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ABSTRACTS COLLECTION

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P1. Biosignatures in LLD and AD Using Circulating Brain-Derived Exosomes

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Background: Chronic inflammation and neurodegeneration are well-characterized pathogenic factors in depression and dementia, especially late-life depression and Alzheimer's Dementia (AD), and a potential therapeutic target for treatment. One important mechanism linking inflammatory and neurodegenerative biomarkers with specific cellular activation and communication is the release of extracellular vesicles (EVs). EVs are nano-sized vesicles, have specific membrane proteins, and contain nucleic acid (microRNA) and protein cargo. EVs can be released from cells under various conditions, including chronic inflammation and stress, demonstrating significant age-dependent differences in their pro-inflammatory profile. In addition, brain cells, such as neurons and astrocytes, release EVs that can be extracted from plasma samples. The EVs can be divided into three groups based on size. The most studied vesicle is the exosome, ranging from 30 to 150 nm. The exosomes are considered cell-specific vesicles, and the evaluation of their content can provide detailed information about the biological changes of specific cell types than the evaluation of whole plasma content.

Exosomes from brain cells, such as neuron- (NDEs) and astrocytes-derived exosomes (ADEs), can easily cross the blood-brain barrier and be identified in the periphery. The NDEs and ADEs role is a new research field and an intricate pathway involving the crosstalk between the CNS, the neuroendocrine, and the immune systems. While studies have advanced in AD, the role of exosomes in LLD is poorly investigated. In addition, LLD is multifactorial and could be a prodromal state associated with neurodegenerative diseases, including AD, frontal, temporal dementia (FTD), and vascular dementia (VD).

Therefore, the signature of the exosomes could share common pathways and molecules between LLD and AD. There are no studies in the literature comparing NDEs and ADEs in those two neuropsychiatric illnesses until the present moment. The aim of this project is to characterize biosignatures in NDEs and ADEs (cell-specific) and plasma (non-cell-specific), creating a molecular profile for LLD and AD.

Methods: Therefore, 46 LLD subjects, 25 AD patients, and 34 healthy elderly controls were recruited, matched by age and gender. After the psychiatric evaluation, the blood was collected and centrifuged to obtain the plasma-free platelet. The sample was collected and stored at -80°C. We used the kit vFC™ vesicle flow cytometry for counting and sizing vesicles.

Results: Individuals with LLD presented lower levels of NDE and ADE compared to controls. For ADE, the opposite was demonstrated in AD Patients, with 2 times more ADE than CT and LLD. Next, we evaluate 49 pro-inflammatory cytokines and neurodegenerative protein levels in total exosomes and plasma samples. There was an overlap in the production of 32 proteins when comparing plasma and exosome evaluation. Twelve proteins were identified only in the plasma sample, and 5 only in the exosomes. CXCL2, GDNF, NF-light, GFAP, DR3, and IL-4 receptor levels in the exosomes increased compared to plasma, showing a cell-specific driving response via exosomes. Overlapping the protein evaluation in the exosomes from LLD and AD individuals, 14 proteins were common for both disorders, 15 were exclusive in LLD exosomes, and none of the proteins evaluated were exclusive in the exosomes of AD participants.

Conclusions: These preliminary conclusions reinforce the importance of the exosomes in cellular communication, and the common biomarkers shared in LLD and AD. This mechanism can contribute to the crosstalk between brain cells and the periphery as a window to directly evaluate the molecular pathology of LLD and AD.

Keywords: Late-life Depression, Alzheimer's Disease, Extracellular Vesicles, Inflammatory Markers, Neurodegeneration

Disclosure: Nothing to disclose.

P2. Profiling the Human Anterior Hippocampus With Spatially-Resolved Transcriptomics

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Background: The three-layered archicortex of the hippocampal formation (hippocampus) has unique patterns of gene expression, morphology, physiology, and connectivity that developmentally change across the lifespan. In rodents and non-human primates, the hippocampus is one of the only brain regions in which neurogenesis has been described throughout the lifespan. Due to its structure and circuitry, the region has been linked to a number of critical behavioral functions, including regulation of learning, memory and mood. Hippocampal neurons are organized in densely packed layers according to dendrite-axon polarity, which reflects the intrinsic connectivity of the hippocampal circuitry. Generally, afferent projections to the hippocampus are received in the dentate gyrus (DG), which then transmits through synaptic relays in the cornu ammonis (CA) areas, with the final efferent projections transmitting information out of the hippocampus via the subiculum (SUB). Synaptic relays within the hippocampus are major sites of structural and functional plasticity across the lifespan that regulate critical functions related to learning, memory, mood and stress regulation. Many important plasticity-related transcripts are localized to the dendritic compartment, and their transcription, transport out of the nucleus, and translation within the dendritic compartment is tightly regulated, both developmentally and by neuronal activity. Recent publications have generated cell-type specific molecular profiles of the human hippocampus in adults and across the lifespan using single-nucleus RNA sequencing; however, these resources lack spatial resolution within the hippocampus and lose transcriptomic information from the cytosolic compartment. Additionally, despite extensive characterization of the functional importance of postnatal neurogenesis in rodent DG, indisputable evidence of adult neurogenesis in the human DG remains elusive and its persistence throughout the lifespan remains controversial. Given the tight correlation between spatial structure and function in the hippocampus, and the particular importance of transcripts localized to the synaptic compartment, molecular profiling technologies with the capacity to address these gaps in our knowledge would be highly useful. We previously generated spatial transcriptomic maps of human dorsolateral prefrontal cortex that captured laminar structure from the white matter to the pial surface, and which included both nuclear and cytosolic gene expression. These data were made publicly available in the form of interactive browsers for the neuroscience community to interrogate the data (Maynard, Collado-Torres, 2021), and here, we describe the generation of a similar resource in the human hippocampus, which also includes developmental stages from infant to adult.

Methods: We used the 10x Genomics Visium Spatial Gene Expression platform, which combines spatially-resolved transcriptomic profiles with high resolution histological images to generate spatial transcriptomic maps of the anterior human hippocampus, analogous to the ventral hippocampus of the rodent, across the lifespan. To first characterize the organization of the adult hippocampus, we scored the tissue blocks and used multiple arrays to fully capture all major subfields (CA1-4, DG, and SUB) in adult donors ($N = 9$ neurotypical donors, both males and females, 159744 spots from 32 capture areas). Concurrently, to investigate hippocampal development over the postnatal lifespan specifically in the DG, we generated spatially-resolved, transcriptome-wide gene expression profiles focusing on this region from neurotypical donors spanning the human lifespan: infant, teen, adult and elderly donors ($N = 3$ donors, 10,503-12,552 spots per group, 12 total capture areas).

Results: We applied spatially-informed, unsupervised clustering algorithms across all donors to identify gene expression signatures that define spatial domains. Then, we compared these spatial domains to independently-derived, manually labeled annotations of anatomically-defined hippocampal subfields, which were visually guided by histological staining and expression of known marker genes. We compared gene enrichment across spatial domains, and identified postnatal developmental markers mapping to a number of biological functions including neurogenesis, plasticity, and apoptosis, among others. To make these data available to the community and most effectively support studies of hippocampal function, we generated a user-friendly, interactive web app for data exploration.

Conclusions: We generated spatially-resolved transcriptomic profiles of the adult human hippocampus that span all major subfields, and within the DG specifically, across the lifespan, which identified both canonical and novel patterns of spatial gene expression. Spatially-resolved techniques to identify molecular profiles within the context of the underlying tissue architecture is particularly important given the tight structure-function relationship of the cytoarchitecture within the developing and adult hippocampus. This resource provides a rich source of information about spatial gene expression in the hippocampus across the lifespan, which can be mined for information that contributes to the understanding of the etiology of human-specific neurodevelopmental disorders and neurodegenerative diseases where the hippocampus is implicated.

Keywords: Hippocampus, Spatial Transcriptomics, Dentate Gyrus

Disclosure: Nothing to disclose.

P3. Sex Moderates the Impact of Development Stress and Age on Mitochondrial Function

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Background: Mitochondrial dysfunction may drive alterations in neural function and behavior and is influenced by aging and early life experiences. Here, male and female rodents were used to determine the influence of sex, age, and adolescent stress on synaptic mitochondrial function and predictive value of peripheral markers.

Methods: C57Bl/6NTac mice were subject to chronic repeated predation stress (CRPS) for 15 days during their adolescent (PND36-50) and early adult (PND57-71) stages. In addition, impact of aging was assessed in Wistar rats. In both studies, synaptic mitochondrial respiration was assessed using the SeahorseXFe24 instrument in the hippocampus (HPC) and prefrontal cortex (PFC). Peripheral markers of inflammation, reactive oxygen, and mitochondrial function were measured.

