increased locomotor activity (repeated measures ANOVA with Day and Treatment as factors; main effect of Treatment $p<0.05$) following treatment with 2.0 mg/kg methamphetamine. Sex was included as a factor in all initial statistical analyses but was removed if it did not contribute significantly to phenotypic variance.

Conclusions: Our assessment of facial grimace suggests potentially unique mechanistic adaptations associated with opioid withdrawal during development that lead to different nociceptive versus affective manifestations of withdrawal. Additionally, transcriptomic network analysis potentially indicates sex-dependent modulation of metabolic function in response to perinatal MOR. Prior neonatal MOR treatment induced persistent disruptions on spatial memory function and anxiety-like behaviors during adolescence. Decreases in psychostimulant-induced locomotion could indicate disruption in the development and function of the dopaminergic mesolimbic reward circuitry in mice treated with neonatal MOR. In future studies, reward-related behavior (intracranial self-stimulation) will be assayed during adulthood (>P50) to assess long-term effects on reward sensitivity and substance abuse susceptibility as a result of neonatal MOR exposure.

Keywords: Addiction Phenotypes, Opioid Addiction, Behavioral Pharmacology

Disclosure: Nothing to disclose.

W162. Glyoxalase 1 Activity Mediates Alcohol Withdrawal Severity in Mice

Amanda Barkley-Levenson*, Amy Lee, Abraham Palmer

University of California, San Diego, La Jolla, California, United States

Background: Alcohol withdrawal can produce significant physiological symptoms including neuronal hyperexcitability and seizures. Acute alcohol withdrawal is most commonly treated with benzodiazepines, but benzodiazepine treatment has been associated with abuse liability, increased risk of cognitive impairments, and greater risk of early relapse to alcohol use. Therefore, identifying new treatment options for acute alcohol withdrawal could improve patient outcomes. The enzyme glyoxalase 1 (GLO1) has been previously implicated in alcohol consumption and depression- and anxiety-like behaviors in mice and rats. This is hypothesized to be due to the GABAergic activity of the substrate of GLO1, methylglyoxal (MG). Here, we investigated whether genetic of pharmacological manipulations of GLO1 activity (and therefore MG levels) could alter alcohol withdrawal seizure severity in mice.

Methods: Male and female mice from a mutant line overexpressing Glo1 on a C57BL/6J (B6) background and wild type littermates (n=4/sex/genotype) were given a 4g/kg alcohol injection and assessed for handling induced convulsions (HIC) during peak withdrawal (6-8hr after injection). In a separate experiment, male mice from another mutant line overexpressing Glo1 on an FVB/NJ (FVB) background and wild type littermates (n=9-12/genotype) were given a 4 g/kg alcohol injection and assessed for HICs during the same peak withdrawal time period. To assess GLO1 inhibitor effects on alcohol withdrawal, male wild type B6 mice were given 10 days of daily 2 g/kg alcohol injections along with the alcohol dehydrogenase inhibitor pyrazole, or saline and pyrazole injections. Starting 1 hr after the final injection, mice were assessed hourly for HICs for 10 hours. At 3.5 hr into withdrawal, mice were given a pretreatment of either 25 mg/kg of a GLO1 inhibitor or vehicle to determine whether GLO1 inhibition reduces peak withdrawal severity (n=12/alcohol group/inhibitor group). Data were analyzed by one- or two-way ANOVA where appropriate.

Results: Mice overexpressing Glo1 on the B6 background showed a trend toward a significant increase in HIC scores during peak withdrawal compared to wild type littermates ($p=0.085$) although minimal withdrawal seizure activity was observed across groups. Mice overexpressing Glo1 on an FVB background showed significantly greater HIC scores during peak withdrawal compared to wild type littermates ($p=0.038$). Neither genotype differed from wild type littermates in baseline seizure susceptibility. The 10 days of chronic alcohol injections produced a significant increase in HIC scores compared to saline-treated animals. Mice in the withdrawal group that received GLO1 inhibitor treatment showed reductions in withdrawal seizure severity ($p=0.1$), although they did not return to baseline levels.

Conclusions: Increased GLO1 activity (due to Glo1 overexpression) appears to be associated with greater handling induced seizure susceptibility during acute alcohol withdrawal in mice. Furthermore, this effect was found to generalize across two different genetic backgrounds. In contrast, decreasing GLO1 activity by treating with a GLO1 inhibitor attenuates alcohol withdrawal seizure severity in a more chronic model of alcohol dependence. This convergence of genetic and pharmacological evidence suggests that GLO1 is a novel target in alcohol withdrawal and GLO1 inhibitors may have potential for medication development for the treatment of neuronal hyperexcitability and seizures during acute withdrawal.

Keywords: Alcohol Withdrawal, Glyoxalase 1, Behavioral Genetics, Transgenic Mice

Disclosure: Nothing to disclose.

W163. High-Throughput Phenotyping Reveals Possible Relationships Between Circadian Rhythms and Cocaine Addiction in Genetically Diverse Mouse Populations

Sam-Moon Kim*, Chelsea Vadnie, Vivek Philip, Leona Gagnon, Kodavali Chowdari, Elissa Chesler, Ryan Logan, Colleen McClung

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States

Background: Circadian clocks regulate reward-related pathways and disruptions of circadian rhythms increase the vulnerability to addiction. Drug of abuse affects core-clock mechanisms, but the genetic mechanisms underlying this mutual interaction is not fully understood. Diverse mouse populations are powerful tools to study the genetics of complex traits because they have tremendously more genetic and phenotypic diversity than conventional inbred mouse strains.

Methods: In order to understand the genetic link between circadian rhythms and cocaine addiction, our study used diversity outbred (DO) and collaborative cross (CC) mouse populations and their eight founder strains composed of five common inbred (A/J, C57BL/6J, 129S1/SvlmJ, NOD/HILtJ and NZO/HILtJ) and three wild-derived (CAST/EiJ, PWK/PhJ, and WSB/EiJ) mice for high-throughput screening of circadian rhythms and cocaine addiction-related behaviors.

Results: We found significant strain and sex differences in period, phase, amplitude and robustness of fibroblast molecular rhythms among the founders with strong heritability. Extreme phenotypic differences were observed between A/J and CAST/EiJ in fibroblast and behavioral rhythms: the longest period in A/J and the shortest period in CAST/EiJ mice. Our preliminary correlation analysis suggested that the mouse strains with longer periods and with reduced amplitude displayed more relapse-like and drug-seeking behaviors in cocaine self-administration (IVSA) respectively. In CC strains, CC004/TauUnc displayed significantly higher addiction-related behaviors in cocaine-sensitization and IVSA relative to CC041/TauUnc. CC041/TauUnc also exhibited a longer circadian period and lower amplitude in molecular and behavioral rhythms relative to CC004/TauUnc mice. Furthermore, we measured molecular rhythms from 329 DO mice and they displayed more circadian phenotypic diversity than the founders possibly due to their genetic heterogeneity. While 80% of molecular rhythms observed in the founders had a period of approximately 24h, only 25% DO mice displayed molecular rhythms with a period of about 24h.

Conclusions: Our study demonstrates that genetic diversity contributes to phenotypic variability in circadian rhythms. This suggests that our high-throughput data will be very powerful for high-precision genetic mapping and provide very useful preliminary data for elucidating the genetic mechanisms underpinning the correlations between circadian rhythms and addiction-related phenotypes.

Keywords: Circadian Rhythms, Collaborative Cross and Diversity Outbred Mice, Fibroblasts

Disclosure: Nothing to disclose.

W164. Opioid Treatment Mobile Application (OPTiMA) to Reduce Relapse Among Adults Receiving Outpatient MAT: Validating Neuroimaging Tasks to Understand Mechanisms of Treatment Response

Andrew James*, Ronald Thompson, Blake Walden, Daphne Jackson, Laura Spell, Natalie Morris, Margaret Healy, Michael Mancino, Clinton Kilts

University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States

Background: We conducted an experimental trial of the smartphone app OPTiMA (Opioid Treatment Mobile Application) that we developed as an adjunctive therapy to reduce relapse among adults receiving outpatient medication assisted treatment (MAT) for opioid use disorder (OUD). This substudy evaluated the use of functional neuroimaging to model brain-behavior relationships encoding attentional bias and craving for personalized drug cues in persons with OUD. Specifically, we sought to determine if these tasks elicited patterns of neural activity comparable to our previous findings in persons with cocaine-use disorder (CUD), thus validating their use for future clinical trials exploring the neural mechanisms underlying MAT treatment response.

Methods: Patients receiving MAT for OUD at the University of Arkansas for Medical Sciences (UAMS) were invited to participate in a three-month non-randomized experimental trial of OPTiMA. Participants attended a one-hour intake session, during which research staff obtained written informed consent, installed OPTiMA on participants' smartphones, and provided training in OPTiMA's use. Participants used OPTiMA to record daily self-reports (via 10-point Likert scales) of craving intensity, withdrawal severity, stress, anger, and depression for the previous day. Participants also recorded illicit opioid use, alcohol consumed (beer-units), and marijuana consumed (gram-units). After completing daily self-reports, participants received personalized feedback to promote continued abstinence. Participants also received daily SMS reminders to complete their self-reports. A subset of participants (N=7) enrolled in a functional MRI study to model neural mechanisms of attentional bias (Counting Stroop) and craving (script-based mental imagery) for personalized opioid-use cues and events.

Results: N=16 participants enrolled in study, with 4 withdrawn for noncompliance (using app once or never). The remaining 12 participants were 100% abstinent of illicit opioid use (determined via urinalysis) for duration of treatment during 3-month study. N=7 participants enrolled in our optional fMRI substudy. We report a strong positive relationship between dorsal anterior cingulate activity (dACC) and attentional bias for opioid cues (rho=0.85, p=0.017), comparable to our previously reported relationship for cocaine cues in persons with CUD (rho=0.36, p=0.02). We also report ventral striatal activity during opioid script-based imagery ($p<0.05$), again comparable to our previous work in CUD.

Conclusions: Our pilot sample of patients with OUD demonstrates patterns of drug cue-induced neural activity comparable to our previous findings among participants with cocaine-use disorder (N>40). This finding supports the use of our fMRI paradigms in a future clinical trial probing the neural mechanisms underlying treatment response for patients receiving MAT with or without adjunctive app-based intervention.

Keywords: Opioid Use Disorder, Opioid Epidemic- Novel Approaches, Smartphone Apps, Functional Neuroimaging

Disclosure: Nothing to disclose.

W165. In Vivo Imaging of 11β-HSD1 With [18F]AS2471907 in Alcohol Use Disorder and Relationships to Drinking Behavior and Stress in the Human Laboratory

Terril Verplaetse*, Shivani Bhatt, Songye Li, Soheila Najafzadeh, Paul Emery, Clara Wong, Ansel Hillmer, Henry Huang, Sherry McKee, Kelly Cosgrove

Yale University School of Medicine, New Haven, Connecticut, United States

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

drinking behavior, as well as stress outcomes in the human laboratory in individuals with AUD.

Methods: To date, we have imaged 9 individuals with moderate to severe AUD (n=6 men, n=3 women; mean age=39 years) and 12 healthy controls (n=7 men, n=5 women; mean age=29 years). Participants received 93.3 ±15.9 MBq [18F]AS2471907 as a bolus injection at high specific activity and were imaged for 180 minutes on the High-Resolution Research Tomograph (HRRT; 2-3 mm resolution). 11β-HSD1 availability was quantified by [18F] AS2471907 volume of distribution (VT; mL/cm3), the ratio at equilibrium of [18F]AS2471907 in tissue to un-metabolized [18F] AS2471907 in arterial plasma. A priori regions of interest included amygdala, hippocampus, ventromedial PFC (vmPFC) and caudate, as these corticolimbic regions are involved in HPA axis regulation, stress pathophysiology, and addiction. Individuals were required to be overnight abstinent from drinking. Participants with AUD (n=8 total; n=3 women; mean age=38 years; 90.6±19.6 MBq injected dose of [18F]AS2471907) completed two laboratory sessions (stress vs. neutral imagery induction) to assess the relationship between 11β-HSD1 levels and the ability to resist drinking (latency to drink), alcohol self-administration, and alcohol craving, as well as self-report measures of stress, negative affect, anxiety, and depressive symptoms.

Results: Individuals with AUD consumed 50.93 drinks/week and had 5.74 drinking days/week. Healthy controls consumed 2.75 drinks/week and had 1.30 drinking days/week. Preliminary data suggest that 11β-HSD1 levels are higher in amygdala, hippocampus, vmPFC, and caudate in individuals with moderate to severe AUD compared to healthy controls ($p<0.03$). Regarding the human laboratory component, there were significant positive correlations between [18F]AS2471907 VT in all regions of interest with baseline anxiety sensitivity scores (Anxiety Sensitivity Index; r=0.73-.081, p=0.01-0.04) during the stress laboratory session only. During the neutral laboratory session, there were significant positive correlations between [18F]AS2471907 VT in caudate, hippocampus, and vmPFC with negative affect at baseline (9am; r=0.71-0.77, p=0.03-0.05) and sedation scores on the Biphasic Alcohol Effects Scale (6pm; r=0.76-0.81, p=0.02-0.03). Significant positive correlations between [18F]AS2471907 VT in all regions of interest and physiologic reactivity (e.g., heart rate, blood pressure) were present throughout the laboratory sessions (9am, 11am, 1pm, 6pm; r=0.72-0.84, p=0.01-0.05). Correlations between [18F] AS2471907 VT and latency to drink, ad-libitum alcohol consumption, and alcohol craving were not significant for either laboratory session ($p>0.05$).

Conclusions: This is the first in vivo examination of the relationship between 11β-HSD1 levels and drinking behavior and in individuals with AUD. These preliminary findings suggest a possible role for 11β-HSD1 in AUD and a possible relationship between [18F]AS2471907 VT and laboratory measures of anxiety and subjective alcohol effects. Future studies will further investigate [18F]AS2471907 as a marker of 11β-HSD1-mediated HPA-axis reactivity (e.g., brain cortisol regulation) in relation to alcohol use, including stress-related drinking behavior.

Keywords: Alcohol, PET Imaging, Stress Reactivity, Cortisol, Alcohol Use Disorder

Disclosure: Nothing to disclose.

W166. Simultaneous PET/MR Imaging of Dopamine Receptor Adaptations Following Repeated Amphetamine Administration in Non-Human Primates

Hanne Hansen*, Martin Schain, Helen Deng, Joseph Mandeville, Bruce Rosen, Christin Sander

Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

Background: Amphetamine is powerful dopamine (DA) releaser and has previously been used in PET studies to study the effects of endogenous neurotransmitter release and consequential receptor internalization (Narendran et al., Synapse, 2007; Skinbjerg et al., NeuroImage 2010). Here, we explore the effects of repeated amphetamine administrations on DA release capacity and receptor adaptations, as reflected by modulation of [11C] raclopride PET/fMRI signals.

Methods: Three anesthetized rhesus macaques were scanned with a simultaneous PET/MR scanner. [11C]Raclopride was administered as a bolus+infusion with a total scan time of 2h. Amphetamine (0.6 mg/kg) was given intravenously after 40 min as a within-scan challenge, during which functional MRI (gradient-echo EPI, with iron oxide contrast agent) was acquired simultaneously with PET. The experiment was repeated either 3h later or 24h later. Furthermore, this experimental design was repeated with the administration of the D1 receptor antagonist SCH 23390 prior to radiotracer injection. FMRI data were analyzed with a general linear model and converted to relative changes in cerebral blood volume (CBV). Receptor availability (BPND) was quantified using a modified SRTM with a time-dependent binding term (Ichise et al., JCBFM, 2003; Sander et al., PNAS, 2013).

Results: The naïve amphetamine challenge caused a reduction in BPND (26.5 ± 9.3%, n=6) together with a short-lasting decrease in CBV. The repeated amphetamine challenge after 3h caused a further reduction in BPND (19.3 ± 11.5%, n=3) and a strikingly different CBV response, showing a long-lasting increase in CBV. The repeated amphetamine challenge 24h after 24h also cause a reduction in BPND (13.9 ± 9.4%, n=5) and a CBV response that had both a short-lasting negative response and a long-lasting positive response. Administration of SCH23390 did not alter the overall shape of the amphetamine-induced changes in CBV at 0h. However, the long-lasting positive CBV response at 3h was reduced to a short-lived positive response and the biphasic CBV response at 24h was reduced to a short-lasting negative CBV response resembling the naïve CBV response.

Conclusions: The naïve amphetamine challenge caused a decreases in [11C]raclopride binding, which was further decreased by the repeated amphetamine challenge 3h later. This decreased binding likely represents a combination of DA release and D2/D3 receptor internalization. At the repeated experiment 24h later, [11C]raclopride BPNDs are back to baseline. However, the capacity for releasing dopamine appear to be decreased, as the peak occupancy is lower. The negative CBV response after the first amphetamine injection suggests that inhibitory D2/D3 receptors dominate the naïve response. After 3h, D2/D3 receptor internalization was persistent, whereas D1 receptors remained available for functional activation: The positive CBV response is consistent with an activation of excitatory D1 receptors, which was effectively blocked by SCH23390. The repeated amphetamine challenge 24h later, reveals that the D2/D3 receptors are again available for activation, but some D1 receptor component is still present. This D1 receptor component was again efficiently blocked by SCH23390.

Using simultaneous PET/MR and amphetamine challenges to elevate extracellular dopamine in the nonhuman primate brain, this study provides in vivo evidence of different receptor internalization mechanism for the D1 and D2/D3 receptors. These data contribute to the mechanistic understanding of how stimulants modulate the dopaminergic system and how this may ultimately lead to substance use disorder.

Keywords: Amphetamine, Receptor Internalization, Trafficking, Dopamine (D2, D3) Receptors, D1 Dopamine Receptors, Hybrid PET/MR

Disclosure: Nothing to disclose.