Results: Stress history did not change oxygen consumption rate (OCR) of synaptic mitochondria in the PFC of either sex ($p > 0.05$). Stress history decreased OCR in the HPC ($p = 0.0045$) of males, but increased OCR in the HPC ($p < 0.0001$) of females. With regard to aging influences on mitochondrial function, in males, age altered overall OCR within synaptic mitochondria of PFC ($p < 0.0001$) and HPC ($p < 0.0001$). Compared to young and aged males, middle-aged males had reduced basal respiration ($p < 0.0001$), maximal respiration ($p = 0.0006$), proton leak ($p = 0.0003$), ATP production ($p < 0.0001$), and spare capacity ($p = 0.005$). Western blot densitometry indicated that aged males had the highest density of mitochondria ($p = 0.01$). In females, PFC OCR was elevated in middle-aged females compared to young ($p = 0.03$) and aged ($p < 0.0001$) females. Middle-aged females had higher basal respiration ($p < 0.0001$), maximal respiration ($p < 0.0001$), ATP production ($p < 0.0001$), and spare capacity ($p < 0.0001$) in both

HPC and PFC than other ages. Density of synaptic mitochondria in aged females was reduced ($p = 0.004$).

Sample size for CRPS study was 10 per group for each sex. Sample size for the aging study was 6 per group per sex per age. Test statistics for results presented above are included here. Stress history decreased OCR in the HPC ($F(1,132) = 8.340$, $p = 0.0045$) of males, but increased OCR in the HPC ($F(1,108) = 17.68$, $p < 0.0001$) of females. With regard to aging influences on mitochondrial function, in males, age altered overall OCR within synaptic mitochondria of PFC ($F(2,180) = 32.96$, $p < 0.0001$) and HPC ($F(2,180) = 60.31$, $p < 0.0001$). Compared to young and aged males, middle-aged males had reduced basal respiration ($F(2,30) = 13.02$; $p < 0.0001$), maximal respiration ($F(2,30) = 9.52$; $p = 0.0006$), proton leak ($F(2,30) = 10.54$; $p = 0.0003$), ATP production ($F(2,30) = 26.63$; $p < 0.0001$), and spare capacity ($F(2,30) = 6.44$; $p = 0.005$). Western blot densitometry indicated that aged males had the highest density of mitochondria ($F(2,12) = 6.85$; $p = 0.01$). Middle-aged females had higher basal respiration ($F(2,30) = 21.63$; $p < 0.0001$), maximal respiration ($F(2,30) = 26.17$; $p < 0.0001$), ATP production ($F(2,30) = 20.59$; $p < 0.0001$), and spare capacity ($F(2,30) = 29.88$; $p < 0.0001$) in HPC and PFC than other ages.

Conclusions: Although both males and females displayed changes in synaptic OCR as a function of early life stress and age, the patterns of the changes differed by sex. Mitochondrial dynamics within the males showed no region specific differences, yet female dynamics were significantly lower in the HPC than the PFC in all dynamic measurements. Ongoing work suggests that estrogen signaling within the mitochondria is a critical factor in these differences and reflected in peripheral metrics.

Keywords: Mitochondrial Respiration, Sex Differences, Synapse, Prefrontal Cortex, Hippocampus

Disclosure: Nothing to disclose.

P4. Defining Neuropeptide Signals That Slow Cognitive Aging

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Background: Cognitive decline is a major deficit that arises with age in humans. Though human life expectancy is increasing, there are currently no treatments to effectively treat or prevent age-related memory decline, creating a growing public health burden. Previous work in mammals and our own work in C. elegans has revealed a correlation between maintaining memory function with age and activity of the memory regulating transcription factor cAMP response-element binding protein (CREB). However, CREB is ubiquitously expressed throughout the brain and body; therefore, identifying tissue- and cell-type specific activators of CREB would reveal promising targets for the development of novel therapeutics that age-related memory loss. We recently found that activation of the highly conserved Gαq signaling pathway in a single sensory neuron (AWC) in C. elegans could enhance molecularly conserved, CREB-dependent long-term memory (LTM) in young animals as well as slow age-related LTM deficits. Maintenance of LTM ability with age by elevated Gαq signaling was found to be due to cell non-autonomous, but cell-specific regulation of CREB activity in "memory center" neurons (AIM) in the worm. We recently determined that LTM phenotypes observed due to increased Gαq signaling resulted from increased neuropeptide release by the AWC sensory neurons. However, the identities of these memory-promoting neuropeptides, their downstream signaling pathways, and their role in age-related cognitive decline remain unknown.

Methods: C. elegans maintenance: Animals were maintained under standard laboratory conditions and fed the E. Coli strain OP50 ad libitum. Synchronized populations for behavior and lifespan assays were generated by standard hypochlorite treatment.

RNAi Treatment: Standard RNAi by feeding was performed to achieve gene knockdown. Briefly, HT115 E. Coli expressing RNAi was fed to C. elegans. To knock down genes specifically in adulthood, animals were fed RNAi at the L4 larval stage, after terminal nervous system differentiation. To achieve RNA-knockdown selectively in neurons, experiments were performed in a transgenic C. elegans strain (egl-30(js126);LC108 [punc-119::sid-1]) that overexpresses a double stranded RNA transporter in all neurons.

Behavior Assays: Standard positive olfactory association assays were performed. These assays pair the neutral odorant butanone with food (E. coli) so that animals form a positive butanone association. Learning and memory were assayed as a training-dependent increase in preference for butanone as measured by population chemotaxis assays (~100 animals per assay) to obtain a chemotaxis index. Memory performance was calculated by Performance Index (Chemotaxis_Index(trained) - Chemotaxis_Index (naive/untrained). For all behavioral assays, 10-15 replicates were used, and either one- or two-way ANOVA followed by Bonferroni post-hoc tests were performed.

Lifespan Assays: Standard C. elegans lifespan assays were performed in the context of RNAi treatment. For each condition, three biological replicates of ~100 worms were used. Data was analyzed by log-rank (Mantel-Cox) method in Kaplan-Meier survival analysis.

Results: We find that genetic manipulations that specifically increase neuropeptide secretion from the AWC neuron in C. elegans slow age-related deficits in associative learning and LTM ($p < 0.05$). Longevity analysis revealed that increased neuropeptide secretion for the AWC, has no detectable effect on lifespan ($p > 0.05$), suggesting that the maintenance of learning and memory ability observed is due to increased neuronal healthspan.

In order to identify these healthspan promoting signals, we generated a list of approximately 40 candidate AWC-expressed neuropeptides using publicly available genomics datasets, many of which have no previously known role in associative behavior or aging. We are performing an RNAi-based screen in the worm to determine if specific neuropeptides are necessary for the neuropeptide-dependent enhanced learning and memory ability of animals with elevated Gαq signaling. We have identified novel peptide signals, including insulin-like peptides (ins) and neuropeptide-like proteins (nlps), that promote associative learning and memory, and in ongoing studies, we are examining other peptides of interest and their role in regulation of CREB activity.

Conclusions: Here we have found that enhancing the activity and secretion of specific neuropeptides slows age-related deficits in associative learning and memory. Identifying new molecules that regulate CREB in a cell-specific manner will be of broad interest as the effects of increasing CREB activity have memory-promoting effects in both invertebrates and mammals, and may present new therapeutic targets for the treatment of cognitive decline.

Keywords: CREB, Aging, Neuropeptides

Disclosure: Nothing to disclose.

P5. Exercise Parameters That Open a 'Molecular Memory Window' for Cognitive Enhancement Shine Light on Key Memory Mechanism in the Adult, Aging, and Alzheimer's Disease Brain

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Background: The ability to learn, consolidate and retrieve information is critical for everyday life and this ability begins to decline with normal aging and is severely exacerbated by Alzheimer's Disease (AD). Basic research and clinical trials universally demonstrate the benefits of exercise for cognitive function. However, age and physical disability-related factors that may affect individuals throughout the lifespan progressively limit and reduce engagement in exercise and associated cognitive benefits. Defining the mechanisms by which exercise leads to cognitive enhancement throughout the lifespan will hold therapeutic benefit for individuals of all ages and abilities.

Methods: In this study, we use exercise as an approach to unlock a novel understanding of the molecular mechanisms that enable and drive consolidation of memory. We identify and utilize specific exercise parameters that allow memory consolidation to occur under inadequate, subthreshold learning conditions, as well as parameters where these cognitive benefits are maintained for specific durations of time. Specifically, adult male mice underwent 14 days of initial exercise, received a sedentary delay period (0-2 weeks), and a brief 2-day period of reactivating exercise, followed by 3 min inadequate, subthreshold training in an object location memory (OLM) task. Those parameters were then used to examine hippocampal long-term potentiation (LTP) using theta burst stimulation in the schaffer collateral pathway. To begin to define the mechanism responsible for driving long-term memory formation and maintaining cognitive benefits, we utilized an unbiased RNA-sequencing approach on dorsal hippocampal tissue taken during memory consolidation, 1 hour following training, in mice receiving the same exercise parameters above. To examine the role of an identified gene target ACVR1C, a type 1 receptor for the TGF-β family of signaling molecules in hippocampus-dependent learning and synaptic plasticity, we used intra-hippocampal delivery of AAV1-ACVR1C point mutant constructs that either enhance or disrupt function and allowed it to express for two weeks before onset of behavior. Next, sedentary mice were trained using either a subthreshold (3 min) or standard (10 min) OLM task and memory was tested the following day. The same mice from behavioral studies were used to assess the impact of Acvr1c manipulation on hippocampal LTP.

Given misregulation of the TGF-β pathway that occurs with age and in AD patients, we next aimed to determine whether enhancing Acvr1c function would regulate long-term memory formation and synaptic plasticity in aging and AD mouse hippocampus and ameliorate impairments. First, we examined whether Acvr1c declines with age. Dorsal hippocampus was obtained from 3 and 20 mo. female and male C57BL/6J mice and processed for RT-qPCR. Additionally, Acvr1c transcripts per million (TPM) values from an RNA-Seq data set obtained through the MODEL-AD consortium were analyzed from 4, 8 and 12 mo. C57BL/6J and 5xFAD female and male mice. To determine whether enhancing ACVR1C function in dorsal hippocampus would ameliorate impairments in memory and LTP in 18 mo. C57BL/6J and 5xFAD males, aforementioned methods were applied.

Results: We demonstrate that 14 days of voluntary wheel-running facilitates hippocampus-dependent memory (One-way ANOVA, Group: $(F(6,64) = 8.13, P < 0.0001$; Tukey test: $P < 0.001$, 14D vs. Sed) and synaptic plasticity (One-way ANOVA, Group: $(F(6,89) = 22.22, P < 0.0001$; Tukey test: $P < 0.0001$, 14D vs. Sed) in adult mice compared to sedentary, effects which can be maintained and re-engaged with brief 2-day re-introduction to exercise following a sedentary delay (Behavior: Tukey test: $P < 0.05$, LTP: $P < 0.0001$, 2-day re-introduction vs SED). We identify a gene coding for a type 1 receptor for the TGF-β family of signaling molecules, Acvr1c as one of few genes (including Bdnf) showing up-regulation in the hippocampus under exercise conditions that enable the formation of long-term memory and synaptic plasticity. We find that disrupted ACVR1C function under adequate learning conditions in adults impairs memory $(t(17) = 4.65, P = 0.0002)$ and synaptic plasticity $(t(18) = 3.512, P = 0.0025)$. Conversely, overexpression of ACVR1C enables learning under inadequate training conditions in adults $(t(18) = 3.303, P = 0.004)$ and enhances LTP $(t(14) = 3.953, P = 0.0014)$. We find Acvr1c levels decrease in the hippocampus with age in C57Bl/6J $(t(26) = 2.72, P = 0.01)$ and 5xFAD female and male mice (Three-way ANOVA, Age: $(F(2,48) = 54.95, P < 0.0001$; Tukey test: $P < 0.0001$ 12 mo. vs. 4 mo.), and demonstrate that Acvr1c over-expression ameliorates age and AD-associated impairments in memory (18 mo. C57BL/6J: $(t(12) = 2.350, P = 0.036)$ and synaptic plasticity (18 mo. C57BL/6J: $(t(14) = 3.953, P = 0.001)$, 18 mo. 5xFAD: $(t(10) = 9.653, P = 0.001)$.

Conclusions: Using a novel exercise-based approach that allows memory formation to occur under inadequate, subthreshold training conditions, we identify ACVR1C as a fundamental driver and bidirectional regulator of memory formation and synaptic plasticity in the adult brain. We also demonstrate a key role for ACVR1C in the aging- and AD-brain. These data suggest that promoting ACVR1C through exercise or pharmacological intervention may protect against age and AD-associated cognitive impairment, providing a potentially powerful and novel disease modifying treatment strategy for AD.

Keywords: Memory, Exercise, Long Term Potentiation, Hippocampus, Alzheimer's Disease

Disclosure: Nothing to disclose.

P6. A Comparison of Pathways Associated With Protein Aggregation in Alzheimer's Disease and Schizophrenia

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Background: Alzheimer's disease (AD) is the most common neurodegenerative disorder, characterized universally by cognitive decline, and heterogeneously by emotional and behavioral changes. Between 40-60% of AD patients experience psychotic symptoms. Recent studies suggest greater cognitive impairment and more rapid cognitive decline in AD patients with psychosis (AD + P).

Schizophrenia (SCZ) affects 1% of the population and is characterized by positive and negative symptoms, as well as cognitive deficits. Psychosis, in the form of hallucinations and delusions, is the most prominent symptom. SCZ is also a heterogeneous disorder in its clinical presentation and progression.

The pathological hallmark of AD is protein aggregation. Protein aggregation likely disrupts important proteins and pathways that can lead to the symptoms of AD, including psychosis and cognitive decline. We have demonstrated protein aggregation in a subset of patients with SCZ. In addition, we have shown using olfactory neurons, that SCZ patients with aggregation have more pronounced cognitive impairment than SCZ patients without aggregation as a mechanism. Further, we and others have previously shown that the protein products of rare genetic variants linked with SCZ in unique pedigrees can aggregate and disrupt the function of critical proteins required for the proper functioning of the cell. While protein aggregation has been linked to neuronal cell death in AD, the protein aggregation we observe in SCZ likely leads to cellular dysfunction and not profound cell loss. While the mechanisms leading to aggregation in both diseases may be partly distinct, there are likely similarities, with proteins that are prone to aggregate overlapping between diseases and relating to the common clinical phenotype of psychosis and/or cognitive impairment. Therefore, we hypothesize

that there will be proteins and pathways in AD that will overlap with some of those identified in SCZ patients with protein aggregation as a mechanism, providing insight into common pathways and mechanisms associated with AD and SCZ.

Methods: A comparative proteomic study of protein mass spectrometry data obtained from insoluble proteins biochemically isolated from Alzheimer's disease as reported in a previous proteomic study (Hales et al., 2016) and schizophrenia post-mortem brains. Proteome Discoverer was used to analyze the proteomic data to maximize the number of proteins identified. Panther pathway analysis was used analyze the enriched protein data to determine proteins and biological processes most relevant to protein insolubility in AD and SCZ.

Results: We have obtained promising preliminary data through a comparative proteomic investigation of the insoluble proteins in a SCZ subtype, characterized by protein aggregation, with proteins identified in a previous similar proteomic insolubility study in AD, that suggests overlapping proteins and pathways between the two neuropsychiatric disorders. These results suggest a common proteomic signature in AD and SCZ.

Conclusions: By comparing the aggregated proteins in AD to aggregated proteins in SCZ, a disease characterized by psychosis and cognitive deficits, we can better understand common pathways implicated in these disorders.

Keywords: Alzheimer's Disease, Schizophrenia (SCZ), Proteomics

Disclosure: Nothing to disclose.

P7. Sex-Specific Association of Amyloid Precursor Protein With Microglial Activation and Modulation by Apolipoprotein E Genotype in Major Depressive Disorder

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Background: Female sex and depression are both associated with increased risk for Alzheimer's disease (AD) but the mechanisms for the increased risk remain poorly understood. Increased brain amyloid deposition and microglial activation have both been implicated in AD and depression. However, to our knowledge, there are no studies which have examined if sex- and depression-related effects on amyloid β-peptide (Aβ) dynamics and microglia activation may contribute to increased risk for AD associated with female sex and depression. Non-amyloidogenic proteolytic cleavage of amyloid precursor protein (APP) by α-secretase results in release of one secreted form of APP (sAPPα), whereas the amyloidogenic cleavage by β-secretase releases a C-terminally truncated form of APP (sAPPβ) plus Aβ. Both sAPPα and sAPPβ have been reported to induce microglia activation. These observations prompted us to examine the relationship between sex, depression, and cerebrospinal fluid (CSF) levels of sAPPα and sAPPβ. CSF levels of the secreted ectodomain of triggering receptor expressed on myeloid cells 2 (sTREM2), which has emerged as a specific marker of microglial activation, was also examined. Additionally, we also investigated the potential influence of APOE genotype on these biomarkers.