W167. The Gut Microbiome and its Metabolites are Critical Regulators of Drug-Seeking and Transcriptional Control in a Translational Model of Cocaine Addiction

Katherine Meckel*, Rebecca Hofford, Arthur Godino, Emily Peck, Erin Calipari, Drew Kiraly

Icahn School of Medicine at Mount Sinai, New York, Nebraska, United States

Background: Psychostimulant use disorder represents a public health crisis leading to tremendous morbidity and mortality. Over the past several decades, there has been tremendous growth in our understanding of the neurobiology and circuitry underlying psychostimulant and other substance use disorders. Despite this, there are currently no FDA-approved medications for treatment of psychostimulant use disorders. This has led to the growth of work examining factors that lie outside the blood-brain barrier as potential translational research strategies. In recent years, it has become increasingly clear that there are important connections between the brain and the resident population of bacteria in the gut, the gut microbiome, which may underlie the pathophysiology of neuropsychiatric disease. Robust literature implicates microbiome compositional changes in modulating disease pathogenesis in animal models of autism, neurodegenerative disease, depression, and addiction. While the mechanisms of this gut-brain signaling are not fully clear, substantial research suggests that neuroactive metabolites produced by the microbiome may play a potential signaling role. The most robust evidence exists for a class of bacterially-derived metabolites known as Short-Chain Fatty Acids (SCFA). Production of SCFA by the microbiome has been shown to alter cellular, molecular, and behavioral effects in models of neuropsychiatric disease. Recent work from our lab has shown that the microbiome and SCFA can significantly affect behavioral and transcriptional responses to short-term cocaine exposure in a conditioned place preference model. In the current study, we used our established microbiome depletion model to examine the effects of microbiome manipulations on drug-seeking behavior in a translational model for drug-relapse behavior. Behavioral studies are coupled with cutting edge transcriptomic, metabolomic, and epigenetic profiling to help determine the mechanisms underlying these gut-brain effects.

Methods: Depletion of gut bacteria and their metabolites was induced in Sprague-Dawley rats via addition of broad-spectrum non-absorbable antibiotics (vancomycin, neomycin & bacitracin) to their drinking water and compared to untreated controls. For experiments examining the specific mechanistic contributions of SCFA, the three primary SCFA produced in the gut (butyrate, acetate, propionate) were replenished via addition to drinking water at normal physiological concentrations. Male rats (n=6/group) were first trained to self-administer cocaine (FR1 0.8mg/kg/inf) followed by testing for drug-seeking behaviors utilizing either: (1) a within-session threshold test to evaluate motivation for cocaine at a range of doses or (2) 21 days of abstinence followed by a cue-induced cocaine-seeking task to model relapse behavior. Following all behavioral testing, nucleus accumbens was isolated and tissue processed for RNA-sequencing analysis. Transcriptomes were compared by differential gene expression analysis via the DeSeq2 analysis package, and functional pathways altered between groups were identified using gene ontology analysis. All experimental protocols in animal studies were approved by the Mount Sinai Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: Antibiotic-treatment did not affect acquisition of cocaine on an FR1 schedule. However, when animals who were already stably administering underwent a within-session dose-response threshold task, antibiotic-depleted animals showed significantly enhanced motivation for low dose cocaine ($p<0.01$). Similarly, microbiome depletion increased cue-induced cocaine-seeking following prolonged abstinence in a relapse model ($p<0.01$). To determine the molecular effects underlying gut-brain signaling in this model, we next performed supplementation of SCFA in animals with and without a depleted microbiome. Supplementation with SCFA fully reversed the effects of microbiome depletion ($p=0.52$). RNA-sequencing analysis demonstrated that microbiome-deficient animals exhibited significant alterations of gene expression in networks known to affect synaptic plasticity compared to both SCFA-treated subjects and water-treated controls. Taken together, these findings suggest that the microbiome and its metabolic byproducts are critical in the regulation of drug-seeking behavior and synaptic plasticity.

Conclusions: Animals lacking a complex gut microbiome show significantly increased cocaine-seeking behaviors and altered expression of synaptic plasticity genes. In the absence of a normal microbiome, repletion of bacterially-derived SCFA metabolites reverses behavioral and molecular changes associated with microbiome depletion. Molecular analyses reveal marked changes in activity-dependent gene expression in antibiotic-treated rats compared to controls. These findings suggest that gut bacteria via their metabolites may serve as homeostatic regulators of gene expression in the brain, and suggest the microbiome has potential as a translational research target.

Keywords: Gut Microbiome, Cocaine Self-Administration and Reinstatement, Cocaine Seeking, Metabolism, Neuroimmune Mechanisms

Disclosure: Nothing to disclose.

W168. Long-Term But Not Short-Term Alcohol Binge Drinking Leads to Changes in the Gut Microbiota and the Fecal Metabolome in Adult Male Baboons

Daria Piacentino*, Silvia Grant-Beurmann, Carlotta Vizioli, Xiaobai Li, Mary Lee, Paule Joseph, Claire M. Fraiser, Elise Weerts, Lorenzo Leggio

National Institutes of Health, Bethesda, Maryland, United States

Background: Binge drinking is a pattern of drinking that brings the blood alcohol level (BAL) to over 0.08 g/dL in about 2 hours. Binge drinking is the most common, costly, and deadly pattern of excessive alcohol (EtOH) use in the United States, where it causes approximately 44,000 deaths/year. Research is lacking on the role of the microbiota-gut-brain axis on EtOH binge drinking. We previously showed that EtOH binge-like exposure induces a significant and detrimental decrease in gut microbial α-diversity in Wistar rats. Here, we investigated whether EtOH binge drinking is associated with significant changes in the gut microbiota and/or in fecal metabolites related to gut microbial dysbiosis in adult male baboons.

Methods: We studied three groups of baboons of the species Papio anubis: S=Short-term EtOH exposure group, self-administering EtOH for 2-3 yrs (N=5); L=Long-term EtOH exposure group, self-administering EtOH for >10 yrs (N=4); C=Control group, self-administering Tang®, an isocaloric nonalcoholic drink, for 8 yrs (N=5). Daily blood draws from the baboons in the L and S groups confirmed that they binge drank 7 dd/week, with BALs constantly >0.08 g/dL (range=0.09-0.15 g/dL) after a 2-hour self-administration period. Fecal samples were collected in two conditions: D=during 3 days of active Drinking (dd 1-3) and A=during 3 days of Abstinence (dd 4-6). Diet did not vary between animals; all the baboons were fed daily with primate chow (50-73 kcals/kg), fresh fruit or vegetables, and a children's chewable multivitamin daily. Gut microbiota analysis consisted of DNA extraction and 16S rRNA gene sequencing, analysis of gut microbial α-diversity (Shannon Diversity Index) and β-diversity

(Bray-Curtis Dissimilarity), which measure, respectively, diversity within and between samples, and comparison of relative taxonomic abundances through Linear Discriminant Analysis Effect Size (LEfSe). Fecal metabolomics relied on Ultra Performance Liquid Chromatography/Mass Spectroscopy (UPLC-MS/MS), which identified 565 metabolites. ANOVA and linear mixed-effects models were used for data analysis.

Results: The baboons in the three groups did not differ significantly in age, weight, and liver function, with the latter being normal, as demonstrated by liver enzyme levels and veterinarians' clinical evaluation. Furthermore, they did not differ significantly in the daily amount of EtOH or Tang consumed under condition D. Microbial α-and β-diversity were significantly decreased in L vs. S and C ($p's<0.001$). At LEfSe, the genera Lactobacillus and Streptococcus exhibited significantly higher relative abundances in L vs. S and C, the genera Faecalibacterium, Parabacteroides, and Oribacterium in S vs. L and C, the genera Clostridium and Butyrivibrio in C vs. S and L ($p's<0.01$). Microbiota-generated metabolites of aromatic amino acids (indol, indolelactate, indolepropionate, phenylacetate, phenylethylamine, tyramine) increased in L vs. S and C ($p's<0.01$). In contrast, S showed few differences vs. T, which could be due to altered gut microbial colonization kinetics dictated by the length of exposure to EtOH. Interestingly, serotonin, a tryptophan-derived neurotransmitter that has roles in mediating mood, behavior, and gut movement, increased significantly in L vs. S and C; this may particularly relevant, given that the gut dysbiosis might contribute to neuroinflammation in the context of EtOH exposure. Secondary bile acids produced by the gut microbiota (deoxycholate, 3-dehydrodeoxycholate, glycodeoxycholate) increased in both L and S vs. C ($p's<0.05$), suggesting differential microbial metabolism of bile acids in EtOH-exposed baboons. In line with long-term EtOH exposure, mucosal damage markers (N-acetylated amino acids) increased in L vs. S and C ($p's<0.05$). The gut microbiota and the fecal metabolome did not significantly differ under conditions D and A.

Conclusions: These novel findings suggest that changes in the gut microbiota and the fecal metabolome occur after long-term (>10 yrs) but not short term (2-3 yrs) EtOH binge drinking. These changes are not affected by acute forced withdrawal from chronic EtOH exposure, as shown by the lack of differences between the drinking and abstinence conditions. Future research will need to examine if prolonged and sustained abstinence restores the normal gut microbiota. Additionally, a deeper investigation into the gut microbiota changes and the microbiota-derived metabolites could gain validity as a translational tool, to be used to restore the gut microbiota homeostasis to treat AUD and other EtOH-related disorders.

Keywords: Alcohol Drinking, Gut Microbiota, Metabolomics, Nonhuman Primate Models

Disclosure: Nothing to disclose.

W169. Inhibition of TrkB Receptor Impairs Exercise-Induced Attenuation of Cocaine Reinstatement

Ursula Gelpi-Dominguez, Roberto Morales-Silva, Gigliola Marrero-Cristobal, Angel Echevarria-Rivera, Marian Sepulveda-Orengo*

Ponce Health Sciences University/Ponce Research Institute, Ponce, Puerto Rico

Background: Aerobic exercise is a promising, non-pharmacological treatment currently under investigation as a therapeutic strategy to prevent drug relapse. Several studies have shown that exercise attenuates drug craving and prolongs relapse time in humans, and preclinical studies with animal models have shown similar results in both male and female rats. However, little is known about the effects of exercise on the cocaine-induced cellular adaptations believed to be responsible for cocaine seeking. One potential mechanism that can be modulated by exercise and cocaine is the BDNF/TrkB pathway, as studies have shown that exercise increases levels of brain-derived neurotrophic factor (BDNF) in the hippocampus, and BDNF infusion into the prefrontal cortex and/or nucleus accumbens attenuates cocaine-seeking behavior. We hypothesize that voluntary aerobic exercise activates the BDNF/TrkB pathway, leading to reduced cocaine-seeking behavior.

Methods: We employed a self–administration, extinction, and reinstatement paradigm of cocaine, which included access to a running wheel during the extinction phase in both, male and female rats. During 12 days of short access (2 hours) cocaine self-administration sessions with two levers, one active and one inactive, rats learned that the active lever was paired with cues, such as light and tone, and with a cocaine infusion. After the last self-administration session, rats were separated into two groups. One had access to a running wheel for 6 hours daily, while the other group was confined to its home cage. In the extinction phase, rats were exposed to the same operant chamber as before, but without any cues or cocaine infusion, for two hours daily, during 16 days. Subsequently, each of the two groups were further subdivided into two groups, which were injected intraperitoneally with either vehicle or the TrkB antagonist (ANA 12, 0.4 mg / kg), and the drug-seeking memory was recalled using a cue-primed reinstatement. Twenty-four hours after reinstatement, levels of the proteins tropomyosin receptor kinase B (TrkB), and BDNF from nucleus accumbens were measured by western blot.

Results: Our data shows that a TrkB antagonist (ANA 12) impairs exercise-induced attenuation of drug seeking behavior during cue-primed reinstatement in male rats. Furthermore, male rats exposed to cocaine and voluntary exercise showed decreased BDNF expression compared to homecaged rats. Interestingly, BDNF expression was not affected by administration of ANA12 in any of the groups. Preliminary data from female rats exposed to voluntary exercise and injected with ANA12 appears to show similar behavioral results as in male rats. However, results may not be significant due to the small number of rats in each group. An experiment with a larger number of female rats in each group is currently underway. Western blot protein analysis to measure TrkB expression in all groups is also in progress.

Conclusions: Our findings show that voluntary exercise modulates BDNF expression in rats exposed to cocaine, and that the TrKB receptor plays a role in voluntary exercise-induced attenuation of cue-primed reinstatement. These findings support the hypothesis that voluntary exercise modulates the BDNF/TrkB pathway in male rats exposed to cocaine. Experiments are underway to determine whether exercise has similar effects in female rats exposed to cocaine. Studies such as the present one are necessary to understand how to fully take advantage of the benefits of exercise as a non-pharmaceutical intervention for cocaine addiction.

Keywords: Cocaine Self-Administration and Reinstatement, Aerobic Exercise, BDNF, TrkB, Nucleus Accumbens

Disclosure: Nothing to disclose.

W170. Attenuating Pain and Opiate Urges With Neuromodulation: Comparing 10 Days of TMS to the DLPFC Versus the Motor Cortex

Julia Imperatore, Daniel McCalley, Jeffrey Borckardt, Kathleen Brady, Colleen Hanlon*

Wake Forest School Medicine, Winston Salem, North Carolina, United States

Background: Poorly controlled chronic pain is a primary factor driving non-prescription use of opiates. In a first step towards developing a non-invasive neural circuit based therapeutic for opiate users with chronic pain, this study was designed to evaluate the feasibility and preliminary efficacy of two cortical

locations as neuromodulation targets for decreasing pain and opiate sensitivity.

Methods: Twenty-two individuals with chronic pain currently using prescription opiates were randomized to receive 10 sessions of 10 Hz transcranial magnetic stimulation (TMS) to either the left dorsolateral prefrontal cortex (DLPFC) or to the motor cortex (MC). Multivariate linear models were used to evaluate the effect of these techniques on quantitative sensory testing, brief pain inventory scores, and ratings of distress and opiate use.

Results: Twenty participants (90%) completed all 10 treatment sessions and follow up visits. There was a significant main effect of stimulation site for both quantitative sensory testing and self-reported pain and opiate usage metrics. While both sites had a moderate effect (Cohen's d: 0.5-0.8) on stress, pain, and discomfort, MC stimulation had larger effects on pain interference and the urge to use opiates than DLPFC stimulation. Overall, 35% of the individuals voluntarily decreased their opiate dose (57% MC group), with a 10% decrease in morphine equivalent units on average.

Conclusions: While these are preliminary data and require future double-blind sham-controlled studies, they suggest that the MC may be a promising target for decreasing opiate dependence and pain interference among chronic pain patients.

Keywords: Pain Therapeutics, Non-Invasive Neuromodulation, Opiate Addiction, Corticospinal Tract

Disclosure: Nothing to disclose.

W171. Recently Abstinent Tobacco Smokers Exhibit Less Amphetamine-Induced Dopamine Release in Ventral Striatum Compared to Nonsmokers: a [11C]PHNO PET Study

Katina Calakos*, Ansel Hillmer, Jon Mikael Anderson, Keunpoong Lim, Nabeel Nabulsi, Jim Ropchan, Gustavo Angarita-Africano, Stephen Baldassarri, David Matuskey, Yiyun Huang, Kelly Cosgrove

Yale University School of Medicine, New Haven, Connecticut, United States

Background: Nicotine, the primary addictive component of tobacco smoking, binds to and activates beta2-subunit containing nicotinic acetylcholine receptors on ventral tegmental area neurons, which facilitates dopamine release in the ventral striatum (Imperato, Mulas, and Di Chiara, 1986; Picciotto et al., 1998). Difficulty in quitting smoking may be attributed to deficits in striatal dopamine release as well as aversive withdrawal symptoms such as anhedonia (Powell et al., 2002; Leventhal et al., 2010). Here, amphetamine-induced dopamine release is compared between recently abstinent smokers and nonsmokers using positron emission tomography (PET) imaging with [11C]PHNO, a dopamine D2/3 receptor agonist radioligand. It was hypothesized that abstinent smokers would exhibit less amphetamine-induced dopamine release in the ventral striatum than nonsmokers. Furthermore, it was hypothesized that abstinent smokers would exhibit worse mood symptomatology than nonsmokers, and that this would correlate with lower magnitude amphetamine-induced dopamine release.

Methods: Lifetime nonsmokers (n=19, 6 female) and recently abstinent smokers (n=18, 6 female, abstinent 11 ± 9 days) participated in two [11C]PHNO scans. A baseline scan was acquired following bolus injection of [11C]PHNO (443.1 ± 25.7 MBq; 1.9 ± 0.2 micrograms). Amphetamine (0.5 mg/kg, PO) was administered three hours before a second [11C]PHNO scan (424.6 ± 27.7 MBq; 2.1 ± 0.2 micrograms). On PET scan day, subjects also completed the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977). PET data were analyzed with SRTM2 (reference region: cerebellum) to estimate [11C]PHNO binding potential (BPND), which is proportional to D2/3 receptor availability, in the ventral striatum, caudate, putamen, pallidum and substantia nigra. BPND was measured at baseline and post-amphetamine. Percent change in BPND before and after amphetamine, an indirect measure of dopamine release, was calculated per region of interest (ROI) per subject. In the a priori ROI the ventral striatum, group differences in baseline BPND and amphetamine-induced percent change in BPND were tested using Welch's two-sample t-tests. Group differences in baseline BPND and amphetamine-induced percent change in BPND in the other regions were assessed separately with two-sample tests. Spearman's rank correlations were computed to analyze associations between scan-day CES-D total scores and PET outcome measures. For all analyses, the significance level was set to $p < 0.05$, and p-values were adjusted with a false discovery rate correction when appropriate for multiple comparisons correction.

Results: There were no group differences in baseline BPND in any regions. Amphetamine-induced percent change in BPND in the ventral striatum was significantly lower in abstinent smokers compared to nonsmokers (nonsmokers: $n = 19$, 26.5 ± 6.5%, abstinent smokers: $n = 18$, 19.6 ± 10.0%; $p = 0.021$; $d = 0.82$). No group differences in amphetamine-induced percent change in BPND emerged in other regions. Abstinent smokers had slightly higher scores on the CES-D than nonsmokers (nonsmokers: $n = 17$, 4.5 ± 3.7; abstinent smokers: $n = 19$, 8.8 ± 7.6; $p = 0.108$; $d = 0.72$). CES-D scores were significantly negatively correlated with percent change in BPND in the ventral striatum for abstinent smokers ($n = 15$, $r = -0.627$, FDR-adjusted $p = 0.025$), but not for nonsmokers ($n = 16$, $r = 0.116$, FDR-adjusted $p = 0.670$).

Conclusions: Abstinent smokers exhibited less amphetamine-induced dopamine release in the ventral striatum than non-smokers. In abstinent smokers, worse mood symptoms were significantly related to less amphetamine-induced dopamine release in the ventral striatum. These findings are consistent with evidence of lower dopamine release in individuals with other addictive disorders compared to controls (Martinez et al., Biol. Psychiatry 2005; Martinez et al., Am J Psychiatry 2007; Trifilieff et al., Semin Nucl Med 2017), and suggest that anhedonia during abstinence is related to dopaminergic dysfunction. Our findings indicate that deficits in both dopamine release and mood exist during early abstinence, which may contribute to difficulty in quitting. Funded by: R01 DA038832.

Keywords: Tobacco Smoking, Dopamine (D2, D3) Receptors, PET Imaging, Nicotine Withdrawal

Disclosure: Nothing to disclose.

W172. Alcohol-Induced Changes in Circulating Endocannabinoids, Mood, and Reward Processing in Healthy Social Drinkers

Elisabeth Paul, Rebecca Boehme, Robin Kämpe, Bijar Ghafouri, Niclas Stensson, Markus Heilig, Leah Mayo*

Linköping University, Linköping, Sweden

Background: The endocannabinoid system is proposed to contribute to the reward-related effects of several drugs of abuse, including alcohol. Individuals with a history of alcohol use disorder experience dysregulation of the endocannabinoid system, including cannabinoid receptor availability, which may contribute to deficits in reward processing. However, less is known about the acute effects of alcohol on the endocannabinoid system and how this may relate to the reward-related effects of alcohol in healthy humans.

Methods: Healthy social drinkers ($N = 30$; 16 each men, women) completed two sessions (placebo; 0.6 g/kg alcohol) in counterbalanced order approximately one week apart. Following drink consumption, participants completed reward processing tasks, including the Monetary Incentive Delay (MID) task and a

probabilistic reversal learning task, in an fMRI scanner. Both tasks assess behavioral and neural reactions to monetary losses and gains. Behavior in the MID task was analyzed using multilevel modeling, while computational modeling was employed in the reversal learning task. Blood samples were collected via an indwelling intravenous catheter for analysis of circulating endocannabinoids (anandamide, AEA; 2-arachidonylglycerol, 2-AG) before and after drink administration. Self-report questionnaires assessed mood and subjective drug effects throughout the sessions.

Results: Results indicate individual variability in the effect of alcohol on circulating endocannabinoids that relates both to mood and reward processing. For instance, individuals experiencing greater increases in AEA following alcohol consumption also reported larger increases positive mood at the alcohol session ($p = 0.009$). Moreover, in the MID task, we found an interaction between trial type (gain or loss) and AEA levels. Specifically, those who experienced greater decreases in AEA following alcohol consumption demonstrated increased behavioral motivation (e.g. faster reaction times) in monetary gain trials ($p = 0.014$). There was no association between AEA or 2-AG and loss trials. Finally, behavior in the reversal learning task was also related to changes in AEA. In particular, decreases in AEA were associated with increased reward sensitivity at the alcohol session ($p = 0.011$), but not at the placebo session. There was no relationship between AEA or 2-AG and punishment sensitivity.

Conclusions: In sum, individual variability exists in the effects of alcohol on circulating endocannabinoids and AEA in particular. Increases in AEA following alcohol consumption are associated with greater positive mood and decreased sensitivity to monetary gains. Current analysis are underway to determine the underlying neural correlates to these behavioral findings. Future work will assess the role of the endocannabinoid system in reward processing within populations with more extensive alcohol use histories.

Keywords: Endocannabinoid System, Alcohol, Monetary Reward

Disclosure: Nothing to disclose.

W173. Sex Differences in Striatal Dopamine Release and Resting Network Connectivity in the Human Brain

Peter Manza*, Ehsan Shokri-Kojori, Corinde Wiers, Danielle Kroll, Dana Feldman, Katherine McPherson, Catherine Biesecker, Dardo Tomasi, Gene-Jack Wang, Nora Volkow

National Institutes of Health, Bethesda, Maryland, United States

Background: There are marked sex differences in the prevalence of neuropsychiatric diseases, many of which, including substance use disorders, implicate the dopamine system. Sex differences have also been noted in the sensitivity to drugs such as stimulants which directly increase dopamine levels. However, in humans there is little conclusive evidence for sex differences in the brain dopamine response to stimulant drugs. Further, recent evidence suggests that brain functional network connectivity is strongly associated with stimulant-induced dopamine release, but the role of sex differences in these processes is unknown.

Methods: Here we report on two independent [11C]raclopride PET brain imaging studies that used a methylphenidate challenge to increase dopamine, with different routes of administration (study 1 = oral 60 mg; study 2 = intravenous 0.5 mg/kg; total n = 95; 65 male, 30 female). Immediately following PET scans, we also collected resting-state functional MRI scans to measure brain network connectivity strength.

Results: We find that females show significantly greater ventral striatal dopamine release than males to both oral ($t = 2.483$, $p = .019$) and intravenous ($t = 2.009$, $p = .049$) methylphenidate, in blinded placebo-controlled designs. This effect was specific to ventral striatum, as there were no significant differences in dopamine release in the dorsal striatal regions (caudate and putamen). Women also showed significantly lower global within-network resting functional connectivity than men ($t = 3.282$, $p = .003$). Resting connectivity mediated the association between sex and ventral striatal dopamine release (mediation effect estimate = -5.623, 95% CI = [-12.214 -1.56], $p = .004$). Associations remained significant after controlling for age, BMI, and head motion.

Conclusions: These data have implications for sex differences in vulnerability to substance use disorder and other neuropsychiatric diseases and for their treatment, including the use of methylphenidate for ADHD, and point to functional network strength as a key determinant of the brain's response to stimulants.

Keywords: Stimulants, PET Imaging, Sex Differences, fMRI Functional Connectivity, Dopamine

Disclosure: Nothing to disclose.

W174. Acute Alcohol Effects on Psychomotor Performance in AUD, Heavy, and Light Drinkers

Andrea King*, Abigayle Feather, Ashley Vena

University of Chicago, Chicago, Illinois, United States

Background: The theory of behavioral tolerance to alcohol posits that greater experience with drinking to intoxication leads to less impaired cognitive and psychomotor performance. However, the nature of development of this acute tolerance over time in humans remains uncertain. In the present study, our goal was to test whether drinkers with a variety of drinking patterns differ in their acute psychomotor and neurocognitive responses to alcohol. It was predicted that excessive drinking would be associated with the most behavioral tolerance to alcohol-induced effects on psychomotor performance.

Methods: Data were examined in N=293 participants (aged 21-35 yrs) from the first three cohorts of the larger Chicago Social Drinking Project. Participants included drinkers with DSM-5 alcohol use disorder (AUD; n=103 (61% severe), non-AUD heavy social drinkers (HD; n=104) and light social drinkers (LD; n=86). All were tested in two random-order, oral beverage sessions with administration of alcohol (0.8 g/kg) or placebo (1% alcohol taste mask). Women received an 85% dose modification due to sex differences in body water. The beverage was consumed over a 15-minute period. A subset of AUD drinkers (n=61) underwent an additional, random-order session with a very high alcohol dose (1.2 g/kg). In all sessions, performance was measured by the Grooved Pegboard (fine motor speed) and Digit Symbol Substitution Test (DSST; motor spend, encoding, and short-term memory). These were given prior to beverage consumption and then repeated at four intervals after consumption in each session. Alcohol minus placebo change scores were calculated at each time point and compared across groups.

Results: Breath alcohol concentrations (BrAC) peaked one hour after consumption with a mean of 0.85g/dl for the high dose and 0.132 g/dL for the very high dose. Alcohol impaired performance on both tasks across all three groups with the largest speed and accuracy impairments during the rising limb to peak BrAC with gradual recovery during declining BrACs. For Pegboard, the high dose produced the largest impairment in LD (>HD=AUD) with AUD showing acute tolerance and the most rapid recovery during the declining limb. However, for AUD, the very high alcohol dose impaired speed on the Pegboard task, i.e., >200% slower relative to the high dose impairment in LD and HD. For DSST, alcohol produced similar impairment across groups during the rising limb, but at peak BrAC, both AUD and HD showed acute tolerance while LD were remained just as impaired and they took longer for recovery of function. Similar to the data with the Pegboard, for

DSST, the very high alcohol dose impaired AUD's performance, i.e., >125% worse performance than the high-dose impairment observed in LD and HD.

Conclusions: This study provided a comprehensive examination of alcohol-induced psychomotor impairment at two intoxicating doses and in three drinker subgroups. Findings confirm that while alcohol impaired speed and accuracy in both tasks, AUD drinkers exhibited acute tolerance with shorter recovery of function than in lighter drinkers. However, after consuming a dose that may be more relevant to their drinking pattern (re: the very high dose), AUD drinkers showed fine motor and encoding impairment greatly exceeding that of the high dose impairment shown in the other groups. Thus, while chronic problematic drinkers may show some behavioral tolerance in terms of less severe performance impairments to alcohol, such effects are time- and dose-dependent and, most importantly, not observed at more excessive drinking levels commensurate with their usual drinking patterns. The results elucidate the nature of acute behavioral tolerance in drinkers and also have potential implications in the prevention of alcohol consequences and lessening the societal burden of hazardous drinking.

Keywords: Tolerance, Alcohol Sensitivity, Psychomotor Speed, Cognitive Impairment, Acute Effects

Disclosure: Nothing to disclose.

W175. Short, Long, and Intermittent Access to MDPV and Cocaine Self-Administration in Male and Female Rats: Interactions With Substance Use Disorder-Like Phenotypes

Michelle Doyle, Agnieszka Sulima, Kenner Rice, Gregory Collins*

University of Texas Health Science Center at San Antonio, San Antonio, Texas, United States

Background: Despite decades of research, the behavioral, pharmacological, and neurobiological determinants of one's vulnerability to develop a substance use disorder (SUD) are not well understood. In rats, short-access to cocaine self-administration tends to result in highly predictable and well-regulated patterns of drug intake; however, manipulating access conditions (e.g., long or intermittent access) can produce neurobiological and behavioral changes thought to be related to SUDs. In contrast to cocaine, short-access to MDPV (3,4-methylenedioxypyrovalerone) self-administration results in a subset or rats developing high levels of dysregulated drug-taking behavior that resemble the compulsive patterns of drug taking often reported in individuals with SUDs. However, it is unclear whether this phenotype would be exacerbated if rats had long-, or intermittent-access to MDPV self-administration. The current studies aimed to test the hypotheses that (1) under short-access conditions, rats self-administering MDPV exhibit higher levels of SUD-like behaviors, including greater drug intake, greater rates of responding during periods of signaled drug unavailability, and reduced sensitivity to punishment by foot shock; and (2) long- and intermittent-access to MDPV or cocaine self-administration will result in greater SUD-like behaviors compared to short-access self-administration.

Methods: Male and female Sprague Dawley rats self-administered MDPV (0.032 mg/kg/infusion; n=66) or cocaine (0.32 mg/kg/infusion; n=66) under short-access conditions and the severity of their SUD-like phenotype was evaluated. Then, rats self-administered the same drug under short-, long-, or intermittent-access conditions prior to re-evaluating the severity of their SUD-like phenotypes.

Results: Though long- and intermittent-access increased total levels of drug intake and resulted in repeated bouts of rapid drug intake, respectively, these access conditions did not appear to systematically alter the SUD-like phenotypes of rats self-administering either cocaine or MDPV. However, rats that self-administered MDPV exhibited higher levels of drug taking and greater rates of responding when drug was not available compared to rats self-administering cocaine, regardless of access condition. Though female rats had greater levels of drug intake than male rats under some conditions, sex did not appear to be a primary factor in the severity of the SUD-like phenotype at baseline or after manipulating access conditions.

Conclusions: These results suggest that the SUD-like phenotype of rats self-administering MDPV may be more robust than the SUD-like phenotypes of rats with a history of self-administering cocaine. Therefore, MDPV self-administration may be a useful method to study individual differences vulnerability to develop substance use disorders.

Keywords: Cocaine Self-Administration, Synthetic Psychoactive Cathinones, MDPV Self-Administration, SUD-Like Phenotypes

Disclosure: Nothing to disclose.

W176. Genetic Deficiency in Plasma Membrane Monoamine Transporter Function Sex-Selectively Influences Cocaine Sensitization

Jasmin Beaver, Lynette Daws, T. Gilman*

Kent State University, Kent, Ohio, United States

Background: Plasma membrane monoamine transporter (PMAT) can transport monoamines, such as dopamine and serotonin, much faster than the more selective dopamine transporter (DAT) or serotonin transporter (SERT), though PMAT has a lower affinity for these substrates than either DAT or SERT. Consequently, PMAT function is hypothesized to predominantly emerge when DAT or SERT function is impaired, under conditions of either heightened release in response to stressors, or pharmacological blockade. However, the contribution of PMAT function to behavioral responses to drugs of abuse that impair monoamine uptake (e.g., cocaine, amphetamine) has not been investigated. Given that no selective inhibitors of PMAT are available, we employed mice constitutively deficient in PMAT to explore this question. We hypothesized that, relative to wildtype controls (+/+), mice with reduced (+/-) or ablated (-/-) PMAT function would exhibit augmented cocaine sensitization. Cocaine inhibits function of DAT, SERT, and norepinephrine transporter, but whether cocaine acts at PMAT has not been thoroughly explored. Blockade of these higher affinity monoamine transporters by cocaine could permit the contribution of the lower affinity PMAT function to be unmasked.

Methods: Adult (≥80 days old) male and female PMAT +/+, +/-, and -/- mice were used for all experiments. Males were always run on separate days from females. Pilot cocaine-induced locomotor data (N=4-5) were generated with one day of sequential injections of saline (10 mL/kg) followed by cocaine (2.5, 2.5, 5.0, 10.0 mg/kg, ip.; final cumulative dose 20 mg/kg) every fifteen minutes, all following a 30 min habituation period. Ongoing experiments (current N=2-8) are using an established cocaine sensitization paradigm (Elliot, 2002, Behavioural Pharmacology 13:407-415). After a 30 min habituation period, mice are injected with saline followed by sequential doses of cocaine every ten minutes (5.0, 5.0, 10.0, 20.0 mg/kg; final cumulative dose 40 mg/kg). This process is repeated for five consecutive days. For all experiments, area under the curve for total distance traveled after each dose (pilot experiments) or day (ongoing experiments) is calculated and analyzed with a three-way mixed-model ANOVA (sex × genotype × dose/day). Experiments are ongoing, with power analyses indicating that a final N=9-16, for females and males, respectively, will provide 80% power with a medium effect size. All experiments were approved by institutional IACUCs, and

were executed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Results: Pilot experiments with low, cumulative doses of cocaine (2.5-20 mg/kg) on a single day did not elicit any PMAT genotype-specific differences in females, but a trend in male PMAT +/- and -/- mice suggested attenuated locomotor responses to cocaine compared to PMAT +/+ males. Ongoing experiments are investigating cocaine sensitization with a higher cumulative cocaine dose range (5-40 mg/kg) over 5 consecutive days. Early results (N=2-8) support the trend for a sex-specific effect of PMAT deficiency. Statistical analyses will be performed once experiments have Ns that were a priori determined to provide sufficient power.

Conclusions: These early findings may indicate a previously unrecognized influence of PMAT function, in a sex-selective manner, on behavioral responses to drugs of abuse that act at monoamine transporters. Consequently, PMAT function might partially explain individual and sex differences in humans in responses to psychostimulants, and possibly influence risk of drug addiction. Future investigations will examine if the rewarding effects of cocaine are altered in PMAT-deficient mice, and explore responses to amphetamine.

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Keywords: Plasma Membrane Monoamine Transporter, Cocaine, Dopamine, Drug Addiction

Disclosure: Nothing to disclose.

W177. Conventional Rodent Nicotine Self-Administration Procedures Over-Estimate Efficacious Exposures of Smoking Cessation Drugs in Human Smokers

Ian Gurrell, Paul Kenny, Amir Inamdar*

AstraZeneca Neuroscience, Cambridge, United Kingdom

Background: The intravenous (IV) nicotine self-administration procedure is considered the most direct and reliable measure of the reinforcing properties of nicotine in laboratory animal including rats. Whether standard nicotine self-administration (0.03 mg/kg/infusion) procedures in rats can reliably predict efficacious exposures of smoking cessation drugs in human smokers is unclear. A factor likely to influence 'translatability' in this regard is whether doses of nicotine consumed by rats have similar pharmacodynamic effects of smoked nicotine in humans. We investigated whether the rat IV nicotine self-administration procedure can reliably predict efficacious exposures of known smoking cessation drugs in human smokers. We also sought to improve the predictive power of this procedure by modifying the dose of nicotine available for consumption by rats to better recapitulate the pharmacodynamic actions of the drug seen in smokers. Finally, we used the modified self-administration procedure to predict efficacious exposures of the novel smoking cessation agent AZD4041, a highly selective and potent orexin-1 receptor (OX1R) antagonist currently in phase 1 studies.

Methods: We reviewed the literature for the effects of drugs known to promote smoking cessation in humans on IV nicotine self-administration behaviour in rats, where a standard unit dose of nicotine (0.03 mg /kg/ infusion) was used. We also compiled literature data for the pharmacokinetics and plasma protein binding properties of these same drugs in rats. In this manner, we could establish the relationship between in vivo unbound exposure and efficacy for each of the smoking cessation drugs in the standard rat nicotine self-administration procedure. Utilisation of these data and an inhibitory effect PK/PD model facilitated an estimation of in vivo IC50 values in for each agent in nicotine self-administering rats. Subsequently, the derived in vivo IC50 values in the self-administering rats were compared with clinically effective (Ceff) exposures in human smokers. In parallel, we performed IV self-administration studies in rats in which the unit dose of nicotine (0.03 or 0.12 mg /kg/ infusion) was modified and the effects of the established smoking cessation agent varenicline on responding for nicotine infusions were examined. We also examined the effects of AZD4041 on responding for the standard and higher doses of nicotine and used these data to estimate efficacious exposures in human smokers.