Methods: Fifty-one cognitively intact subjects (31 subjects with major depressive disorder and 20 age-matched healthy controls) aged 60 years and older, completed a 3-year longitudinal study and an optional lumbar puncture. Independent-samples Mann-Whitney-U tests were used to examine group differences by diagnosis, sex, and APOE genotype for sAPP-α and sAPPβ.

Results: There were no significant differences between subjects with late-life major depression (LLMD) and Controls in mean (± SEM) CSF sAPPα (551 ± 48 vs 616 ± 50 pg/mL) or sAPPβ (164 ± 15 vs 187 ± 14 pg/mL) levels. Irrespective of diagnosis, females had significantly higher levels of both sAPPα ($p = 0.016$) and sAPPβ ($p = 0.010$) compared to males. sAPPα and sAPPβ were significantly correlated with each other (rho = 0.930, $p < 0.001$). Mean CSF sTREM2 concentrations were 3507 ± 490 pg/mL in LLMD subjects and 5096 ± 640 pg/mL in Controls ($p = 0.075$). In the whole sample, sTREM2 correlated with both sAPPα (rho = 0.462, $p = 0.001$) and sAPPβ (rho = 0.504, $p < 0.001$). When examining the relationships as a function of diagnosis, sAPPα was found to be associated with sTREM2 in Controls only (rho = 0.474, $p = 0.035$) while sAPPβ correlated with sTREM2 in LLMD only (rho = 0.370, $p = 0.048$). When examining the relationship as function of sex, we found that sTREM2 significantly correlated with both sAPPα (rho = 0.629, $p = 0.002$) and sAPPβ (rho = 0.673, $p = 0.001$) in females but not in males (sAPPα: rho = 0.310, $p = 0.109$; sAPPβ: rho = 0.341, $p = 0.076$). Finally, as a function of APOE genotype, significant associations were found only for APOEe4 non-carriers. sTREM2 correlated with both sAPPα (rho = 0.567, $p < 0.001$) and sAPPβ (rho = 0.579, $p < 0.001$) in the e4 negative group, but not for e4 positive (sAPPα: rho = 0.182, $p = 0.516$; sAPPβ: rho = 0.193, $p = 0.491$).

Conclusions: We did not find significant differences in mean CSF sAPPα and sAPPβ levels between Controls and LLMD or between APOE ε4 carriers and non-carriers. We found significant associations of sTREM2 with both sAPPα and sAPPβ in females but not in males. sAPPα and sAPPβ correlated significantly only in non-APOE ε4 carriers but not in APOE ε4 subjects. These observations suggest that APP expression may influence neuroinflammatory response mainly in female subjects and in non-APOE ε4 carriers and should thus be explored as a potential molecular therapeutic target for the prevention and treatment of AD in these populations.

Keywords: CSF sTREM2, sAPPβ, sAPPα, Microglia, Depression

Disclosure: Nothing to disclose.

P8. The Pink Brain Project: Cognitive and Immunological Effects of Yoga Compared to Memory Training in Older Women at Risk for Alzheimer's Disease

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Background: Subjective cognitive decline (SCD) and mild cognitive impairment (MCI) accompanied by cardiovascular risk factors (CVRFs) are known risk factor for developing dementia. Yoga has been identified as a safe practice with positive effects on cognitive functions in healthy elderly and older adults at risk for cognitive decline. While yoga commonly involves postures (=asanas), focus on breath (=pranayama), postural alignment, and movement, brief meditative practices are also beneficial for brain health and cognition. Kirtan Kriya (KK), a yogic meditation, is associated with improved mood, cognition, and quality of life in SCD and MCI.

Methods: We conducted a randomized, controlled trial to assess the efficacy of Kundalini yoga paired with Kirtan Kriya meditation (YOGA) and memory enhancement training (MET) on mood and cognitive functioning in a group of older women with CVRFs and SCD (clinicaltrials.gov=NCT03503669). INTERVENTIONS: The YOGA intervention consisted of weekly, 60-minute in-person classes with a certified instructor for 12 weeks. In addition, each

participant received a CD containing a 12-minute guided KK recording for daily homework practice. MET involved 12 weekly in-person group classes with 12 minute daily homework exercises. OUTCOMES: Memory performance was the primary outcome. A delayed recall domain score was computed from the Hopkins Verbal Learning Test, Wechsler Memory Scale, and Rey-Osterreith Complex Figure 3-min Delay tests. An executive function domain score was calculated from the Stroop Interference and Trails B tests. Secondary outcomes included depression (BDI, Beck Depression Inventory), anxiety (HAM-A, Hamilton Anxiety Rating Scale), stress (PSS, Perceived Stress Scale), resilience (CD-RISC, Connor-Davidson Resilience scale), subjective memory (MFQ, Memory Functioning Questionnaire; Factor 1=Frequency of Forgetting; Factor 2: Seriousness of Forgetting), and health-related quality of life (SF-36, 36-Item Short Form Survey). Outcomes were measured at 12-weeks and 24-weeks follow-up. BIOLOGICAL SAMPLES: Peripheral whole blood samples were collected at baseline, 12-weeks, and 24-weeks follow-up for RNA sequencing and cytokine/chemokine assays. RNA-SEQ: RNA Sequencing was performed on 2 Illumina NovaSeq 6000 S4 lanes for 2x150 cycles in paired-end mode, generating 150 bp sequencing reads (mean ~30 million reads/sample). Differential gene expression was tested using negative binomial generalized linear models (DeSeq2 and edgeR, R 4.1). CYTOKINE/CHEMOKINE ASSAY: Human 38-plex magnetic cytokine/chemokine kits (EMD Millipore, HCYTMAG-60K-PX38, Burlington, MA) were used per manufacturer's instructions. STATISTICAL ANALYSIS: Outcomes (clinical and cytokine/chemokine concentrations) were analyzed using a mixed effects general linear model (SAS 9.4), including intervention group, time, and the interaction between time and intervention group. Age, sex, and education were used as covariates (only for cognitive outcomes). Post-hoc analyses determined the significance of specific pair-wise group differences and within-group changes. Effect sizes (Cohen's d) were calculated for clinical outcomes.

Results: CLINICAL OUTCOMES: A total of 79 participants (YOGA = 40; MET = 39) were randomized and 63 completed the 24-week follow-up (YOGA = 65% completion rate; MET = 95%; $\chi2(1)=10.9$, $p < 0.001$). Mean age of participants was 66.5 (SD = 9.2) years and mean MMSE was 28.4 (SD = 1.4). At 12-weeks and 24-weeks follow-up, both interventions demonstrated improvement in frequency of forgetting (MFQ-Factor 1). Between group differences were not significant ($F(1, 76)=0.2$, $p = 0.7$). At 24-weeks, YOGA participants demonstrated between- and within-groups improvements in seriousness of forgetting/MFQ-Factor 2 (YOGA mean change (SD) = 0.65 (1.25), $t(76)=2.1$, $p = 0.04$; MET mean change (SD) = -0.31(1.35), $t(76)=-0.9$, $p = 0.4$; $F(1, 76)=4.9$, $p = 0.03$; effect size (95% confidence interval)=-0.73 (-1.26, -0.19)). YOGA participants demonstrated between- and within-groups decline in delayed recall scores at 24-weeks (YOGA mean change (SD) = -0.31(0.37 $t(76)=-3.8$, $p = 0.0003$; MET mean change (SD = 0.02 (0.55), $t(76)=0.5$, $p = 0.6$; $F(1, 76)=10.3$, $p = 0.002$; effect size (95% confidence interval=0.19 (-0.32, 0.70)). Executive functioning, however, showed no between- or within-groups differences ($F(1, 76)=0.8$, $p = 0.4$). GENE EXPRESSION: Compared to MET, at 12- and 24-weeks follow-up, YOGA uniquely modulated targets related to interferon signaling and innate and adaptive immunity. CYTOKINE/CHEMOKINE CONCENTRATIONS: Compared to YOGA, MET participants displayed higher Eotaxin-1 levels ($F(2,67)=3.94$, $p = 0.02$) at 12- and 24-weeks follow-up.

Conclusions: At 24-weeks follow-up, YOGA yielded a significant, large effect size improvement in subjective cognitive impairment compared to MET. On a transcriptional level, YOGA is a robust mediator of psycho-neuro-immune pathways, including suppression of several typically pro-inflammatory molecules. Eotaxin-1 levels increased over time in MET but not YOGA participants. Eotaxin-1 levels have been shown to increase with age and cognitive decline. These results suggest clinical and biological benefits of YOGA for SCD in menopausal women at risk for Alzheimer's disease due to cardiovascular disease, linking changes in cognition to anti-inflammatory effects of yoga.

Keywords: Clinical Trial, Cognitive Decline, Transcriptomics, Neuroimmunology, Prevention of Alzheimer's Disease

Disclosure: Nothing to disclose.