Results: We found that drugs known to promote smoking cessation in humans generally decreased IV nicotine self-administration in rodents. Specifically, the nicotinic acetylcholine receptor partial agonist varenicline, the CB1 receptor antagonist rimonabant, the 5-HT2c receptor agonist lorcaserin and the triple re-uptake inhibitor bupropion decreased nicotine intake by rats across multiple laboratories. However, in vivo IC50 values of these drugs, each with different pharmacological mechanisms of actions, were consistently higher (2-5-fold) than Ceff values from human smokers. Notably, in our rat self-administration study, we found that increasing the unit dose of nicotine available for consumption from the standard dose typically used to a higher dose that recapitulates in rats some of the pharmacodynamic actions of nicotine in human smokers (from 0.03 to 0.12 mg /kg/ infusion) resulted in a 'leftward' shift in the in vivo IC50 value of varenicline in rats to a range similar to its Ceff value in smokers. The predicted efficacious exposures of AZD4041 in human smokers were dramatically lower when modelled on data from rats consuming the higher nicotine dose compared with the standard dose, and were in line with established exposures for orexin antagonists used to treat other human indications (i.e., facilitation of sleep).

Conclusions: These findings suggest that the rat IV nicotine self-administration procedure can reliably detect compounds that have efficacy in promoting smoking cessation in humans. However, standard iterations of the self-administration procedure that employ a unit dose of nicotine (0.03 mg /kg/infusion) that sustains maximal rates of responding by rats consistently over-estimates the exposures of compounds necessary to detect efficacy in human smokers. Recently, it was shown self-administration of doses of nicotine higher than 0.03 mg /kg/ infusion, which reside on the descending portion of the dose-response curve (0.12 mg /kg/ infusion) and activate the habenula region of the brain, increase blood glucose levels in rats similar to the actions of cigarettes in human smokers. Therefore, efficacy in the self-administration procedure employing these higher doses may be more predictive of efficacious exposures of smoking cessation agents in human smokers. Indeed, we found that varenicline was far more effective at suppressing nicotine intake in rats responding for the higher dose of nicotine compared with those responding for the standard dose. Notably, the predicted efficacious exposures for the AZD40401 were markedly lower when modelled using data from rats responding for the higher dose compared with the standard dose of nicotine. Together, these findings suggest that the rat IV nicotine self-administration procedure can reliably detect the beneficial actions of compounds that are efficacious smoking cessation drugs in human smokers. However, the translatability of this procedure to predict efficacious exposures in humans may depend on the dose of nicotine consumed by the rats.

Keywords: Nicotine, Self-Administration, Smoking Cessation, Animal Models

Disclosure: AstraZeneca: Employee (Self), AstraZeneca: Stock / Equity (Self), GlaxoSmithKline: Stock / Equity (Self)

W178. Sex Differences in the Impact of Dopamine D3 Receptor Antagonism on Fentanyl Self-Administration and Analgesia in Male and Female Rats

Chloe Jordan*, Bree Humburg, Zheng-Xiong Xi, Amy Newman

National Institute on Drug Abuse, Rockville, Maryland, United States

Background: Throughout the COVID-19 pandemic opioid abuse and overdose rates have continued to rise. The opioid epidemic remains a major public health crisis with massive personal and economic costs. Fentanyl is a potent synthetic opioid that has been crucial for pain management in hospital settings, but is increasingly misused, diverted, and synthesized clandestinely to substitute for heroin in illegal drug markets. Identifying non-opioid-based pharmacotherapies for the treatment of pain and opioid abuse is desperately needed. We recently discovered that highly selective dopamine D3 receptor (D3R) antagonists decrease oxycodone self-administration and augment analgesia in male rats. However, whether D3R antagonists alter behavioral responses to more potent opioids of abuse such as fentanyl remains unclear, while the impact of D3R antagonists in female subjects is almost completely unknown. Here we sought to determine whether the highly selective D3R antagonist, R-VK4-116, is able to reduce fentanyl-taking and fentanyl-seeking behavior with or without alteration in fentanyl analgesia in both male and female rats. We employed a range of behavioral approaches, first characterizing the impact of acute R-VK4-116 treatment on intravenous fentanyl self-administration, followed by examination of the outcome of acute vs. chronic R-VK4-116 treatment on fentanyl-primed reinstatement, and finally observation of the effects of R-VK4-116 with on fentanyl-induced analgesia.

Methods: Adult male (n=24) and female (n=26) Long-Evans rats were allowed to intravenously self-administer fentanyl (2.5 ug/kg/infusion) under a fixed ratio (FR) 2 schedule in daily, 2-hour sessions. Once stable self-administration was achieved (defined as less than 20% variability in responding and an active: inactive lever response ratio of 2:1 or higher for at least 3 consecutive days), animals were treated with vehicle or one of two doses of R-VK4-116 (10 or 20 mg/kg, i.p.) 30-min prior to self-administration sessions. Thereafter, rats underwent extinction training for 12 daily, 2-hour sessions, during which time lever responding was recorded but had no scheduled consequences. Rats were randomly assigned to receive either vehicle or 10 mg/kg R-VK4-116 (i.p.) every day 30-min prior to extinction sessions. Body weight was monitored daily. Once extinction was achieved (defined as active lever responses less than 15% of the self-administration baseline), rats were given a fentanyl priming dose (0.05 mg/kg, i.p.) prior to a reinstatement test in the absence of R-VK4-116 pretreatment. During the reinstatement test active lever responses produced previously fentanyl-paired cues (cue light, syringe pump running), but no fentanyl infusions could be earned. Rats then underwent one additional week of extinction training, followed by a second fentanyl-primed reinstatement test, this time following acute vehicle or R-VK4-116 pre-treatment. Lastly, nociceptive responses on the hot plate test to vehicle, fentanyl (0.05, 0.1, or 0.2 mg/kg, i.p.), or R-VK4-116 (10 or 20 mg/kg, i.p.) alone or in combination were observed in both sexes.

Results: Overall, female rats earned more fentanyl infusions and made more active lever responses for fentanyl than male rats during the self-administration phase. Acute R-VK4-116 pretreatment dose-dependently reduced fentanyl self-administration in both males and females ($P<0.05$). During the first 1-2 days of extinction training, R-VK4-116 treatment reduced active lever responses in both sexes ($P<0.05$); all rats reached extinction criteria within 12 daily sessions. Body weight did not differ between the vehicle and R-VK4-116 treatment groups at any time. After extinction, fentanyl priming produced robust reinstatement responses in both male and female rats (chronic vehicle group, $P<0.01$, compared to extinction levels). Chronic R-VK-4116 treatments during the extinction phase significantly suppressed fentanyl-primed reinstatement in male ($P<0.05$), but not in female rats. However, when R-VK4-116 was given on the reinstatement test day in rats after additional week of extinction, R-VK4-116 prevented fentanyl-primed reinstatement responding in both male and female rats. In the hot plate test, females were less sensitive to fentanyl and required significantly higher doses than males to produce analgesic effects. R-VK4-116 itself did not induce any changes in nociceptive response in either sex. However, pretreatment with R-VK4-116 significantly increased fentanyl analgesia in females only ($P<0.001$, compared to fentanyl alone).

Conclusions: Females self-administered more fentanyl and were less sensitive to fentanyl-induced analgesia than males. While D3R antagonism by R-VK4-116 acutely reduced fentanyl self-administration and fentanyl-primed reinstatement in both males and females, chronic R-VK4-116 treatment during the extinction phase reduced fentanyl seeking in males only. Conversely, D3R antagonism by R-VK4-116 enhanced fentanyl-induced analgesia only in female rats. To explore the neural substrates by which R-VK4-116 and fentanyl exert sex-dependent effects, future studies will examine the levels of mu opioid receptor and D3R mRNA in reward- and pain-related regions of both males and females using RNAscope in situ hybridization.

Keywords: Fentanyl, Sex Differences, Dopamine D3 Receptors, Opioids, Intravenous Drug Self-Administration

Disclosure: Nothing to disclose.

W179. Ethanol Intake, Preference, and Relapse-Like Behavior are Differentially Regulated by Gonadal Status and Sex Chromosome Complement

Elizabeth Sneddon-Yepez*, Natalie Cavalco, Asa Jaymes, Noah Ostlie, Lindsay Rasizer, Anna Radke

Miami University, Oxford, Ohio, United States

Background: Recent research indicates that the diagnosis of alcohol use disorder (AUD) in women is on the rise in the United States. Rodent models show behavioral sex differences in different aspects of ethanol (EtOH) drinking behaviors such as escalation, reinstatement, and relapse. The mechanisms associated with increased vulnerability for these behaviors in females are still unclear. The four-core genotype (FCG) mouse model allows investigation of how sex chromosome complement and gonadal status influence behavior independently and together. Here, we used the FCG mouse model to investigate the influence of gonadal hormones and sex chromosomes on EtOH intake and relapse susceptibility.

Methods: FCG mice (n = 40, XX/ Sry- = 10, XY/ Sry- = 10, XX/ Sry+ = 10, XY/ Sry+ = 10) were given access to EtOH in their home cage about 6 hours into the light cycle (i.e., 11:30 AM). Mice had access to two bottles for 24 h. One bottle contained reverse-osmosis (RO) drinking water and the other bottle contained EtOH in RO water (v/v). Two "dummy" cages were fitted with bottles to account for evaporation and spillage. EtOH concentrations increased from 5%, 10%, 15%, and 20%. Mice were exposed to each concentration of EtOH for a total of 5 drinking sessions and all mice and bottles were weighed every 24 h. After the last 20% EtOH session, mice underwent a 6-day EtOH deprivation period. After the deprivation period, mice were reintroduced to 20% EtOH for 24 h. This cycle of deprivation and re-exposure was then repeated for a total of 5 deprivation sessions. At least two weeks following deprivation, water consumption was measured in one 24 h session and 2.5% sucrose consumption was assessed over 5

24 h drinking sessions. Animals were cared for in accordance with the guidelines set by the National Institutes of Health and all procedures were approved by the Institutional Animal Care and Use Committee at Miami University.

For consumption, preference, and deprivation session data, a Three-Way Analysis of Variance (ANOVA) was used with gonadal status and sex chromosome complement as between-subjects factors and session as the within-subject factor. For total water consumption and total sucrose consumption, a Two-Way ANOVA was used with gonadal status and sex chromosome complement as between-subjects factors. To assess effect size, an eta squared was used on all significant findings. The alpha level was set at $p < 0.05$.

Results: Mice with female gonads (Sry-) and XX chromosomes consumed greater amounts of EtOH. A Three-Way ANOVA identified that gonadal status and sex chromosome complement are associated with EtOH consumption with a main effect of the Sry gene, $F(1, 35) = 4.71$, $p = 0.037$, $\eta2 = 3.38\%$, chromosomes, $F(1, 35) = 9.84$, $p = 0.004$, $\eta2 = 7.07\%$, and EtOH concentration, $F(3, 105) = 55.05$, $p < 0.001$, $\eta2 = 36.07\%$, and an interaction of EtOH concentration X gonadal status, $F(3, 105) = 3.16$, $p = 0.028$, $\eta2 = 2.07\%$, and EtOH concentration X chromosomes, $F(3, 105) = 2.84$, $p = 0.042$, $\eta2 = 1.86\%$. Mice with XX chromosomes preferred EtOH to a higher degree than mice with XY chromosomes. A Three-Way ANOVA revealed that sex chromosome complement is associated with preference for EtOH over water with a main effect of chromosomes, $F(1, 35) = 8.13$, $p = 0.007$, $\eta2 = 5.78\%$, EtOH concentration, $F(3, 105) = 47.19$, $p < 0.001$, $\eta2 = 35.17\%$, and an interaction of EtOH concentration X gonadal status, $F(3, 105) = 3.17$, $p = 0.027$, $\eta2 = 2.36\%$. Mice with XX chromosomes drank more EtOH during deprivation sessions when consumption was expressed as a percent of baseline. A Three-Way ANOVA identified a main effect of chromosomes, $F(1,35) = 6.25$, $p = 0.017$, $\eta2 = 7.13\%$, and session, $F(4,140) = 9.31$, $p < 0.001$, $\eta2 = 10.13\%$. Mice with female gonads (Sry-) consumed greater amounts of water. A Two-Way ANOVA showed that gonadal status is associated with water consumption during EtOH sessions, $F(1, 35) = 0.81$, $p < 0.001$, $\eta2 = 25.71\%$, and non-EtOH sessions, $F(1, 23) = 13.38$, $p = 0.001$, $\eta2 = 34.81\%$. A Two-Way ANOVA did not identify any significant differences associated with gonadal status or sex chromosome complement on sucrose intake.

Conclusions: We found that female gonads (Sry-) and XX chromosomes are associated with greater amounts of EtOH intake. Interestingly, we also discovered that mice with XX chromosomes have a higher preference for EtOH and are more prone to relapse than mice with XY chromosomes. This result indicates individuals with XX chromosomes may have a predisposition to prefer alcohol and have a higher susceptibility to relapse than individuals with XY chromosomes. Water consumption was increased in mice with female gonads (Sry-) during EtOH drinking sessions and this effect persisted on non-EtOH sessions. We did not see any association between gonadal status or sex chromosome complement on sucrose intake which indicates that the observed differences are specific to EtOH intake. These findings indicate that EtOH intake, preference, and relapse-like behavior may be differentially regulated by gonadal status vs. sex chromosome complement and suggest novel, future avenues for investigating the neural mechanisms of female vulnerability to alcohol.

Keywords: Ethanol, Gonadal Hormones, Sex Chromosomes, Four-core Genotype Mouse, Sex Differences

Disclosure: Nothing to disclose.

W180. The Histone Methyltransferase G9a Mediates Stress-Regulated Alcohol Drinking

Ethan Anderson*, Marcelo Lopez, Howard Becker, Christopher Cowan

Medical University of South Carolina, Charleston, South Carolina, United States

Background: The epigenetic modifier G9a is a histone methyltransferase that dimethylates lysine 9 on histone 3 (H3K9me2) and is decreased in the nucleus accumbens (NAc) following cocaine or morphine exposure. Mimicking this reduction of NAc G9a levels decreases stress-induced reinstatement of cocaine seeking; however, the role of G9a in alcohol use behavior is not known. We hypothesized that chronic ethanol use downregulates G9a in the NAc, and this limits stress-regulated alcohol drinking.

Methods: To test for chronic ethanol-dependent regulation of G9a expression, male C57BL/6J mice received chronic intermittent ethanol (CIE) vapor or air exposure in inhalation chambers for four weeks. Tissue was harvested 72-96h later and analyzed by western blot for G9a and Histone H3K9me2 levels. To test the role of NAc G9a on ethanol-related behaviors, adeno-associated virus (AAV) expressing a G9a shRNA or a scrambled control was infused bilaterally into the NAc of adult male mice. Separate cohorts of mice underwent experiments to test G9a's effects in NAc on voluntary ethanol intake, CIE-induced escalation of drinking, and three types of stress-regulated drinking (kappa opioid receptor agonist (U50,488)-induced pharmacological stress, predator odor exposure stress, and repeated forced-swim stress). Finally, repeated or acute systemic administration of a G9a inhibitor was tested on kappa opioid agonist-induced alcohol drinking behavior.

Results: Our findings reveal that CIE exposure decreased G9a and H3K9me2 levels in the NAc. Interestingly, reducing NAc G9a levels did not alter two-bottle choice alcohol drinking or CIE-induced escalation of alcohol drinking, but it blocked all three forms of stress-regulated alcohol drinking. Finally, we also observed that repeated, but not acute, systemic administration of a G9a inhibitor blocked stress-induced alcohol drinking.

Conclusions: These data strongly suggest that alcohol-induced reduction of G9a levels in NAc serves to limit stress-regulated alcohol drinking, and that antagonizing G9a function could be a novel therapeutic approach to treat alcohol use disorder.

Keywords: Epigenetic, Alcohol Use Disorder - Treatment, G9a, Stress Models, Kappa Agonist

Disclosure: NeuroEpigenix: LLC, Patent (Self)

W181. Evaluation of the Addiction Neuroclinical Assessment (ANA) Framework Through Deep Phenotyping of Problem Drinkers

Steven Nieto*, Erica Grodin, ReJoyce Green, Lara Ray

University of California Los Angeles, Los Angeles, California, United States

Background: To advance the development of a neuroscience-informed understanding of alcohol use disorder (AUD) through the Addictions Neuroclinical Assessment (ANA) framework, the present study reports on deep-phenotyping of a large sample of problem drinkers.

Methods: Participants ($n = 1,679$) were primarily heavy drinkers with and without AUD, who completed a phenotypic battery of well-validated scales and behavioral measures of alcohol use and problems, mood, attention, and impulsivity. These scales were subjected to sequential factor analytic work in order to derive a factor solution that explains biobehavioral variation in the sample. To assess the construct validity of the resulting factor solution, scores on each factor were associated with demographic and clinical indicators.

Results: Factor analysis techniques using indicators of alcohol use and problems, mood, attention, and impulsivity implicated

four functional domains that compliment and extend the proposed ANA domains: negative alcohol-related consequences, incentive salience, negative emotionality, and executive function. Demographic and clinical variables significantly predicted scores on all ANA domains.

Conclusions: This study provides an independent test of the recently proposed neuroscience-based ANA framework. Results largely support the novel approach in identifying four core constructs in problem drinkers. Future studies can deepen our understanding of how these domains are relevant to AUD by incorporating biomarkers.

Keywords: Alcohol and Substance Use Disorders, Addiction Phenotypes, Translational Neuroscience

Disclosure: Nothing to disclose.