P9. Network Controllability: A Potential Targeting Approach for TMS With Important Considerations for Aging

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Background: Repetitive Transcranial Magnetic Stimulation (rTMS) is a non-invasive brain stimulation technique widely used in both clinical practice and research studies, due to its ability to modulate neuronal activation. However, rTMS suffers from an overall moderate efficacy, and improving targeting approaches may significantly increase rTMS effects. Indeed, while early studies used imprecise scalp-based coil placement to define the stimulation target, more recent studies rely, instead, on fMRI results for neuronavigated targeting. This is done either by having participants perform a task and extracting the peak activation within a specific region of interest, or by extracting the region with strongest connectivity value during a task or resting state scan. While these approaches have enhanced rTMS efficacy, they rely on identifying bivariate relationships between a single seed and target node, even though the brain consists of assemblies of large-scale networks that communicate in a highly multivariate fashion. This problem is especially relevant for rTMS targeting in older adults, as the brain undergoes a number of large-scale network changes. Thus, a central challenge is in identifying a relevant multivariate network measure that summarizes these complex network dynamics and their age-related change. One such measure is network controllability, which measures the strength of influence of one cortical node on the global network. Central to this approach, is the hypothesis that applying energy (e.g., via rTMS) to nodes with high modal controllability might push the brain into hard-to-reach states. We recently demonstrated that young adult participants benefited the most from rTMS when the stimulation site coincided with the node of higher modal controllability. The current study focused on how controllability changes with age, and how these changes can impact the effectiveness of rTMS in aging populations.

Methods: Imaging data from 52 young adults (23.8 ± 4.8 years old, 29 females) and 29 older adults (67.9 ± 5.7 years old, 17 females) were used. After neuroimaging (including diffusion-weighted imaging), subjects received active or sham rTMS over the left dorsolateral prefrontal cortex or the left parietal cortex during four subsequent visits. DWI data were preprocessed using a standard DWI processing pipeline with FSL and MRtrix software, to generate a high-dimensional structural connectome in each participant. The number of white matter streamlines connecting each pair of regions was used to compute the modal controllability of each node. Modal controllability values were then compared between the two groups: across the whole brain and for four networks of the Yeo atlas. In addition, since modal controllability is derived from DWI data, we also computed the degree, the number of links connecting one node with every others, to test whether the results were specific to controllability or were simply a reflection of structural connectivity changes with aging.

Results: Results demonstrated significant modal controllability differences due to aging. Specifically, paired sample t-tests showed that older adults have significantly higher modal controllability for each of the four tested networks (DMN, Fronto-Parietal, Visual and Somoto-Motor) compared to younger adults ($p < 0.001$ for all comparisons, CIs = [0.009; 0.02]; [0.01; 0.03]; [0.01; 0.02]; [0.02; 0.03] for each network respectively). Young adults were found to have higher modal controllability in parietal and medial prefrontal regions, as expected by network controllability theory which states that most of those nodes are in cognitive control network. However, older adults did not display this pattern and instead had higher modal controllability within the SFG and premotor regions. Finally, the degree analysis did not reveal any significant differences associated with aging in those regions of interest, providing evidence that the results were specific to controllability.

Conclusions: Taken together these results suggest that while network controllability can be used as a potential targeting approach for rTMS, and has been associated with some promising results, some important consideration needs to be given regarding the effect of aging on what targets should be chosen for maximum effect. Contrary to assumptions based on executive control, for older adults the modal controllability nodes were not found in the fronto-parietal network but instead in sensory and premotor regions. Further, the fact that aging differences on controllability were not explained by structural connectivity changes reinforces the idea that this measure reflects more than just connectivity.

Keywords: rTMS, Diffusion Tensor Imaging (DTI), Aging

Disclosure: Nothing to disclose.

P10. Disentangling the Relationship Between Age, Sleep, and Alcohol Consumption: Examination Among Healthy Community-Residing Men and Women

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Background: Epidemiological evidence demonstrates that the prevalence of current drinking among older adults has increased dramatically over the previous two decades. The significance of this change is amplified by the fact that aging is generally accompanied by increased vulnerability to alcohol's negative effects on multiple biobehavioral processes, including sleep. Notably, regardless of alcohol use, sleep problems are commonly reported in even healthy aging adults. Thus, the intersection of age, alcohol use and sleep constitute a significant area of study. Here, we examined relationships between sleep and alcohol use in a sample of healthy, community-residing older adults, focusing on potential sex differences in these effects. Together, evolving patterns of alcohol use, shifts in populations age distributions, and robust data regarding the sensitivity and relevance of sleep to well-being, reinforce the relevance of this area of study.

Methods: 56 (36 women) healthy current drinking adults between the ages of 65 and 90 ($M = 74.02 \pm 5.86$) were recruited to take part in an initial study examining potential effects of psychosocial stressors on alcohol use. For our current objective, we focus on sleep disruption/problems. Composite scores for typical (QFI) amount of absolute alcohol consumed was derived. Participants completed the Pittsburg Sleep Quality Index (PSQI), a self-report measure of sleep to evaluate subjective sleep quality with global scores greater than 5 reflecting poor sleep. General linear models were used to assess the relationship between sleep disturbance and alcohol use (dependent measure). Sex and its potential interaction with sleep was included in the model. Additional analyses utilizing NIAAA criteria for low risk and binge drinking were also conducted.

Results: Significant effects of sex [$F(1,108)=6.76$, $p = .01$] and sleep disturbance [$F(1,108)=16.07$, $p < .0001$] were noted. As expected, men endorsed greater alcohol consumption than women. Contrary to our initial expectations, higher indices of poor sleep predicted less alcohol use. However, these effects are qualified by a significant interaction between sleep and sex [$F(1,108)=4.10$, $p = .05$]. Interrogation of the interaction revealed a stronger negative relationship between higher levels of sleep disruption and lower alcohol among men ($r = -0.44$, $p = .004$) than for women ($r = -.27$, $p = .02$). There were no sex differences in rates of adherence to low risk drinking guidelines ($p = .11$). Higher indicators of sleep disruption predicted greater likelihood of adherence to proposed low risk guidelines [$F(1,108)=4.04$, $p = .05$], with no sex by sleep interaction. Higher indices of sleep disruption were also positively related to numbers of binge drinking episodes [$F(1,108)=11.36$, $p = .001$]. The presence of a significant interaction between sex and sleep quality [$F(1,108) =11.55$, $p = .001$] for binge drinking episodes shows a robust negative relationship among men ($r = -.45$, $p = .003$), compared to that for women ($r = .15$, $p = .20$).

Conclusions: Taken together, our results highlight differences in drinking related to sleep quality/disturbances offering compelling evidence that poor sleep may be predictive of less alcohol use and thus less likelihood of exceeding proposed limits for low risk drinking in healthy community-residing older adults. Inversely, older adults exceeding NIAAA levels for low risk and binge drinking generally reported better sleep. Thus, while unexpected, these results may reflect unique socio-demographic characteristics in our sample (e.g., well-educated, predominantly white non-Hispanic, high income). Additionally, observed sex-specific differences partially align with current evidence suggesting more robust associations between use patterns and sleep in men overall. Given the dramatic increase in the aging population, rise in drinking prevalence, and common reports of severe sleep deficits/disruptions among older groups, disentangling patterns of use and how they may interact with sleep and other biobehavioral processes remains an important area of future research.

Keywords: Sleep Disturbances, Older Adults, Alcohol

Disclosure: Nothing to disclose.

P11. Longitudinal Impact of Midlife Metabolic Health on Memory Function: Role of Sex and Reproductive Aging

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Background: In early midlife, men and women undergo different aging processes. In addition to chronological aging, women undergo reproductive aging whereby they experience a depletion of sex steroid hormones, including estradiol. Estradiol is not only neuroprotective but regulates glucose metabolism and mitochondrial function, including oxidative stress production. With menopause, there is a 15-20% decrease in glucose metabolism and mitochondrial function. As such, menopause presents a window of vulnerability in women that may increase susceptibility to neurodegeneration and cognitive impairment. Here, we assessed the longitudinal impact of metabolic and mitochondrial function, in relation to sex and reproductive aging, on memory performance in early midlife.

Methods: Participants were assessed at three time points in midlife: at ages 40-50, 45-55, and 55-63 years old. 103 participants (48M:55F) underwent metabolic and memory assessments at ages 40-50. At ages 45-55, as part of a larger cohort study, the same participants underwent a 5-year follow-up assessment of verbal and associative memory and mitochondrial function was assessed using 8-hydroxydeoxyguanosine (8-OHdG), a measure of oxidative DNA damage. Finally, in a subsequent 8-year follow-up study, 76 participants (32M:44F) from the larger cohort, now ages 55-63 years old, completed the same memory tasks to assess memory decline in midlife. Generalized estimating equations adjusting for age, education, baseline memory performance, and follow-up years were used to assess associations between metabolic function and memory outcomes. Models were run both stratified by sex and with the inclusion of an interaction term.