W182. A Conserved Cortical Serotonergic Mechanism Underlying Binge Consumption of Alcohol and Aggression

Melanie Pina*, Thomas Kash

University of North Carolina School of Medicine, Chapel Hill, North Carolina, United States

Background: Previous findings have demonstrated that aggressive behavior is heavily modulated by central serotonin (5-HT) systems, and that heightened aggression is observed following low doses of alcohol and during acute alcohol withdrawal. A large body of work has suggested that forebrain 5-HT via action at 5-HT1A receptors may serve as a critical neural substrate underlying alcohol-induced aggression. Notably, the relationship between alcohol and aggressive behavior is complicated by the impact of heavy and protracted alcohol use on 5-HT systems, which dampens 5-HT levels and alters the expression of 5-HT receptors. While a majority of studies have focused on the prefrontal cortex as a mediator of alcohol-related aggression, recent work has identified a distinct and robust 5-HT projection from the dorsal raphe nucleus (DRN) to the orbitofrontal cortex (OFC), another site that is critically involved in aggression. Given that several lines of evidence show that repeated alcohol exposure occludes 5-HT1A-mediated inhibitory control over the OFC, this suggests a potential conserved signaling mechanism that may underlie both aggression and alcohol consumption. Here, we examine the role of the DRN and OFC 5-HT signaling in driving heavy volitional alcohol intake and aggression in mice.

Methods: We modeled binge alcohol intake using a two-bottle choice drinking in the dark (DID) procedure, where mice were given three cycles of limited access to alcohol. Following DID, whole-cell patch clamp electrophysiology was used to assess alcohol-induced plasticity in the DRN and OFC, and fluorescence in situ hybridization was used to quantify 5-HT1A receptor expression in the OFC. To test the causal role of 5-HT1A receptors in binge alcohol intake and aggression, we generated a Htr1a floxed mouse line that allows for conditional site-specific deletion of 5-HT1A in OFC pyramidal neurons via injection of AAV encoding a cre-recombinase under the control of the CAMKIIa promoter. Following viral injection into the OFC, Htr1afl/fl mice were tested for baseline aggression via the resident-intruder (R-I) task. Mice then underwent DID and were tested for alcohol-withdrawal induced aggression at 24 h following their final binge alcohol session.

Results: Repeated cycles of DID decreased the excitability of DR 5-HT neurons and increased the intrinsic excitability of OFC pyramidal neurons. In water-exposed control mice, bath application of 5-HT robustly hyperpolarized OFC pyramidal neurons. However, this effect was absent in DID mice confirming that binge-like alcohol intake impairs inhibitory 5-HT signaling in this region. Using in situ hybridization, we found that DID reduced 5-HT1A receptor expression in the OFC ($p<0.05$ DID vs water), suggesting a potential mechanism for the loss of 5-HT-induced inhibition we observed. Conditional knockout of 5-HT1A in OFC pyramidal neurons increased baseline aggression and heightened alcohol withdrawal-induced aggression. Moreover, we found that 5-HT1A deletion in the OFC increased binge-like alcohol intake ($p<0.01$ knockout vs control) and aversion (quinine)-resistant alcohol drinking ($p<0.05$ vs control) but did not impact overall fluid consumption or intake of a 2% sucrose solution.

Conclusions: Our findings indicate there is a conserved cortical 5-HT mechanism that underlies binge-like alcohol intake and aggression in mice. Specifically, we show that binge alcohol intake can dampen DRN 5-HT neuronal activity and alter 5-HT1A receptor expression and signaling. In addition to promoting compulsive and binge-like alcohol drinking, our results show that 5-HT1A receptor signaling in the OFC drives aggression at baseline and following alcohol withdrawal. Thus, this work indicates that 5-HT signaling in the OFC may be a promising target for the treatment of alcohol use disorder and pathological aggression.

Keywords: Alcohol and Substance Use Disorders, Aggression, Binge-Drinking

Disclosure: Nothing to disclose.

W183. Cell-Type Specific Encoding of Cocaine-Seeking and Refraining From Seeking in the Nucleus Accumbens Core

Reda Chalhoub*, Constanza Garcia-Keller, Rusty Nall, Jasper Heinsbroek, Ana-Clara Bobadilla, Peter Kalivas

Medical University of South Carolina, Charleston, South Carolina, United States

Background: The nucleus accumbens core (NAc) plays a central role in drug addiction. Its constituent D1- and D2- expressing GABAergic medium spiny neurons (D1- and D2-MSNs) integrate cortical and limbic inputs to regulate drug seeking behavior. However, the activity dynamics of identified MSNs, and the information encoded by these distinct subpopulations during drug seeking and refraining from seeking, remain largely elusive.

Methods: We recorded single cell $Ca2+$ dynamics in D1- and D2-MSNs of freely behaving D1- and D2-cre transgenic mice using a miniature microscope (nVista) and virally expressed Cre-dependent $Ca2+$ indicator (GCaMP6f). Mice were trained to self-administer cocaine in daily 2 hours-sessions during which a single nose-poke (FR1) resulted in an intravenous cocaine injection and presentation of drug-contingent cue. Following a brief abstinence period (7-10 days), drug seeking was assessed during a post-abstinence test (PA), wherein cues, but not cocaine, were present. Drug seeking behavior was then extinguished to the cocaine-associated context before undergoing a second cue-induced reinstatement session (RST). Calcium activity was recorded throughout representative sessions of the entire behavioral paradigm; recordings were normalized, and single cell calcium activity was isolated and aligned to behavioral responses (i.e. active and inactive nose-pokes).

Results: On a population level analysis, extinction training attenuates the overall activity of D1-MSNs (decreased firing rate), while it potentiates the activity of D2-MSNs (increased firing rate). We next examined neuronal activity around active nosepokes, our primary measure of drug seeking activity. During PA seeking test, both types of neurons exhibit both excitatory and inhibitory calcium activity immediately after drug-seeking nosepokes. Extinction training was associated with a decrease of significantly time-locked neurons around cocaine seeking event, exclusively in D1-MSNs. Furthermore, hierarchical clustering analysis was used to study the heterogeneity of MSN activity around a seeking event and revealed multiple functional clusters across both subtypes.

Conclusions: Our preliminary data show that drug seeking activity is associated with distinct heterogenous activity across

both D1- and D2- MSNs. Furthermore, extinction training is associated with degraded temporal encoding of drug-seeking events in D1-MSNs but not in D2-MSNs. Further experiments are needed to understand the distinct heterogeneous activity seen in both neuronal subtypes around seeking events and reveal encoding patterns of functional clusters. Such insights into the cocaine-seeking activity dynamics in the nucleus accumbens, a neural substrate of reward processing, can provide a foundation towards the understanding the neurobiological basis of relapse.

Keywords: Nucleus Accumbens, Medium Spiny Neuron, In Vivo Calcium Imaging, Cocaine Seeking, Extinction Learning

Disclosure: Nothing to disclose.

W184. Electrophysiological Characterization of Medium Spiny Neurons in the Nucleus Accumbens of Sign- and Goal-Trackers, a Model of Individual Variation in Incentive Learning

Cristina Maria Rios*, Geoffrey Murphy, Jonathan Morrow

University of Michigan, Ann Arbor, Michigan, United States

Background: As a central component of the "motive circuit", the nucleus accumbens (NAc) has been pivotal in understanding the mechanisms underlying cue-driven motivated behaviors. Its central involvement in these processes implies that individual variations in NAc functioning could lead to maladaptive behaviors like those associated with addiction. Vulnerability to addiction varies greatly from one individual to the next, and increased sensitivity to reward cues has been identified as a risk factor for addictive behaviors. However, linking these predisposing factors with the responsible neurobiology has been challenging. To address this, our lab utilizes a rat model of individual variation in Pavlovian learning known as the "sign- and goal-tracking model". Compared to "goal-trackers" (GTs), "sign-trackers" (STs) not only use reward cues as predictors, but also attribute them with incentive salience, resulting in increased motivation towards and fixation on these cues. As expected, STs are more susceptible to cue-induced reinstatement or "relapse" of drugs of abuse when compared to GTs, making this model suitable for studying predisposition to addiction-like behaviors. Interestingly, STs and GTs exhibit different cue- and reward-evoked patterns of activity in the NAc during Pavlovian learning, but it remains to be determined how these different NAc activity patterns arise or how they may account for the different behavioral patterns that define STs and GTs.

Methods: We explored the possibility that differences in the intrinsic neuronal properties of NAc medium spiny neurons (MSNs) within the shell and core sub-regions contribute to the ST/GT phenotype. Male Sprague Dawley rats (8-10 weeks old) underwent six daily sessions of a Pavlovian conditioning approach (PCA) procedure. Each session consisted of 25 presentations of a retractable lever (CS) that extended into the chamber for 10 seconds, and 25 deliveries of a sucrose-free banana pellet (US) into the pellet magazine. Animals were classified based on whether their conditioned responses were preferentially directed toward the lever-cue (STs) or toward the food-cup (GTs). Seven days after PCA training was completed, acute coronal brain slices were prepared and whole-cell patch-clamp recordings were performed in the NAc of STs ($n = 10$), GTs ($n = 7$), intermediate responders (IRs) ($n = 20$), and unpaired controls (UN) ($n = 20$). Passive membrane properties were measured (resting potential, capacitance, input resistance) as well as active action potential firing properties, from resting, in response to a step current injection protocol (-500 to 500pA in 25pA increments). Overall baseline excitatory synaptic input was also measured (MSNs were clamped at -80mV) by recording miniature excitatory post-synaptic currents (mEPSCs) in the presence of GABA and voltage-gated sodium channel blockers (picrotoxin and TTX).

Results: Consistent with previous literature, we found regional differences between the core and shell sub-regions. Cells in the shell exhibited lower cell capacitance (Two-way ANOVA: main effect of location: $F(1, 100) = 32.8$, $P<0.0001$) and higher input resistance (Two-way ANOVA: effect of location: $F(1, 100) = 23.3$, $P<0.0001$) when compared to those in the core. We found no significant differences between STs and GTs in either passive or active MSNs excitability properties. However, both STs and GTs exhibited decreased firing in the NAc core compared to unpaired controls (Two-way ANOVA RM: main effect of phenotype: $F(2, 55) = 3.556$, $P<0.0353$; Sidak's post hoc test: ST vs UN: $t(242) = 5.952$, $P<0.0001$; GT vs UN: $t(216) = 5.436$, $P<0.0001$) and IRs (data not included), suggesting that an extreme bias in behavior might require a distinctive lower baseline state in the NAc core. For baseline excitatory synaptic input, no significant differences were found in the shell, and as with active intrinsic properties in the core, we found no significant differences between STs and GTs, but STs (Unpaired t-test: ST vs UN: $t(25) = 2.71$, $P = 0.0120$; ST vs IR: $t(33) = 2.98$, $P = 0.0054$) and GTs (Unpaired t-test: GT vs UN: $t(24) = 2.277$, $P = 0.0320$; GT vs IR: $t(32) = 2.773$, $P = 0.0092$) had decreased mEPSC amplitude compared to unpaired controls and IRs. No differences were found in mEPSC frequency or number of events, suggesting that the synaptic transmission differences are due to receptor-mediated post-synaptic responses to excitatory transmission.

Conclusions: Our findings suggest that the ST/GT phenotype does not correlate with intrinsic passive or active excitability properties of MSNs in the NAc core and shell, nor with their overall baseline excitatory synaptic properties. Collectively, these data suggest that the different NAc activity profiles associated with STs and GTs are most likely the result of differences at the neural circuit level, involving modulation from key input regions to the NAc. Together, this work will shed light on the neurobiological basis of increased susceptibility to cue-driven psychopathologies such as addiction.

Keywords: Nucleus Accumbens, Pavlovian Conditioning, Whole-Cell Patch Clamp Recording, Intrinsic Excitability, Incentive Salience

Disclosure: Nothing to disclose.

W185. Assessment of Nucleus Accumbens Adenosine Signaling During Motivated Appetitive Behaviors

Kyle Brown*, Kayla Siletti, Michael Saddoris, Ryan Bachtell

The University of Colorado Boulder, Boulder, Colorado, United States

Background: The adenosine (Ado) neurotransmitter system is implicated in regulating motivated behaviors. Adenosine receptors are expressed densely within the striatum, including the nucleus accumbens (NAc), where they co-localize with dopamine (DA) receptors. Pharmacological manipulation of DA and Ado receptors often produce inverse behavioral effects. Ado release likely coordinates with DA and provides important contributions to reward-guided learning. Yet, limitations in our ability to measure Ado in behaving animals with precise temporal and spatial resolution have precluded our knowledge of the complex interplay between these neurotransmitter systems in the regulation of motivated appetitive behaviors.

While phasic DA release has been well-characterized in reward-based learning, investigation of the Ado system has been limited to pharmacological and genetic manipulations, which do not provide the ability to measure endogenous synaptic release of Ado within the NAc. In order to address this issue, our laboratory has developed a method to detect sub-second Ado release via fast-scan cyclic voltammetry (FSCV). In addition, we have taken advantage of a recently developed Ado fluorescent biosensor. The present experiments employ electrochemistry and fiber

photometry to compare the release of DA and Ado in vivo following evoked ventral tegmental area (VTA) activity and during behavior for Pavlovian and goal-directed sucrose seeking. These techniques may yield novel insights into endogenous Ado release during motivated appetitive behaviors.

Methods: Carbon-fiber microelectrodes were calibrated in flow cell preparation using known concentrations of Ado and DA, such that we could later convert output current to extracellular concentrations detected in vivo. The triangular voltage waveform used to oxidize and reduce DA (-0.4V to 1.3V, and back, versus Ag/AgCl) was optimized to detect adenosine (from -0.4V to 1.5V, and back, versus Ag/AgCl). The current measured on each sweep then underwent chemometric regression to determine the individual contributions of Ado and DA to the signal. We anticipated that the spatiotemporal sensitivity of FSCV would allow us to detect co-release of DA and Ado in NAc core of awake male Long-Evans ($n = 3$) rodents following electrical VTA stimulation. Release and reuptake kinetics of Ado and DA (Δcurrent (nA)/time) were compared with Student's t-tests.

We then tested whether FSCV could be used to detect behaviorally relevant DA and Ado signals in the NAc core of male Long-Evans ($n = 3$) during a task in which 2-sec cue lights predicted the insertion of a lever that could be pressed to obtain sucrose reinforcement. On certain trials, expected rewards were omitted to compare responses to positive (reinforced) and negative (omitted) reward prediction errors. Baseline subtracted changes in detected DA and Ado current (nA) during cue presentation, lever press, and reward delivery were analyzed with ANOVAs.

Finally, ongoing work is exploring the use of a genetically-encoded Ado-sensitive biosensor, AAV9-hSyn-GRABAdo, obtained from the laboratory of Yulong Li. To determine the applicability of this sensor for detecting behaviorally relevant signals, we have obtained chronic, fiber photometric recordings of NAc core activity in male and female Long-Evans rodents ($n = 6$) over multiple days of a Pavlovian conditioning task. Fiber photometric data are being analyzed in MATLAB to calculate z-score normalized ΔF/F.

Results: Optimization of the applied voltage waveform allowed us to isolate oxidation peaks of DA (+0.54V) and Ado (+1.05V) in flow cell preparation. Moreover, the current detected within the NAc core following VTA electrical stimulation demonstrated unique kinetics for these two signals. Namely, Ado release (rise velocity) ($n = 6$, $p<.01$) and reuptake (Vmax) ($p<.01$) were notably slower than those of DA.

During behavior, task-relevant activities of DA and Ado were separable. During lever press, the signals diverge, wherein DA increases ($p<.01$) and Ado decreases ($n = 3$, $p<.05$) compared to their baseline levels. During the time of expected reward delivery, NAc core DA decreased regardless of outcome ($p<.01$); intriguingly, Ado significantly increased only during reward omission ($p < .05$).

Finally, work is ongoing to validate the use of GRABAdo to detect phasic, behaviorally-relevant activity. Immunohistochemical amplification of GFP has demonstrated membrane-bound expression of the sensor in NAc neurons. Transient fluctuations in fluorescence detected in male and female rats during the Pavlovian conditioning task suggest congruence between our FSCV and fiber photometry signal. Ongoing work is being conducted to optimize and quantify event-related changes in fluorescent signal.

Conclusions: Our FSCV results suggest that we can detect and quantify phasic sub-second DA and Ado signals. Moreover, our recordings suggest that Ado plays a dissociable role from DA during appetitive behavior. In particular, the increase in Ado during unexpected outcomes suggests that Ado may correspond with prediction error updating.

Despite our successes with FSCV, this technique demonstrates notable resistance to our goal of scalable, chronic, and simultaneous recordings of DA and Ado. Given the potential of the GRABAdo sensor to provide not only chronic recordings over the course of learning but also simultaneous, multiplexed recordings with other sensors, we are continuing our work to improve GRABAdo recordings during reward-seeking behavior.

Keywords: Adenosine Signaling, Nucleus Accumbens, Motivation

Disclosure: Nothing to disclose.

W186. Cocaine Cue-Induced Mesostriatal Activation in Active Cocaine Users: Associations With Impulsivity and Prior Stimulant Drug Use

Valérie D'Amour-Horvat*, Sylvia Cox, Alain Dagher, Theodore Kolivakis, Marco Leyton

McGill University, Montreal, Canada

Background: The ability of drug-paired cues to activate mesostriatal pathways is thought to underlie their incentive motivational properties and elicit drug-seeking behaviors. Both clinical anecdotes and studies in laboratory animals suggest that drug ingestion can augment these appetitive effects, especially in highly impulsive individuals. Here, we tested these hypotheses in humans using functional magnetic resonance imaging (fMRI) in active cocaine users and cocaine-naïve healthy volunteers.

Methods: Fourteen active cocaine users (mean age = 25.07, SD = 2.97; 11M/3F) and ten healthy volunteers (mean age = 29.05, SD = 5.70; 5M/5F) underwent two separate fMRI sessions one hour after the administration of placebo and dextroamphetamine (0.3 mg/kg, p.o.) respectively, in a fixed order. During each scanning session, participants were exposed to two functional runs with 30-second cocaine ($n = 12$) and neutral videos ($n = 10$). Parameter estimates for the contrast "cocaine cues – neutral cues" were extracted from a priori selected regions of interest (ROIs) including limbic (LS), associative (AS) and sensorimotor (SMTS) striatum, and midbrain. All participants completed the Barratt Impulsiveness Scale (BIS-11) and the Substance Use Risk Profile Scale (SURPS). Cocaine wanting was measured with visual analog scales at baseline and after each cue presentation.

Results: Craving: Compared to the healthy controls, the cocaine users reported higher cocaine wanting scores both at baseline ($p = 0.005$) and in response to the cocaine cues ($p < 0.001$). Among the cocaine users only, the wanting scores increased from the first to second run of drug cue exposures ($p = 0.001$). Amphetamine administration was associated with greater absolute cocaine wanting scores in the cocaine users, but not greater drug cue-induced increases relative to the session's elevated baseline ($p = 0.777$). Brain activations: Across sessions, exposure to cocaine cues minus neutral cues induced larger fMRI BOLD responses in the cocaine users compared to healthy controls in the AS ($p = 0.019$), LS ($p = 0.057$) and midbrain ($p = 0.015$), with the latter effect specific to the second functional run. The effect of amphetamine was limited to cocaine cue-induced responses in the SMTS: on this session, cocaine cue-induced BOLD responses increased from the first to the second run in cocaine users ($p = 0.025$), but not in healthy controls or in either group on the placebo session. Finally, in the cocaine users, greater cocaine cue-induced responses in the AS were correlated with higher BIS-11 Nonplanning scores ($r = 0.55$, $p = 0.042$) and lower SURPS Anxiety Sensitivity Scores ($r = -0.54$, $p = 0.044$), and activations in all three striatal subregions correlated with their lifetime use of stimulant drugs (AS: $rs = 0.65$, $p = 0.012$; LS: $rs = 0.59$, $p = 0.027$; SMTS: $rs = 0.57$, $p = 0.034$). A similar trend with lifetime stimulant use was seen on the amphetamine session (AS: $rs = 0.47$, $p = 0.088$).

Conclusions: The modest sample size noted, the present results suggest that impulsive cocaine users have larger striatal responses to cocaine-related cues. With repeated drug use, these responses

become larger and, once a stimulant has been ingested, particularly resistant to habituation. Together, these effects might increase the risk for stimulant drug use problems.

Keywords: Drug Cues, fMRI, Incentive Salience, Impulsivity, Substance Use Disorders

Disclosure: Nothing to disclose.

W187. Corticotropin-Releasing Factor Receptor Type 1 Signaling in the Basolateral Amygdala Facilitates Context-Cocaine Memory Reconsolidation in Male and Female Rats

Jobe Ritchie, Jennifer Walters, Justine Galliou, Christopher Ibarra, Shayna Grogan, Rita Fuchs*

Washington State University, Pullman, Washington, United States

Background: Exposure to drug-associated environmental stimuli results in the retrieval of drug-associated memories that can elicit drug craving and relapse in cocaine users. Upon retrieval, drug-associated memories become destabilized and require memory reconsolidation to maintain their strength over time. Thus, interference with memory reconsolidation may weaken drug-associated memories and promote drug abstinence. The basolateral amygdala (BLA) is a critical brain region for cocaine-memory reconsolidation. Corticotropin-releasing factor receptor type 1 (CRFR1) is densely expressed in the BLA, and CRFR1 stimulation activates intra-cellular signaling cascades that are necessary for memory reconsolidation. Hence, we tested the hypothesis that BLA CRFR1 stimulation is requisite for cocaine-memory reconsolidation.

Methods: Using an instrumental rat model of drug relapse, male (N = 47) and female (N = 43) Sprague-Dawley rats were trained to self-administer cocaine intravenously in a distinct environmental context during 10 daily 2-hour sessions. This was followed by extinction training in a distinctly different context over 7 daily 2-hour sessions. Next, the rats were re-exposed to the cocaine-paired context for 15 minutes, to retrieve and destabilize cocaine memories and trigger reconsolidation. Immediately or 6 hours after memory retrieval (i.e., during or after memory reconsolidation, respectively), the rats (n = 12-14/group) received bilateral microinfusions of antalarmin (CRFR1 antagonist; 500 ng/hemisphere), corticotropin-releasing factor (CRF; 30 or 500 ng/hemisphere), or dimethyl sulfoxide vehicle (100%, 0.5 µL/hemisphere), into the BLA. Twenty-four hours later, daily extinction training sessions resumed in the extinction context and were followed by a single test of cocaine-seeking behavior in the cocaine-paired context (index of cocaine-memory strength) on the third day post treatment. Potential off-target effects of the manipulations on extinction memories and locomotor activity were also evaluated. Cocaine intake, lever responses, and locomotor activity data were analyzed using analyses of variance (ANOVA) with sex, treatment, testing context, and time as factors, where appropriated. Post-hoc comparisons were conducted using Sidak's or Tukey's post-hoc tests. Alpha was set at 0.05 for all analyses.

Results: Females responded more than males during cocaine self-administration training (ANOVA sex main effect, $p = 0.0006$) and extinction training (ANOVA sex x time interaction, $p = 0.002$); however, the effects of the CRFR1 manipulations on cocaine-memory reconsolidation were not sex specific (all ANOVA sex main and interaction effects, $p > 0.05$). Antalarmin administered into the BLA immediately after cocaine-memory reactivation (i.e., during memory reconsolidation), but not six hours later (i.e., after memory reconsolidation), attenuated lever responding in the cocaine-paired context three days later, with no effects on responding in the extinction context, relative to vehicle (ANOVA treatment x context interaction, $p = 0.008$). CRF administration into the BLA immediately after cocaine-memory reactivation had similar effects on lever responding at test (ANOVA treatment x context interaction, $p = 0.01$). Furthermore, neither antalarmin nor CRF altered locomotor activity three days after administration (ANOVA treatment main and treatment x time interaction, $p > 0.58$).

Conclusions: Antalarmin administration into the BLA immediately after cocaine-memory reactivation reduced subsequent cocaine-seeking behavior in a memory reactivation-dependent manner, suggesting that it interfered with the reconsolidation of cocaine memories or reduced cocaine-memory strength. CRF administration into the BLA immediately after cocaine-memory reactivation reduced subsequent cocaine-seeking behavior without altering locomotor activity following the same treatment-to-test interval. Thus, CRF either impaired cocaine-memory strength by triggering rapid CRFR1 internalization during cocaine memory reconsolidation or it elicited a protracted effect on motivation to seek cocaine. Ongoing research will assess whether the effects of CRF are dependent on memory reactivation, consistent with the former possibility. Overall, our findings indicate that CRFR1 signaling in the BLA facilitates cocaine-memory strength during reconsolidation and may be of interest as a target for anti-relapse treatment strategies.

Keywords: Cocaine, Self-Administration, Memory Reconsolidation, Amygdala, Corticotropin-Releasing Factor (CRF)

Disclosure: Supernus Pharmaceuticals Inc.: Consultant (Self)

W188. Female Rats are More Susceptible to High-Risk Addictive Behaviors Than Male Rats

Timothy O'Neal*, Zackari Murphy, Derek MacDougall, Susan Ferguson

University of Washington, Seattle, Washington, United States

Background: Opioid addiction is a chronic, relapsing disorder, characterized by bouts of drug taking and drug seeking. The ongoing opioid epidemic in the United States has resulted in nearly half a million deaths since 1999 (NCHS, National Vital Statistics System, Mortality), and overdose-related deaths during the early months of COVID-19 increased more than 40% from the same time in 2019 (ODMAP). Although men have historically been more likely to die of a fatal opioid overdose, the death rate has been rising faster in women than men, though whether this is due to longer-term transitions from prescription opioids to highly potent opioids (e.g., fentanyl) or innate differences in vulnerability to the addictive properties of opioids is unknown. Of note, reports suggest women may escalate drug intake at a faster rate and be more prone to relapse than men. Importantly, however, only a subset of individuals transitions from casual drug use to addiction, so whether women are generally more susceptible to addiction or whether a greater subset of women progress to addiction is unknown. The historical exclusion of female subjects from preclinical research has contributed to the incomplete understanding of sex differences in addiction; moreover, recent efforts to begin studying sex differences have focused almost exclusively on psychostimulant addiction. Addiction develops in part from disruptions within the cortico-basal ganglia circuit (C-BG), a network integral for learning, decision-making, and motivation. However, whether these disruptions differ between females and males and contribute to differences in addictive behaviors remains to be studied.

Methods: To examine the impact of sex differences on vulnerability to opioid addiction, separate groups of female and male Sprague-Dawley rats were tested in classical addiction models. In experiment 1, rats (n=54) were trained to self-administer heroin (0.075mg/kg per infusion) using an intermittent-access procedure that produces variability in addiction severity and allows extraction of six notable addictive behaviors: drug intake, intake consistency, drug seeking, motivation, extinction, and cue-induced-reinstatement. Individual

addictive behaviors were combined into an overall severity score that was used to classify individual subjects as low-risk or high-risk for addiction. In experiment 2, rats (n=34) were trained on a heroin conditioned place preference (CPP) procedure to assess sensitivity to heroin reward. After an initial day of exploration (15min), rats received four days of conditioning, with saline in the morning and heroin (1mg/kg or 3mg/kg) in the afternoon. The following day, rats were again allowed to freely explore the box to assess preference for the two contexts. In experiment 3, rats (n=32) underwent locomotor sensitization to assess sensitivity to the sensitizing effects of heroin. After three days of habituation (30min, saline), rats received nine days of sensitization (30min, 2mg/kg heroin) in chambers equipped with infrared beam-breaks to monitor locomotor activity. Immediately after completion of behavioral testing, brains were extracted and processed for cFos immunohistochemistry (experiment 1: cue-induced reinstatement; experiment 2: post-test) in key nuclei of the C-BG.

Results: Intermittent-access heroin self-administration produced subsets of low-risk and high-risk rats that significantly differed in daily heroin intake, total heroin consumption, drug seeking, motivation, extinction, and cue-induced reinstatement (all $p < 0.001$), though female and male rats within each subset did not differ in any of the six behaviors (all $p > 0.05$). However, female rats were significantly more likely to be classified as high-risk than male rats ($p < 0.05$), suggesting greater vulnerability to the development of heroin taking and heroin seeking behaviors in females. Conversely, females and males did not differ in their development of heroin CPP at either dose tested, nor did they differ in development of heroin sensitization (all $p < 0.05$). Interestingly, cFos immunohistochemistry revealed divergent activation in the nucleus accumbens and the prefrontal cortex between females and males, suggesting a sex difference in the encoding of heroin cues.

Conclusions: Epidemiological research has shown women to be more vulnerable to the negative consequences of opioid addiction, yet preclinical research has focused almost exclusively on male subjects for decades. The data presented here demonstrates increased vulnerability to high-risk addictive behaviors in female rats as well as non-overlapping patterns of neuronal activation following presentation of drug-paired cues and contexts. Experiments are currently underway to further explore sex differences in drug- and cue-encoding by the C-BG, including fiber photometry recordings from the nucleus accumbens during heroin CPP and proteomic analysis of nucleus accumbens synaptosomes following heroin sensitization. Together, these studies will clarify how C-BG network dynamics shift during the development of heroin addictive behaviors and will explore whether the encoding of heroin cues follows divergent pathways in females and males.

Keywords: Opioid Addiction, Nucleus Accumbens, Sex Differences, Heroin

Disclosure: Nothing to disclose.

W189. Hierarchical Control of Mesolimbic Dopamine and Striatal Encoding of Reward-Paired Cues Governs Behavioral Flexibility

Kurt Fraser*, Val Collins, Fiona Pat, Patricia Janak, Benjamin Saunders

Johns Hopkins University, Baltimore, Maryland, United States

Background: The ability to resolve uncertainty surrounding reward-associated cues is essential for the proper organization and generation of reward-seeking. Dopamine neurons are reported to be sensitive to the likelihood of reinforcement yet how dopamine neurons, dopamine release, and the activity of their striatal targets are related to the coding and resolution of uncertainty is unclear. Here we make use of pharmacology, in vivo electrophysiology, calcium imaging, and optogenetics to determine the contributions of the mesolimbic dopamine system to the hierarchical organization of reward-seeking.

Methods: Long-evans rats (n=56) were trained to discriminate when a conditioned stimulus would be followed by sucrose reward by exploiting the prior and non-overlapping presentation of a separate discrete stimulus, an occasion setter. Only when the occasion setter's presentation preceded the conditioned stimulus did the conditioned stimulus predict sucrose delivery.

Results: We found that either reversible inactivation or dopamine antagonism within the nucleus accumbens prevented rats from properly estimating when the conditioned stimulus would be rewarded. We recorded single neurons within the nucleus accumbens (n=295) and observed that the magnitude of conditioned-stimulus evoked inhibition was greater when the conditioned stimulus would be followed by reward, than when it would not. Moreover, we observed a population of neurons in the nucleus accumbens that dynamically altered their firing to the conditioned stimulus, being excited when this cue was not predictive and inhibited when it predicted reward. We monitored dopamine release in the nucleus accumbens making use of fiber photometry and the fluorescent dopamine sensor dLight 1.3b. Dopamine release tracked the presentation of the occasion setter and dynamically scaled with the predictability of the conditioned stimulus predicated on the prior presence or absence of the occasion setting cue. In preliminary experiments we find that optogenetic stimulation of dopamine neurons in the ventral tegmental area can substitute for an occasion setter.

Conclusions: Together these results reveal a mechanism dopamine release in the nucleus accumbens to dynamically control striatal encoding of ambiguous reward-predictive cues and appropriately generate reward-seeking.

Keywords: Incentive Motivation, Dopamine, Nucleus Accumbens, Uncertainty, Behavioral Flexibility

Disclosure: Nothing to disclose.

W190. The Rostral Intralaminar Thalamus Regulates Striatal Pathway Output for Action Reinforcement

Kara Cover, Abby Lieberman, Brian Mathur*

University of Maryland School of Medicine, Baltimore, Maryland, United States

Background: The dorsal striatum (DS) mediates the selection of actions for reward acquisition necessary for survival. Striatal pathology contributes to several neuropsychiatric conditions, including aberrant selection of actions for specific rewards in addiction. A major source of glutamate driving striatal activity is the rostral intralaminar nuclei of the thalamus (rILN). Yet, the information that is carted to the striatum to support action selection is unknown.

Methods: Here we fiber photometrically monitor and optogenetically manipulate the rILN-to-DS projection in mice performing an action sequence task reinforced by sucrose reward. We found that the rILN-to-DS projection stably signals at two time points: action initiation and reward acquisition.

Results: Optogenetic activation of this pathway increased the number of successful trials while optogenetic inhibition slowed action initiation and decreased the number of successful trials.

Conclusions: Together, these data demonstrate that the selection of an action requires a dual, and critical, rILN-to-DS signal for action initiation and the reward achieved by that action. Given this role, the rILN-to-DS pathway represents a node for pathological disruption contributing to action dysfunction in myriad neuropsychiatric illnesses.

Keywords: Addiction Circuitry, Long-Term Depression, Basal Ganglia, Neuronal Tracing, Circuit Optogenetics

Disclosure: Nothing to disclose.

W191. Impaired Emotion Regulation as a Transdiagnostic Domain: The Capacity to Effectively Cope With Spoken Negative Personal Critiques

Harold Koenigsberg*, Kurt Schulz, Jin Fan, Maria Lopez, Bryan Denny, Hannah Lindenmeyer, M. Mercedes Perez-Rodriguez, Erin Hazlett, Antonia New, Daniel Rosell

Icahn School of Medicine at Mount Sinai, Bronx, New York, United States

Background: Emotional instability, the vulnerability to intense emotional reactions to events of daily life, is a seriously maladaptive and destabilizing feature of diverse psychiatric disorders. It leads to unstable, even tumultuous, interpersonal relationships and the inability to maintain consistent life goals and values. It is often accompanied by depression, anger, mood oscillation and threats of self-harm. We have shown that in borderline personality disorder (BPD), emotional instability is associated with an inadequate capacity to engage the highly adaptive emotion regulatory process of cognitive reappraisal and a concurrent failure to adequately recruit pre-frontal cortical regions known to be implicated in reappraisal (Koenigsberg et al, Bio Psych 2009). Little work has been done, however, to examine the extent to which impairments in the capacity to engage reappraisal are associated with emotional instability across diagnoses. In addition, studies of cognitive reappraisal have been seriously limited by use of emotional probes of limited ecological validity (emotional pictures or scripts). In the present study we test whether faulty cognitive reappraisal is a transdiagnostically relevant domain and exploit negative spoken personal comments as a more ecologically valid emotional stimulus.

Methods: We present data on a preliminary sample of unmedicated subjects recruited independent of diagnosis (excluding psychotic, neurological and substance use disorders), covering a wide range of affective instability. Subjects listened to standardized emotionally negative and neutral comments spoken by a contemporary and were instructed either to downregulate their negative reactions by reappraising-by-distancing or to simply listen and experience the feeling evoked by the sentences as 3T BOLD images were obtained. Following each sentence, subjects rated their affect on a -4 to +4 scale.

Results: The sample (n = 39) spanned a wide range of emotional instability (Affective Lability Scale (ALS) scores ranged from 0 to 106 (mean 44.0 ± 29.2)). Whole brain analyses demonstrated that the degree to which subjects manifested emotional instability was inversely correlated with reappraisal-related activation in the R precuneus (x=14, y = -58,z=46, p<0.001, k= 1318 voxels), R dorsolateral prefrontal cortex (DLPFC) (x=36, y=36,z=22, p,0.001, k=287 voxels), and L ventrolateral prefrontal cortex (VLPFC) (x=34, y=52, z=18, p<0.001, k=99 voxels), regions known to be engaged during cognitive reappraisal.

Conclusions: Our examination of neural processing during cognitive reappraisal-by-distancing demonstrates that, across diagnoses, lower levels of emotional instability were associated with a greater capacity to engage neural regions known to implement cognitive reappraisal. In addition to its transdiagnostic perspective, a particular strength of this study is its use of a novel ecologically valid spoken word emotional probe.

Keywords: Emotion Regulation, Human Neuroimaging, Transdiagnostic

Disclosure: Nothing to disclose.