Results: At ages 40-50, there were no significant relationships between metabolic health and memory measures in both men and women ($p > 0.05$). However, women with prediabetic/diabetic levels of HbA1c (>=5.7) at ages 40-50 performed significantly worse on memory tasks 5 years later compared to those with lower levels of HbA1c ($b = -6.32$, $p < 0.01$) and differed significantly from men ($p < 0.01$). In men, we did not find any associations between HbA1c levels and memory performance. Examining the impact of menopause, we found that women with higher HbA1c levels who transitioned to postmenopause during the follow-up period had worse memory performance 5 years later ($b = -1.22$, $p < 0.01$) compared with those who remained in pre/perimenopause ($p = 0.05$). In addition, specifically in women, poor metabolic health (HbA1c) at ages 40-50 was also related to higher levels of oxidative DNA damage (8-OHdG) measured 5-years later ($b = 1.04$, $p = 0.01$; men: $b = 0.17$, $p = 0.54$). Elevated levels of 8-OHdG at ages 45-55, in turn, were related to greater verbal memory decline over the subsequent 8-year follow-up period ($b = -1.54$, $p = 0.01$), again specifically in women ($b = -4.12$, $p = 0.02$; women vs. men: $p = 0.05$). In women, effects again were predominantly driven by those who transitioned from pre/peri- to postmenopause during the 8-year follow-up period ($b = -5.92$ $p < 0.0001$) compared to those that were postmenopausal at both time points.

Conclusions: Results suggest that midlife metabolic health is related to mitochondrial function and has a greater longitudinal impact on memory performance in women compared to men. Further, results suggest that midlife metabolic health may impact memory decline in women during the transition through menopause. However, over time, the healthy menopausal brain may compensate, resulting in attenuation of memory decline.

Keywords: Memory, Sex Differences, Menopause, Oxidative DNA Damage, Metabolic Function

Disclosure: Nothing to disclose.

P12. Metabolic Factors Underlying Individual Differences in Cognition and Brain Structure in Healthy Postmenopausal Women

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Background: The cognitive changes that occur at menopause have not yet been connected to late life risk for pathological aging and Alzheimer's disease (AD). Understanding the neurobiological factors related to individual differences in cognition at menopause is critical for understanding normal cognitive aging and identifying risk factors for pathological aging in women. We used data from a subset of subjects from the Cholinergic Health After MenoPause (CHAMP) Study (clinicaltrials.gov NCT04129060) to examine how blood pressure, cholesterol, and blood glucose influenced relationships between brain structure and cognitive performance in healthy postmenopausal women.

Methods: At screening all women gave blood samples, had vital signs measured, and completed neuropsychological assessments including Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). On the first study day of the larger clinical trial, subjects had a brain MRI where structural and functional scans were obtained. Structural MRI data were analyzed with FreeSurfer version 7.1.1 to extract volumes for hippocampal subfields as well as limbic regions including the basal forebrain, fornix, hypothalamus, and mamillary bodies that were then corrected for total intracranial volume. We examined relationships between brain structure, performance on the neuropsychological assessments, and the metabolic measures including systolic and diastolic blood pressure, fasting glucose and hemoglobin A1C, and HDL and LDL cholesterol.

Results: Forty-five women contributed data to this analysis and were an average age of 59.3 years (SD 5.1 y). They were all postmenopausal, medically healthy, and cognitively normal as defined by our study enrollment criteria. Inclusion criteria allowed medication for controlled high blood pressure (nine women), hyperlipidemia (four women), and Type 2 diabetes (one woman). We examined relationships between variables of interest and then used partial correlations to examine the cognition and brain structure relationships that remained after controlling for the metabolic factors. First, age was correlated with systolic blood pressure ($r = .22$, $p = .03$) but not other metabolic, cognitive, or brain volume data. By contrast, metabolic measures that were consistently positively correlated with cognitive performance and brain volume from the limbic regions and hippocampal subfields included systolic blood pressure, hemoglobin A1C, and LDL. We used a partial correlation approach to examine relationships between neuropsychological performance and brain structure after controlling for metabolic factors. We found consistent relationships between immediate memory from the RBANS and the volume of brain regions in the limbic areas including the left mamillary body ($r = .51$, $p < .001$) and left fornix ($r = .38$, $p = .02$) after controlling for systolic blood pressure, hemoglobin, and LDL. Relationships remained between immediate memory and the right hippocampal body ($r = .52$, $p < .001$) and right hippocampal tail ($r = .48$, $p < .003$) after controlling for systolic blood pressure, LDL, and hemoglobin A1C. Relationships that were somewhat weaker were also observed between delayed memory on the RBANS with some of the hippocampal subfield measures when controlling for systolic blood pressure, hemoglobin A1C, and LDL ($ps < .05$).

Conclusions: These data show that controlling for some metabolic variables allowed us to observe relationships between memory performance and limbic and hippocampal volumes in healthy, cognitively normal, postmenopausal women. These relationships were observed at smaller levels before controlling for metabolic measures. We interpret these findings as evidence that there are relationships between cognitive performance and brain volumes of brain regions relevant for those cognitive processes that are stronger after controlling for subclinical metabolic factors. These data have implications for understanding cognition and brain relationships in women after menopause and highlight the importance of examining metabolic processes that influence brain aging. These data also highlight the need for good control of blood pressure, cholesterol, and blood sugar in women as they age.

Keywords: Menopause, MRI, Cognition

Disclosure: Nothing to disclose.

P13. Regional Grey Matter Volume Predicts Symptom Improvement in a Randomized Clinical Trial of Tai Chi in Geriatric Depression

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Background: Geriatric depression (GD) is associated with significant medical comorbidity, cognitive impairment and brain atrophy, premature mortality, and suboptimal treatment response. More efficacious treatment to improve mood, cognition and quality of life in GD are therefore needed. Mind-body interventions like Tai Chi are promising adjunct treatments to antidepressants. We tested the effect of three months of Tai Chi vs. health education control on grey matter volume (GMV) change and whether baseline GMV predicted symptom and cognitive outcome differentially in the two groups.

Methods: Forty-nine older adults over 60 (38 women, 11 men) diagnosed with major depressive disorder undergoing concurrent antidepressant treatment were randomized to either Tai Chi training ($N = 26$) or health education control ($N = 23$). We administered clinical scales for depression and anxiety and participants were evaluated with a comprehensive neuropsychological battery at baseline and follow-up. In addition, participants underwent an MRI scan at baseline and follow-up. We used general linear models (GLMs) to test time-by-group interactions on these clinical and cognitive scores. Freesurfer version 6.0 was used to process T1-weighted images and perform voxel-wise whole-brain GLMs of group-by-time on symmetrized percent GMV change. Furthermore, we tested group differences in the relationship between baseline GMV and symptom change. All models included age and sex as covariates.

Results: The groups did not differ in demographics or clinical scores at baseline ($p > .05$) and there were no group differences in symptom change from baseline to follow-up ($p > .11$). There were no group differences in treatment-related GMV change. However, while lower baseline GMV in several clusters in the Tai Chi group was associated with larger improvements in anxiety, the health education control group did not show such effects. This pattern was similar for depressive symptoms in a cluster in the right precuneus.

Conclusions: Three months of Tai Chi may be insufficient time to lead to clinical improvements and structural brain changes in GD. However, individual GMV may predict symptom improvements. Longer trials are needed to investigate the long-term effects of Tai Chi on clinical symptoms and neuroplasticity.

Keywords: Late Life Depression, Geriatric, Cognition, Neuroimaging

Disclosure: Nothing to disclose.

P14. Speech Biomarkers for Delirium and Cognitive Impairment in Hospitalized Older Adults

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Background: Up to 50% of hospitalized older adults (75+) experience delirium, which is associated with increased morbidity, mortality, and resource utilization, as well as a 12-fold increased risk for new-onset dementia. Yet, 75% of delirium remains undiagnosed. Few studies have systematically studied speech and language disturbances in hospitalized older adults with delirium, and to our knowledge, no previous study has attempted to objectively quantify these phenomena with computational methods. The objectives of this study were to 1) systematically evaluate and describe speech and language disturbance in hospitalized older adults with and without delirium, 2) evaluate whether automated speech analysis could be feasibly implemented for acutely ill hospitalized patients, and 3) explore whether computational speech and language features could be used to diagnose patients with delirium.