W192. Neural Correlates Underlying Directed Exploration in Humans Revealed via Model-Based fMRI

Jeremy Hogeveen*, Teagan Mullins, John Romero, Elizabeth Eversole, Vincent Costa

University of New Mexico, Albuquerque, New Mexico, United States

Background: To optimize decision making in an uncertain world, it is often necessary to test novel and unfamiliar actions at the expense of the foregone value of familiar actions. Managing these competing demands is known as the 'explore-exploit' tradeoff in reinforcement learning, and it has been argued that aberrant exploratory behavior represents a powerful framework for characterizing pathological decision making in psychiatry (Addicott et al, 2017, NPP). Humans are thought to engage in exploration through two distinct strategies: directed exploration to seek information about the environment, versus random exploration when decision noise is high. Recent studies using targeted recordings from amygdala, ventral striatum, and orbitofrontal cortex have demonstrated encoding of the value computations that facilitate directed exploration in nonhuman primates. These results complement evidence in humans that frontopolar cortex biases the use of directed versus random exploration when making decisions under uncertainty. Here, we translated the same task used to study novelty seeking as a form of directed exploration in nonhuman primates to a brain-wide analysis of directed exploration in humans using model-based fMRI.

Methods: We used computational model-based decomposition of functional MRI during a 3-armed novelty bandit reinforcement learning paradigm in humans (N=38; N=24 female; M=26.7 years). Participants made speeded choices between three neutral images which were rewarded at a low (p=0.2), medium (p=0.5), or high (p=0.8) probability. Occasionally, a novel stimulus insertion trial occurred: a novel image with a randomly assigned reward probability was inserted in lieu of one of the existing options. In the immediate trials after each novel insertion, participants faced the difficult decision to either exploit the most valuable familiar option, or explore the novel option to learn its reward probability. An optimal decision policy was derived using a Partially-Observed Markov Decision Process model (POMDP). In the POMDP, the action value of each option is determined by the sum of its immediate expected value (IEV) and future expected value (FEV). IEV represents an estimate of the likelihood that a given option will be rewarded based on prior decision feedback, whereas FEV reflects the sum of potential rewards to be earned in the future. Critically, discrete decisions to engage in directed exploration are thought to be primarily shaped by an information bonus that can be derived by comparing the relative FEV associated with choosing a particular option. We used the IEV, FEV, and BONUS parameters to model behavioral task performance, as well as trial-by-trial fluctuations in fMRI activation at the time of choice.

Results: Observed explore-exploit behavior was well-modeled by the IEV and BONUS parameters, suggesting that human decision-making profiles closely aligned with POMDP-derived normative performance. These decision making profiles appeared to be relatively stable traits, as a subset of our participants (N=29) completed the task twice enabling us to establish the test-retest reliability of both IEV (a=0.85) and BONUS (a=0.75) parameters. POMDP-based analysis of the fMRI data suggested that IEV was encoded in dorsal anterior cingulate cortex and dorsolateral prefrontal cortex, whereas dorsolateral frontopolar cortex and precuneus regions encoded the FEV of the reward environment. Most importantly, BONUS was encoded in amygdala, striatum, and ventrolateral frontopolar cortex, replicating prior findings in humans and monkeys and indicating that subcortical motivational circuits regulate directed exploration in both humans and

nonhuman primates. Analysis of choice event-related activation within these POMDP-defined regions-of-interest found that dorsolateral frontopolar cortex regions that encode FEV demonstrated decreased activation during exploratory relative to exploitative choices. In contrast, the left ventrolateral frontopolar cluster that encoded BONUS demonstrated increased activation during exploratory relative to exploitative decision events.

Conclusions: Prior neuroimaging studies of explore-exploit decision making in humans have often involved drifting options to maintain high levels of uncertainty, likely assaying random exploration. Here, by making exploration explicit through periodic novel stimulus insertions, we were able to probe the neural correlates of directed exploration for the first time in human subjects, translating a paradigm with established efficacy for investigating the neurophysiology of directed exploration in nonhuman primates. The current data suggests that ventrolateral frontopolar cortex and subcortical circuits directly encode the latent BONUS signals that motivate directed exploration. In contrast, dorsolateral frontopolar cortex encodes the richness of the current reward environment, and given its deactivation during exploration, may play a role in encoding the foregone value of counterfactual choice options during exploration. Such prefrontal systems for encoding both the anticipated consequences of a selected choice option–as well as the anticipated consequences of unselected choice options–would be critical in enabling primates to flexibly update and regulate sophisticated decision strategies, and may underlie our remarkable ability to defer goals in order to explore alternatives.

Keywords: Explore-Exploit Dilemma, Functional MRI (fMRI), Reinforcement Learning, Decision Making, Prefrontal Cortex

Disclosure: Nothing to disclose.

W193. Mapping the Heterogeneous Functions for DACC in Effort-Based Decision-Making Using Computational and Resting State fMRI

Amanda Arulpragasam*, Shabnam Hossein, Kristi Kwok, Jessica Cooper, Michael Treadway

Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire, United States

Background: Effort-based decision-making (EBDM) has emerged as an important translational paradigm for studying motivational deficits in psychiatric populations. While a number of preclinical and clinical studies have identified the dorsal anterior cingulate cortex (dACC) as critical for EBDM, the specific computations performed by this region remain unclear. Prior work suggests that dACC may be encoding the subjective value of a given option, the need to alter an existing strategy (e.g., switching from one option to another), or the difficulty of choosing between two similarly valued options. Importantly, these processes are often highly collinear in many decision-making paradigms, making it difficult to determine which may be most responsible for dACC activity. Determining the specific role for dACC in the context of EBDM is a critical step towards a greater understanding of dACC abnormalities in clinical populations.

Methods: Here, we present data from three studies in a total sample of 58 healthy controls (24 males, Mage = 23.4, SDage = 4.5). Participants completed one of three variants of a sequential effort-based decision-making paradigm while undergoing functional magnetic resonance imaging (fMRI). Primary analyses centered on data from a modified EBDM task specifically designed to isolate neural signals encoding choice difficulty, subjective value, and strategy shifting behavior so as to determine the precise computations performed by dACC subregions during EBDM. This was achieved through a hierarchical trial structure that alternated semi-structured blocks of trials with either ascending and descending reward values that converged on an individual's indifference point. Resting state functional connectivity (rsFC) was also assessed.

Results: Across all three studies our results suggested that when subjective value and choice difficulty are experimentally dissociated, neighboring subregions of dACC responded to choice difficulty ($x = 0$, $y = 26$, $z = 38$, $t = 6.17$, cluster pFWE <0.001) and prediction error ($x = 2$, $y = 26$, $z = 40$, $t = 4.29$, cluster pFWE =0.001). Moreover, our results suggest that previously reported associations between dACC and subjective value are likely driven by the common collinearity between subjective value and choice difficulty, rather than a discrete subjective value computation performed by dACC. Resting state functional connectivity using these two functionally defined dACC seeds revealed distinct patterns of connectivity between these two neighboring seed regions. This finding was replicated in a larger independent sample.

Conclusions: While the dACC has been found to signal subjective value, prediction error and choice difficulty during EBDM, previous studies have failed to adequately disentangle these variables. The current multi-study data suggest that when such decision-making variables are experimentally dissociated, neighboring sub-regions of dACC respond primarily to choice difficulty and expectation violation, but not subjective value. Importantly, we observed that expectation violations frequently occurred in preparation of a behavioral strategy alteration (e.g. switching from choosing high effort to no effort), suggesting that the context of the decision signals a distinct choice/action process. Despite the spatial overlap of these neighboring subregions, rsFC provides evidence that these regions are distinct and contribute to different neural networks. Taken together, these results may prove critical in furthering our understanding of disrupted decision-making observed in psychiatric disorders and informing target selection for treatment studies of motivational impairment.

Keywords: Decision Making, Effort Based Decision Making Task, Human Neuroimaging, Anterior Cingulate Cortex (ACC), Goal-Directed Behaviors

Disclosure: Nothing to disclose.

W194. Embedded Temporal Patterns in the Feedback Signal Differentially Predict VTA Neurofeedback-Mediated Learning to Self-Regulate Motivation

Shabnam Hakimi*, Jeffrey J. MacInnes, Kathryn C. Dickerson, Kelsey McDonald, R. Alison Adcock

Center for Cognitive Neuroscience, Duke University, Durham, North Carolina, United States

Background: The ventral tegmental area (VTA) and its dopaminergic projections are central to volitional behavior. We previously demonstrated that individuals can learn to use motivational imagery to self-activate the VTA only after training with veridical VTA neurofeedback. Individuals who received random noise feedback or neurofeedback from another mesolimbic region, the nucleus accumbens (NAc), did not demonstrate learning success. The mechanism of VTA-mediated learning to self-regulate motivation is unknown. Here, we investigate how the temporal structure of neurofeedback training predicts transfer, as evidenced by VTA self-activation achieved in the absence of feedback.

Methods: In a one-session micro-intervention, participants were trained to use self-generated motivational imagery to volitionally upregulate neural response. Briefly, the participants completed a pre-test, three training runs, and a post-test. During training, participants received real-time fMRI neurofeedback from the VTA via a graphical thermometer updated every 1 s (N=19) over a total of 15 20-s trials (three per run). Another group (N=20) did the same task, but received feedback from the NAc. The analytical

approach was blind to the strategies used, instead focusing on the impact of the learning context. We first extracted mean VTA response during training (i.e., feedback signal) at the single-participant level. Next, we used both parametric and non-parametric (Gaussian process) regression models to parameterize the slope of feedback time series in three temporal contexts: individual trial, scanner run, and full training session, producing two sets of parameters reflecting learning during each context. This approach allowed us to test how assumptions about the evolution of the learning process impact prediction of transfer. Outliers were defined as parameter estimates greater than two standard deviations from the mean and were excluded. We then used mixed effects models to examine, at the group level, how well the parameters for each context predicted training transfer, as indicated by the change in average VTA or NAc activation from pre- to post-test.

Results: The slope of VTA activation change over the entire course of training was significantly associated with transfer only in the VTA feedback group ($p<0.01$); no other temporal context predicted transfer or differentiated the feedback groups. Further, the parametric approach was favorable for transfer prediction relative to the non-parametric approach (adjusted R2, parametric > non-parametric: 0.126 > 0.0718).

Conclusions: These data suggest that, regardless of strategy, successful learning from VTA neurofeedback is most associated with the training context as a whole, with longer-term linear trends also explaining feedback group differences in learning. Relative to non-parametric estimates, parametric estimates of feedback change over time were less noisy and more reliably predicted transfer learning, suggesting that the assumption that learning is linear in time is appropriate for modeling neurofeedback learning in most cases. Future work will further characterize relative contributions of the three temporal contexts in predicting transfer.

Keywords: Real-Time fMRI Neurofeedback, Motivation, Self-Regulation, Learning and Memory, Mesolimbic Reward Circuitry

Disclosure: Nothing to disclose.

W195. Unpacking "Concentration Difficulties": Early Life Stress Mediates the Association Between Anxiety and Impairments of Selective and Divided Attention With Specific Neural Circuit and Neurophysiological Correlates

Arielle Keller*, Bailey Holt-Gosselin*, Ruth Ling*, Leanne Williams

Stanford University School of Medicine, Palo Alto, California, United States

Background: Paying attention to important information is essential for achieving our goals. Although 'concentration difficulties' are a common symptom and diagnostic criterion across many psychiatric disorders, we do not understand its neurobiological basis or the impact of environmental stressors. Leveraging statistical methods and recent findings from cognitive neuroscience, we sought to characterize signatures of impairments in goal-directed attention subtypes in a transdiagnostic sample using measures of behavior, self-report, neural circuit, and neurophysiology.

Methods: We recruited n=35 participants with depression and anxiety symptoms and n=21 healthy controls. Sub-constructs of attention (FSA: feature-based selective attention, SSA: spatial selective attention, TDA: divided attention among tasks, SDA: divided attention among stimuli) were assessed using behavioral tasks during fMRI and EEG recording. Symptoms were assessed using the Depression and Anxiety Stress Scales (Lovibond & Lovibond, 1995) and the Barratt Impulsiveness Scale (Patton et al., 1995). We evaluated relationships among behavior, self-reported symptom, neural circuit and neurophysiological data using linear and linear mixed-effects models. We also tested whether early life stressors mediate the association between attention impairments and self-reported symptoms, co-varying for age and biological sex.

Results: Slower reaction times on the selective and divided attention tasks was associated with higher anxiety severity (FSA: $r(56)=-0.235$, $p_{trend}=.08$; SSA: $r(56)=0.339$, $p=.010$; SDA: $r(56)=0.343$, $p=.011$), while poorer feature-based selective attention accuracy was associated with self-reported concentration difficulties ($t(54) = 2.313$, $p=.025$, Cohen's $d=.658$). As hypothesized, poorer accuracy and reaction times with feature-based selective attention to object categories (faces, houses, bodies, and words) was associated with reduced intrinsic functional connectivity between the right hemisphere fronto-parietal network and object-selective regions of ventral temporal cortex ($\beta=-0.066$, $SE=.019$, $p<.001$) as well as lower induced posterior alpha power in the EEG ($\beta=-0.174$, $SE=0.008$, $p=.046$). Also confirming our hypothesis, slower divided attention reaction times were characterized by lower fronto-central theta power (TDA: $\beta=-0.043$, $SE=0.018$, $p=.028$; SDA: $\beta=-0.03$, $SE=.015$, $p_{trend}=.056$). The total number of early life stressors reported by each participant was found to fully mediate the relationship between anxiety symptoms and task performance on the FSA, SSA and SDA tasks ($p's < .05$) after accounting for age and biological sex.

Conclusions: By leveraging tools and theories from cognitive neuroscience, we have shown that concentration difficulties in anxiety may be parsed into specific deficits on sub-types of goal-directed attention with distinct behavior, self-report, neural circuit, and neurophysiological signatures. We have additionally identified early life stress as a potential mediator of the relationship between anxiety and attention impairments.

Keywords: Attention, Anxiety, Functional MRI (fMRI), EEG/ERP Electrophysiology, Early Life Stress

Disclosure: Nothing to disclose.

W196. Post-Error Recruitment of Frontal-Sensory Cortical Projections Promotes Attention in Mice

Kevin Norman*, Hiroyuki Koike, Justin Riceberg, Julia Bateh, Sarah Lopez, Keaven Caro, Ana Liang, Kazuhiko Yamamuro, Meghan Flanigan, Yury Garkun, Elisa Nabel, Daniel Brady, Christina Cho, Masato Sadahiro, Kohei Yoshitake, Priscilla Maccario, Michael Demars, Scott Russo, Mark Baxter, Matthew Shapiro, Peter Rudebeck, Hirofumi Morishita

Icahn School of Medicine at Mount Sinai, New York, New York, United States

Background: Flexible goal-directed behavior, such as attention, requires a cognitive control system which not only monitors contextually relevant internal states and external events, but also implements strategic adjustment in informational processing and behavior. Across species, attention is regulated by direct projections from the prefrontal cortex to sensory areas. Here, we aim to identify the specific conditions that recruit frontal-sensory projections from the anterior cingulate area to the visual cortex (ACAvis) to causally influence attention behavior in mice.

Methods: We integrated circuit-based techniques, fiber photometry and optogenetics, to monitor and manipulate ACAvis neural activity in male mice performing freely moving attention behavior during the 5-choice serial reaction time task (5CSRTT) with a translational automated touchscreen system. The 5CSRTT requires mice to sustain and divide their attention across five response windows during a 5sec delay in anticipation of the random presentation of brief stimulus at one of the five locations. Male and female mice show similar 5CSRTT performance. All animal protocols were approved by the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount Sinai.

Results: First, we sought to characterize the time course and circumstances of ACAvis neuron recruitment during attention. Unexpectedly, ACAvis neuron activity did not vary depending on whether the current trial was a correct choice, when attention was properly allocated, or an error (incorrect choice or omission, two-way RM ANOVA, F1,7 =3.592, P=0.0999, n=8 mice). Instead, ACAvis neuron recruitment depended on the result of the previous trial as ACAvis neurons demonstrated elevated activity during both the intertrial interval delay (ITI) and when the stimulus was presented on the trial immediately following error trials compared to correct trials (two-way RM ANOVA, F1,7 =2.561, *P=0.0244, Holm-Sidak multiple comparisons at ITI and stimulus period, ***P<0.001, ***P<0.001, n=8 mice), suggesting that ACAvis neurons encode an error monitoring signal that persists after an error occurs. In order to determine whether overall ACAvis activity correlated with improved attention after an error was made, we further classified trials into four groups based on the combined outcome of the previous and current trial. While there was a significant difference among trial combinations (two-way RM ANOVA, F3,21 =7.632, **P=0.0012, n=8 mice), there was no significant difference in ACAvis activity between correct trials after an error trial compared to consecutive error trials.

As the summation of ACAvis neuron activity alone cannot fully explain error correction behavior, we examined whether specific patterns of ACAvis neuron activity contribute to post-error performance. We then performed circuit-selective optogenetic modulation of channelrhodopsin-expressing ACAvis neurons while mice performed the 5CSRTT. Delivering 30Hz stimulation prior to stimulus presentation significantly improved 5CSRTT performance on trials that followed an error (general linear mixed model, effect of treatment (light on vs. off), P=0.6467, effect of frequency (5 vs. 30HZ), P = 0.0016, planned Tukey post hoc tests: 5Hz:C+1 light off vs on, P=1.000, E+1 light off vs on, P=0.1963, 30Hz: C+1 light off vs on, P=1.000, E+1 light off vs on, *P=0.01410). If the previous trial was correct, however, 30Hz stimulation did not affect performance (general linear mixed model, effect of treatment (light on vs. off), P = 0.5546, effect of frequency (5 vs. 30Hz, P = 0.1779). Overall, these findings demonstrate the frequency-specific effect of ACAVIS activity prior to stimulus presentation in improving attention, but only after error trials.