Methods: Hospitalized older adults aged 75 years and older ($n = 33$) were recruited across three medicine units at an academic hospital. Patients with documented diagnosis of dementia were excluded. Delirium assessment was completed by trained research assistants and verified by a delirium expert (LS), and included: Orientation, 3-item recall, confusion assessment method (CAM) long form; and Richmond Agitation and Sedation Scale (RASS). Diagnosis was based on these datapoints and the DSM-5 criteria for delirium. Clinical ratings for language disturbance were completed using the Scale for the Assessment of Thought Language and Communication (TLC). Audio recordings were collected for open-ended prompts, a paragraph reading, and fluency tasks, then transcribed verbatim. Recordings and transcripts were processed separately for each task using an automated pipeline to extract acoustic (prosody and voice quality, speaking tempo and pauses) and textual features (semantic coherence, dysfluencies and speech errors, lexical characteristics, parts-of-speech, speech quantity). To reduce the feature space, we first selected only the features which showed a trend-level correlation with the total CAM score ($p < 0.10$), then visually inspected correlation plots to remove redundancy. The final feature set included 26 measures. Group effects were compared using ANOVA and chi-squared tests, correlations were measured with Pearson coefficients, and category fluency totals were compared to normative data using one-sided t-tests. We used binomial elastic net regression models to predict delirium status (delirium(-) vs. delirium(+)), training on the full dataset with 10-fold internal cross-validation.

Results: Of the 33 participants, 10 met criteria for delirium (Delirium(+)) and 23 did not (Delirium(-)). The Delirium(+) group scored significantly higher on total TLC score ($p = 0.05$, $d = 0.81$) and incoherence ($p = 0.001$, $d = 1.41$). Participants with delirium scored lower on category fluency compared to those without delirium ($p = 0.02$, $d = -0.97$), and both groups scored lower than the normative population (Delirium(-): $p < 0.001$, $d = -1.22$; Delirium(+): $p < 0.001$, $d = -2.20$). Higher CAM score was correlated with total TLC score ($r = 0.41$, $p = 0.02$), incoherence ($r = 0.58$, $p < 0.001$), loss of goal ($r = 0.36$, $p = 0.04$) and lower category fluency ($r = -0.41$, $p = 0.02$). Delirium status was predicted with demographics alone, demographics and clinical speech ratings, demographics and computational speech/language features, or all of these. The model with demographics and computational speech/language features performed best, classifying delirium status with accuracy of 78%, kappa of 0.4, and area under the curve of 0.90. All 23 Delirium(-) participants were correctly identified, in addition to 8/10 Delirium(+) participants. In this model, presence of delirium was most highly predicted by speech errors during paragraph reading, transcribed symbols (including punctuation, restarts, and incomplete words) in the open-ended narratives, use of determiners in the family narrative task, and semantic diversity (lexical ambiguity) of words given during the fluency tasks. Absence of delirium was most highly predicted by filled pauses during the family narrative task (e.g., "um," "uh"), use of adverbs in open-ended narratives, use of adjectives during the picture description, and variance in jitter (fluctuations in voice amplitude) during fluency tasks.

Conclusions: Hospitalized older adults with delirium demonstrate significant impairments in speech and language. In particular, delirium is associated with incoherence, loss of goal, and decreased category fluency scores. Hospitalized older adults without delirium may also demonstrate a subtle cognitive impairment relative to the normative population, as reflected in lower category fluency scores. Automated speech and language analysis was feasibly completed in the acute care setting, and computational features were highly informative for predicting delirium status, providing a proof of concept for this approach.

Keywords: Delirium, Older Adults, Natural Language Processing (NLP), Cognitive Impairment, Automated Natural Speech Analysis

Disclosures: Winterlight Labs: Consultant, Contracted Research (Self); North Shore Therapeutics: Board Member, Founder (Self)

P15. Biological Aging in Alcohol Use Disorder: A Multi-Method Investigation in Blood and Brain

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Background: Alcohol Use Disorder (AUD) is associated with increased mortality and a variety of diseases, such as neurodegenerative diseases and cancer. Biological aging is defined as the gradual deterioration of biological functions, whereas chronological age describes the time since birth. Accelerated biological aging could provide an explanation for the increased mortality and morbidity in AUD. There are multiple biomarkers to estimate biological aging (BioAge-markers), such as epigenetic clocks, telomere length (TL), and mitochondrial DNA copy numbers (mtDNAcn), which are also used as a marker for inflammation. Epigenetic clocks estimate different age-related phenotypes, such as chronological age (Horvath), biological age (Levine), and telomere length based on DNA methylation levels. The acceleration of aging can be calculated by regressing chronological age on epigenetic age and analyzing the residuals. So far, no analysis has investigated multiple BioAge-markers in the context of AUD in peripheral blood and it is unclear how they relate to AUD in other tissues. While AUD has an impact on the entire body, addiction is thought to develop in the brain. Recent studies point towards changes in DNA methylation and gene expression in neuroinflammation-related pathways in the brain, which could be related to mechanisms of biological aging. TL declines naturally with aging, although the effect is much smaller in the brain than in whole blood leukocytes because there are fewer cells that differentiate/maturate. At the same time, increased levels of neuroinflammation in AUD could lead to a reduction in telomere length and altered mtDNAcn (and levels of heteroplasmy). It is hypothesized that biological aging is accelerated in AUD and that this effect is more pronounced in whole blood than in brain samples.

Methods: BioAge-markers were measured in two cohorts of individuals with AUD and controls. The first cohort consisted of 179 individuals, 84 of which had a diagnosis of AUD, in whom whole blood samples were collected at the start of withdrawal treatment. The second cohort consisted of postmortem human brain samples. The brain tissue was obtained from the New South Wales Brain Tissue Resource Centre at the University of Sydney. A total of 91 samples from Brodmann Area 9 (N cases = 43), 94 from the caudate nucleus (N cases = 44), and 63 from the ventral striatum (N cases = 33) were included in the present analysis.

For both cohorts, telomere length and mtDNAcn were measured by quantitative real-time polymerase chain reactions (qRT-PCR) and methylation levels were analyzed with the Illumina Infinium EPIC BeadChip. Levine's, Horvath's, and the telomere epigenetic clocks were calculated using the R package methylclock. For Levine's and Horvath's clocks, the age acceleration, controlled for cell-type composition, was used as an outcome, while the raw estimate was used for estimated telomere length to ensure comparability with measured TL. We performed linear regression analyses with the BioAge-markers as outcomes and included sex, AUD status, an AUD status*age interaction term, smoking, and age (for measured and estimated TL and mtDNAcn) as covariates. To explore the association between the BioAge-markers, we calculated Pearson correlation between the markers in each sample.

Results: In peripheral blood, we identified significant associations between the BioAge-markers and AUD status, all pointing towards accelerated biological aging in AUD. All BioAge-markers were associated with chronological age in the expected directions and small to medium correlations between the BioAge-markers were observed. The highest correlation was observed for measured and estimated telomere length with $r = 0.26$.

In postmortem human brain samples, a significant association of measured TL and AUD ($p = 0.047$) and a trend for the AUD*age interaction ($p = 0.061$) was observed in the caudate nucleus. Only small correlations between the BioAge-markers were observed in postmortem brain samples.

Conclusions: The present study is the first to investigate telomere length, epigenetic clocks, and mtDNAcn from postmortem brain and whole blood samples in individuals with AUD compared with healthy controls. We found evidence for increased biological aging in AUD, as shown by significant associations between epigenetic age acceleration, decreased telomere length and decreased mtDNAcn with AUD in whole blood. The investigation of biological aging in AUD seems to be most appropriate in blood samples. The present study cannot distinguish whether there is no AUD-related increase in biological age in the brain or whether BioAge-markers do not capture age acceleration equally well in brain tissue. Levine's and Horvath's epigenetic clocks are designed to be multi-tissue biomarkers and should therefore perform similarly in brain tissue. Although the telomere clock was trained on DNA methylation in blood, we observed only a small association between estimated and measured telomere lengths in the present study. Further studies investigating brain and blood tissue from the same individual are needed to draw conclusions about the overlap of molecular mechanisms of biological aging in blood and brain.

Keywords: Biological Aging, Alcohol Use Disorder, Telomere Length, Mitochondrial DNA Copy Numbers, Epigenetic Clock

Disclosure: Nothing to disclose.