We then aimed to dissect the temporal requirement of ACAvis projections during error correction behavior in the 5CSRTT independently of local ACA activity. We performed projection-selective optogenetic suppression of ACAvis axonal terminals in visual cortex at key time points during 5CSRTT using the inhibitory opsin halorhodopsin. ACAvis projection inhibition during the 3sec immediately prior to stimulus presentation (-3:0sec) impaired attention (general linear mixed model, effect of treatment (light on vs. off), **P = 0.0032, planned Tukey post hoc tests: C+1 light off vs on, P=0.8976, E+1 light off vs on, **P=0.0054). Shifting inhibition just 2 seconds earlier (-5:-2sec, general linear mixed model, effect of treatment (light on vs. off), P = 0.8802) or during stimulus presentation (0:1sec, general linear mixed model, effect of treatment (light on vs. off), P = 0.7054) spared post-error attention, indicating a narrow time-locked requirement of ACAvis activity. Thus, ACAvis projections are causally important for heightened attention prior to stimulus presentation following an error.

Conclusions: Our data identify prior error history as a key internal condition to recruit top-down frontal-sensory cortical projections and drive post-error attention enhancement. Our findings may provide circuit-based insight into the pathophysiology and intervention strategy for impaired visual attention in neuropsychiatric disorders.

Keywords: Cognitive Control, Gamma Oscillation, Attention, Top-Down, Anterior Cingulate Cortex

Disclosure: Nothing to disclose.

W197. Effects of Developmental Omega-3 Fatty Acid Deficiency on Physiological and Behavioral Responses to Stress in Male and Female Rats

Ruth Asch*, Jennifer Schurdak, Robert McNamara

Yale University School of Medicine, New Haven, Connecticut, United States

Background: An individual's response to acute or chronic stress is determined by numerous factors, including genetics, early life experience, environmental conditions, sex and age. An important neural mediator of stress responsivity is the hypothalamic-pituitary-adrenal (HPA) axis, which initiates the release of glucocorticoid hormones in response to physical or psychological challenge. HPA axis activity and its downstream physiological, metabolic, and immunological/inflammatory effects are tightly regulated, with inputs from the prefrontal cortex (PFC) and amygdala playing an important role. Although dysregulation of the HPA/corticolimbic circuitry and maladaptive responses to stress have been implicated in the pathophysiology of mood and anxiety disorders, associated etiological risk and resilience mechanisms remain poorly understood. Increased risk for mood and anxiety disorders, which frequently initially manifest in adolescence, has been associated with adverse early life experiences (e.g. maltreatment and neglect) as well as deficiencies in essential omega-3 fatty acids (n-3), particularly docosahexaenoic acid (DHA, 22:6n-3) which is the most abundant n-3 in the mammalian brain. Moreover, previous rodent studies have demonstrated that naturally occurring reductions in maternal licking/grooming and arched-back nursing of pups during the first postnatal week alter HPA axis development along with physiological and behavioral responses to stress in adulthood. We previously reported that perinatal dietary n-3 deficiency reduces maternal licking/grooming and arched-back nursing compared with rats maintained on a standard diet fortified with n-3. To extend these findings, the present study investigated the effect of perinatal n-3 deficiency on HPA axis function and anxiety-like behavior in adolescent male and female offspring, and fear conditioning and extinction was used as a behavioral index of corticolimbic function in adult offspring.

Methods: A perinatal feeding paradigm in which female rats received an n-3 deficient (DEF, n=7) or control (CON, n=6) diet 30 days prior to mating through gestation and lactation was used. At postnatal day 21 (P21) male and female pups were weaned onto the same diet as their mother. Adolescent (P40) male (DEF, n=14; CON, n=10) and female (DEF, n=10; CON, n=10) offspring were exposed to 30-minute restraint stress and plasma corticosterone and inflammatory cytokine (IL-1B, IL-6, IL-10, TNFa) levels assessed at 0, 15, 30, 60, and 120 min. In a parallel cohort of male (DEF, n=14; CON, n=12) and female (DEF, n=14; CON, n=12) offspring, we investigated anxiety-like behavior in the elevated plus maze (EPM) in adolescence (P40), and subsequently fear conditioning and extinction in adulthood (P90). Postmortem erythrocyte and PFC membrane fatty acid levels were measured by gas chromatography. Experimental procedures were approved by the University of Cincinnati IACUC and adhere to NIH guidelines.

Results: Adolescent and adult DEF offspring exhibited significantly lower erythrocyte (p≤0.0001) and cortical (p≤0.0001) DHA levels compared with same-age CON rats. Adult, but not adolescent, females had higher erythrocyte DHA levels (p≤0.0001) relative to adult males. Adolescent females, regardless of diet, exhibited greater basal levels of corticosterone (p=0.003) and TNFa (p=0.03) levels relative to males. In response to 30 min restraint stress, increases in corticosterone (time: p≤0.0001) and TNFa (time: p≤0.0001), but not IL-1B, IL-6, or IL-10, were observed in males and females. Additionally, adolescent females exhibited

greater stress-induced increases in corticosterone (sex: p=0.0001, sex x time: p=0.02), IL-6 (sex: p=0.03, sex x time: p=0.04), and TNFa (sex: p=0.003, sex x time: p=0.02) relative to males. No main effects or interactions with diet were observed for changes in cytokine or corticosterone levels. On the EPM, adolescent DEF rats exhibited less time in the open arms (p=0.02) and more time in the closed arms (p=0.04) compared with CON rats. There were no main effects sex, or sex by diet interactions for any EPM parameter measured. Fear acquisition rates were comparable between male and female DEF and CON adult offspring. DEF rats exhibited enhanced fear memory (p=0.04) and impaired fear extinction (diet: p=0.02, diet x sex x time: p=0.02) compared with CON rats. Furthermore, adolescent anxiety-like behavior in the EPM was predictive of freezing behavior during fear extinction in adulthood (r=0.54, p≤0.0001).

Conclusions: The present results suggest sex, but not n-3 fatty acid status, significantly influences the physiological and immunological/inflammatory response to acute stress in adolescent rats. Elevated anxiety-like behavior was observed in adolescent male and female DEF rats, who also displayed impaired fear extinction in adulthood. These findings suggest that the enhanced anxiety and fear behaviors observed in DEF offspring cannot be wholly attributable to an altered physiological and immunological/inflammatory response to acute stress in adolescence. Furthermore, these data suggest insufficient n-3 intake during development may contribute to elevated adolescent anxiety and corticolimbic network dysfunction in adulthood, and that elevated anxiety in adolescents may precede and predict maladaptive responses to stress or trauma later in life.

Keywords: Omega-3 Fatty Acids, Hypothalamic-Pituitary-Adrenal Axis, Stress and Anxiety Behavior

Disclosure: Nothing to disclose.

W198. Systemic Inflammatory Signature and Resting State Connectivity of the Default Mode Network in Idiopathic Psychosis

Paulo Lizano*, George Ling, Sarah Keedy, Shashwath Meda, Adam Lee, Olivia Lutz, Leah Rubin, Carol Tamminga, Brett Clementz, Godfrey Pearlson, Elliot Gershon, John Sweeney, Matcheri Keshavan, Jeffrey Bishop

Beth Israel Deaconess Med. Ctr. & Harvard Medical School, Boston, Massachusetts, United States

Background: Markers of systemic inflammation, whether individually or as a subgroup of inflammatory markers, are reliably elevated in the blood of a subset of individuals with schizophrenia or bipolar disorder. We recently identified a subgroup of idiopathic psychosis subjects (36%) displaying an elevated group of inflammatory marker levels (CRP, IFNγ, IL1β, IL8, IL10, TNFα, and VEGFA). This subgroup performed worse on cognitive measures of visuo-spatial working memory and response inhibition, displayed elevated subcortical volumes, and exhibited evidence of gray matter thickening compared to the psychosis group with low inflammation. While systemic inflammation has been shown to alter amygdala-ventromedial prefrontal cortex (vmPFC), corticostriatal, and default mode network activity, it remains unclear whether indicators of inflammation relate directly to alterations in resting state functional connectivity in a subgroup of psychosis individuals with higher systemic inflammation.

Methods: Serum and resting state functional magnetic resonance imaging (rsfMRI) was examined in 95 psychosis probands (Schizophrenia/Schizoaffective, n=50; Psychotic Bipolar, n=45) and 42 healthy controls (HC) recruited from the Bipolar-Schizophrenia Network of Intermediate Phenotypes (B-SNIP1) Chicago site. A combination of unsupervised exploratory factor analysis and hierarchical clustering was used to identify inflammation subtypes (Proband-Low n=65, Proband-High n=30, HC-Low n=34, HC-High n=8). Two approaches were used to determine whether systemic inflammation co-varied with resting state functional connectivity. In the first approach, empirical multivariate linear regressions were performed using inflammatory subgroups and nine resting-state rsfMRI networks previously found to be reduced in our B-SNIP1 psychosis subjects. The second approach was data-driven and involved (i) using Spearman's rank correlation to identify connectivity features that significantly correlated (p<0.0005) with inflammatory subtypes and then (ii) used canonical discriminant analysis to define a low-dimensional representation of the selected functional connectivity features. Post hoc analyses were carried out to compare canonical factor scores between inflammatory groups. All analyses were covaried for confounding variables such as age, sex, race, and framewise displacement. The False Discovery Rate (FDR) method was used for multiple comparison correction for group comparisons and a p<0.1 was considered significant.

Results: The anterior default mode network was significantly reduced in the Proband-High group compared to the Proband-Low (Cohen's d=-0.64, p=0.009, pFDR=0.088) and HC-High (d=-0.95, p=0.032, pFDR=0.095) groups, but there were no differences compared to the HC-Low group. When examined as a continuous measure, greater inflammation factor scores were significantly associated with lower anterior default mode network connectivity (r=-0.18, p=0.037, pFDR=0.095). In the data-driven approach, three canonical discriminant functional connectivity factors were identified and consisted of somatomotor, subcortical, default mode, visual, dorsal attention and ventral attention connectivity networks. Scores for the first functional connectivity factor (lower anterior and posterior default mode connectivity and increased subcortical and visual connectivity) were significantly different for all of the 6 group contrasts (pFDR<0.001) with the HC-High group having the highest scores and the Proband-Low group having the lowest scores.

Conclusions: Using complementary approaches (empirical and data-driven), we found that inflammatory subgroups have reduced anterior default mode connectivity in the Proband-High compared to the Proband-Low group. The data-driven approach appeared to be more sensitive in identifying other functional networks that may be potentially implicated with increased inflammatory signatures, and they include visual-ventral attention and subcortical networks. The findings in this study are consistent with the alterations noted with systemic inflammation in prior studies of healthy volunteers exposed to an immune challenge, as well as in depressed individuals. These data provide further support for systemic inflammation as a distinct contributing factor to network dysfunction in idiopathic psychosis.

Keywords: Systemic Inflammation, Functional MRI, Psychosis

Disclosure: Nothing to disclose.

W199. Irritability Mediates the Association Between Pain and Depression: Findings From the EMBARC and STRIDE Studies and the VitalSign6 Project

Manish Jha*, Abu Minhajuddin, Cherise Chin Fatt, Madhukar Trivedi

Icahn School of Medicine At Mount Sinai, New York, New York, United States

Background: Pain and depression are the two leading causes of disability in the United States. They are frequently comorbid and are associated with greater severity of depression, poorer quality of life, and increased likelihood of suicide-related outcomes. This report evaluates the association between irritability and pain, and whether irritability mediates the association between pain and depression.

Methods: Participants of two randomized controlled trials, the Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care (EMBARC) study (n=251) and the STimulant Reduction Intervention Using Dosed Exercise (STRIDE) study (n=302), and an ongoing quality-improvement project (VitalSign6) of patients seeking treatment at primary care clinics (n=4370) were included. Pearson's correlation coefficients were estimated to evaluate the association between irritability [5-item irritability domain of Concise Associated Symptom Tracking scale (CAST-IRR)] and pain [Pain Frequency, Intensity, and Burden Scale (P-FIBS)]. Baron and Kenny's method was used to evaluate if irritability mediated the effect of pain on depression [16-item Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR) in EMBARC, QIDS Clinician-Rated (QIDS-C) in STRIDE, and 9-item Patient Health Questionnaire (PHQ-9) in VitalSign6].

Results: Irritability was significantly correlated with pain in EMBARC (r=0.22, p <0.001), STRIDE (r=0.29, p <0.001), and VitalSign6 (r=0.26, p<0.001). Irritability significantly mediated the association between pain and overall depression [Sobel's Test estimate = 3.03 in EMBARC (p = 0.002), 4.41 in STRIDE (p <0.001), and 9.59 in VitalSign6 (p <0.001)]. Irritability mediated 65.5%, 64.6%, and 38.5% of the effect of pain on depression in EMBARC, STRIDE, and VitalSign6 respectively.

Conclusions: Pain in adults is significantly associated with irritability which in turn significantly mediates the effect of pain on depression. Future studies are needed to elucidate the neurocircuit mechanisms linking pain to irritability.

Keywords: Depression, Irritability, Pain, Substance Use Disorders, Major Depressive Disorder

Disclosure: Acadia Pharmaceuticals: Grant (Self), NACCME: Honoraria (Self), Global Medical Education: Honoraria (Self)

W200. Acute Serotonin 2A Receptor Activation Impairs Behavioral Flexibility in Mice

Susan Powell*, Omron Hassan, Landon Klein, Adam Halberstadt, Dionisio Amodeo

University of California, San Diego, La Jolla, California, United States

Background: Serotonin 2A (5-HT2A) receptors are the primary site of action of hallucinogenic drugs and the target of atypical antipsychotics. 5-HT2A receptors are also implicated in executive function, including behavioral flexibility. Previous studies showed that 5-HT2A receptor blockade improved behavioral flexibility in rodent models related to autism spectrum disorder and schizophrenia. The current study was conducted to examine the impact of acute 5-HT2A receptor activation on behavior flexibility in C57BL/6J mice. Because of the therapeutic potential of serotonergic hallucinogens and the unknown impact of many of these compounds on cognition, the present study examined how the 5-HT2A/2C agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and the more selective 5-HT2A agonist 25CN-NBOH impacted behavioral flexibility in C57BL/6J mice.

Methods: Male C57BL6/J mice were tested on a probabilistic spatial discrimination and reversal learning task after an intraperitoneal injection of vehicle, 2.5 mg/kg DOI, 1.0 mg/kg 25CN-NBOH, 1.0 mg/kg of the 5-HT2C receptor antagonist SER-082 or combined treatment with SER-082 (1.0 mg/kg) and 2.5 mg/kg DOI before testing of probabilistic reversal learning. Mice were first trained to navigate the maze, followed by probabilistic spatial acquisition learning and reversal learning. These tasks were conducted in a T-maze with equal sized arms (36 cm long X 12 cm wide X 12 cm tall) made of back Plexiglas. Briefly, after 2-3 days of training, mice were tested in the probabilistic reversal learning test. All mice received only vehicle injections before acquisition of the initial spatial discrimination. During acquisition testing, the mouse was allowed to choose between the two choice arms – one of which was baited with a ½ piece of cereal in each trial. One choice location was pseudorandomly designated as the "correct" spatial location and contained cereal on 80% of trials. For the other 20% of trials, the 'incorrect' location was baited with cereal. Acquisition criterion was achieved when a mouse chose the "correct" location for 6 consecutive trials. Reversal learning was tested 24 h after acquisition testing after injection of vehicle (n = 17), 2.5 mg/kg DOI (n = 8), 1.0 mg/kg SER-082 (n = 8), SER-082/DOI (n = 9), or 1.0 mg/kg 25CN-NBOH (n = 9). All aspects of the reversal learning test were identical to those in the acquisition phase except that reinforcement contingencies were switched from what was presented in acquisition. Reversal learning criterion was identical to the criterion used during acquisition: 6 consecutive correct choices. For assessment of locomotor activity, mice were treated with vehicle or with one of 5 doses of 25CN-NBOH (0.3, 1, 3, 10, 30 mg/kg) 10 min before testing. All studies were conducted at the University of California San Diego (UCSD) in facilities accredited by the Association of Assessment and Accreditation of Laboratory Animal Care International (AAALAC) under UCSD Institutional Animal Care and Use Committee (IACUC) approved animal protocols.

Results: Mice demonstrated comparable performance learning the initial spatial discrimination ($F[4,46] = 1.55$, NS). All treatment groups showed comparable retention of the initial spatial discrimination before the reversal phase ($F[4,46] = 0.53$, NS). Thus, all mice were able to recall the previous spatial discrimination before the reversal phase. Comparisons indicate that there was a significant difference for trials to criterion between the treatment groups during the reversal phase ($F[4,46] = 3.77$, $p < 0.05$). Mice treated with SER-DOI combination or 1.0 mg/kg of 25CN-NBOH required more trials to reach criterion compared to vehicle treated mice ($p < 0.5$). Thus, the 25CN-NBOH and SER-082/DOI combination group significantly impaired probabilistic reversal learning. Analysis of perservative errors committed indicates that treatment groups significantly differed ($F[4,46] = 3.47$, $p < 0.05$). The SER-082/DOI combination treatment group committed significantly more perseverative errors compared to vehicle-treated mice on reversal learning ($p < 0.05$). Analysis of regressive errors committed indicates that treatment groups significantly differed ($F[4,46] = 5.24$, $p < 0.01$) as well. Only the 1.0 25CN-NBOH group committed significantly more regressive errors.

For distance traveled, two-way ANOVA comparisons demonstrate a main effect of treatment ($F[5,74] = 32.41$, $p < 0.01$) and a main effect of 10-min time block ($F[4,281] = 73.06$, $p < 0.01$). An interaction of treatment and time block was also found ($F[25,370] = 8.76$, $p < 0.01$). Dunnett's post hoc comparisons indicate that treatment with 10 or 30 mg/kg 25CN-NBOH significantly reduced locomotor activity compared to vehicle-treated mice. However, 25CN-NBOH at 1.0 mg/kg, as tested in probabilistic reversal learning, did not significantly alter locomotor activity compared to vehicle-treated mice.

Conclusions: Taken together, these studies indicate that acute treatment with specific 5-HT2A receptor agonists at these doses impair cognitive flexibility as measured by probabilistic reversal learning. Limitations of the study include use of a single dose or each drug and the use of only male mice in these initial studies. Future studies will need to examine the effects of 5-HT2A agonists on reversal learning using a broader range of doses and whether these initial findings replicate in female mice.

Keywords: Hallucinogen, Probabilistic Reversal Learning, Serotonin 5-HT2A Receptor, Cognitive Flexibility, 25CN-NBOH

Disclosure: Nothing to disclose.