P16. Cholinergic Integrity in Postmenopausal Women Measured by [18F]FEOBV PET: Relationship to Age, Cholinergic Anatomy, Cognitive Performance and Alzheimer's Disease Risk Factors

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Background: Women appear at higher risk for Alzheimer's disease. This increased risk may be linked to loss of estradiol (E2) support to basal forebrain cholinergic systems. The cholinergic system has

been implicated in many aspects of the cognitive effects shown after E2 administration including attention, working memory, and effort-demanding tasks such as verbal memory. The Cognitive Health After MenoPause (CHAMP, NCT04129060) study is examining cholinergic functional integrity in normal postmenopausal women by measuring working memory performance, functional brain activation, cholinergic basal forebrain structure, and uptake of the cholinergic PET radiotracer [18F]-fluoroethoxybenzovesamicol ([18F]-FEOBV) and whether individual differences in AD biomarkers including amyloid and tau fluid markers (plasma, CSF), amyloid PET, and markers of neurodegeneration (nFL) are related to cognition, brain activation after anticholinergic challenge, cholinergic anatomy and functional integrity.

Methods: One hundred twenty healthy cognitively normal postmenopausal women aged 50-70 years undergo a multimodal assessment including sleep and activity measures, cognitive performance, blood/plasma for genetics and AD biomarkers, and lumbar puncture for CSF biomarkers. In addition, participants undergo MRI scan during anticholinergic drug challenge (mecamylamine) to examine working memory-related brain activity as well as cholinergic basal forebrain structure/volume. Participants underwent a 30 min static PET scan starting three hours after injection with 6.5 MCi ±10% of [18F]-FEOBV. White matter mask from T1 MRI was applied to the [18F]-FEOBV PET as reference region for the calculation of the standardized uptake value rations (SUVRs) in global GM composite ROI (parietal, frontal, temporal, occipital, cingulate, hippocampus) as well as within individual ROIs. Basal forebrain (BFB) volumes for each hemisphere were extracted from T1 MRI for the sub regions Ch1-3 and the Nucleus Basalis of Meynert (NBM; Ch4). We examined how regional and whole-brain [18F]-FEOBV PET uptake correlated with age, Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) cognitive performance and amyloid PET using florbetapir.

Results: Preliminary data show that ten healthy postmenopausal women (mean age: 57.3 ± 5.1 years) have thus far completed cholinergic PET. Global SUVR was observed to decrease with increasing age ($r = -0.52$, $p < .1$). Larger GM volume of the cholinergic NBM for both hemispheres LH: $r = .51$, RH: $r = .65$, $p = .06$) was associated with higher global [18F]-FEOBV SUVR. Global [18F]-FEOBV uptake was positively associated with global cognition as measured by the RBANS Total ($r = 0.41$). Finally, [18F]-FEOBV uptake was lower in participants who showed clinically elevated amyloid levels by florbetapir PET. Assessment of the relationship of cholinergic integrity via [18F]-FEOBV, BFB volume and cholinergic functional imaging to reproductive and menopause history and AD biomarker data is continuing.

Conclusions: Decline of cholinergic integrity post menopause may increase the risk of future cognitive decline and [18F]-FEOBV may be a useful marker of future cholinergic decline along with AD-related biomarkers. The relationships between cholinergic integrity and other predictors in this pilot sample support the hypothesis of a menopausal increase in risk for AD-related pathology in some women, which may be accelerated by loss of cholinergic tone through estradiol depletion following the menopause transition.

Keywords: Acetylcholine, Women's Health, Alzheimer's Disease, Positron Emission Tomography Imaging

Disclosure: Nothing to disclose.

P17. Insulin Resistance and Accelerated Cognitive Aging

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Background: Insulin resistance is an early sign of metabolic dysfunction with the potential to lead to neuropsychiatric sequelae in the long term. Early detrimental effects of IR on brain function have been demonstrated with both indirect and direct measures of IR. However, the trajectory of pathological brain aging and its mediators and moderators are not well-defined. In order to identify whether insulin resistance in otherwise healthy young and middle-aged adults is associated with preclinical signs of neuropsychiatric impairment, we recruited 126 overweight but nondiabetic, non-depressed individuals who completed an insulin suppression test for direct measure of insulin resistance as well as a battery of cognitive and neuropsychiatric measures. We describe the study design and cohort and the relationships among baseline metabolic function and cognitive and psychiatric outcomes, including both subtle cognitive changes as measured by neuropsychiatric testing, and subclinical mood symptoms.

Methods: Participants were recruited through flyers distributed on-campus and in communities surrounding Stanford University. Potential participants were screened for the following eligibility criteria: 23 to 61 years of age, body mass index (BMI) of 21 to 41 kg/m2, at least 12 years of education and a Mini Mental Status Exam (MMSE) score of >27, and adequate visual and auditory acuity for cognitive testing. Insulin-mediated glucose uptake was directly measured by a modified and validated version of the insulin suppression test (IST). The plasma glucose and insulin concentration values during the last 30 min of the infusion were averaged to determine the steady-state plasma glucose (SSPG) and steady-state plasma insulin (SSPI) concentrations, respectively. Plasma insulin, leptin, and cortisol were measured at the Core Laboratory for Clinical Studies at Washington University School of Medicine. Average relative telomere length was measured by quantitative PCR using a method adapted from the original published method by Cawthon et al. and expressed as the ratio of telomere abundance vs. a single copy gene (human b-globin) abundance (T/S ratios). Depressive symptoms were rated using the 24-item Hamilton Depression Rating Scale (HDRS). A cognitive battery of four hippocampus-linked tests was used in all participants in a fixed or randomized order.

Results: Insulin resistance was associated with impaired performance on a visuomotor task (Purdue Pegboard) as well as increases in subclinical symptoms of depression. Furthermore, the direct measure of insulin resistance (SSPG concentration), but not fasting plasma insulin, fasting plasma glucose, or BMI, was associated with higher levels of depressive symptoms in subjects without clinical depression. Only the association of SSPG concentration with depressive symptoms passed multiple testing correction. The association of SSPG concentration with depressive symptoms was robust to adjustment for age, education, BMI, and fasting glucose. Telomere length, obtained as a measure of chronic physiologic stress or allostatic load, showed weak negative correlations with SSPG and fasting glucose, and stronger ones with age and dyslipidemia.

Conclusions: This study of young and mid-life adults explores premorbid changes in metabolic function, mood, and cognition that may presage the development of overt psychiatric illness or cognitive decline. We found that even in this population of young, healthy but overweight adults, there is a range of insulin sensitivity, where greater degrees of insulin resistance were associated with higher BMI, hypertriglyceridemia, low HDL cholesterol, and increased circulating levels of fasting insulin, C-peptide, and leptin. Furthermore, higher degrees of IR were associated with greater degrees of subclinical depressive symptoms and with impaired performance specifically in a visuomotor task, though not in cognitive assessments across the board. Additionally, the significance of the association between IR and both visuomotor performance and subclinical depressive symptoms were attenuated when BMI and fasting glucose were included in the model, suggesting etiological overlap among

these factors. We hypothesize that cognitive and psychiatric declines related to IR likely have a long subclinical prodrome that precedes overt impairment by many years. These data provide a baseline by which this hypothesis may be tested in future analyses.

Keywords: Insulin Resistance, Telomeres, Cognitive Decline

Disclosure: Nothing to disclose.

P18. Effects of Chronic Oral THC Self-Administration on Working Memory Across the Lifespan

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Background: Individuals over the age of 65 have become the fastest-growing demographic of cannabis users. As the number of older adults in the US is expected to reach 90 million by 2050, it is imperative to understand the potential cognitive impacts of cannabis/cannabinoid use in this age group. Cannabis and cannabinoids such as delta-9-tetrahydrocannabinol (THC, the major psychoactive component of cannabis) generally tend to impair cognitive performance, but almost all studies of cannabis and cannabinoids have been conducted in young adult subjects. Given that many aged individuals already exhibit cognitive deficits, it is important to determine how cannabis/cannabinoids affect cognition in this population.

Methods: To address this issue, we evaluated the effects of chronic oral THC self-administration on performance in a working memory task in rats. Young adult (5 months, $n = 20$) and aged (23 months, $n = 18$) Fischer 344 x Brown Norway F1 hybrid rats of both sexes were trained in operant chambers on a delayed response working memory task that depends critically on the prefrontal cortex. In this task, rats were trained in daily 60 min sessions (over 100 trials/day) to remember the left/right position of a response lever over short (0-24 s) delays to earn food rewards. Upon reaching stable performance on the task, rats were given 3 weeks of daily 60 min access to either plain gelatin or gelatin containing 1.0 mg/kg THC in their home cage in the afternoons, while testing in the working memory task continued in the mornings. In a second experiment, behaviorally-naïve aged rats were given daily access to either plain gelatin or gelatin containing 1.0 mg/kg THC in their home cage, and blood samples were collected after 3 weeks for analysis of circulating inflammatory factors. Behavioral data were analyzed via multi-factor ANOVA, with drug condition, age, and sex as between-subjects variables and working memory delay as a within-subject variable. Cytokine data were analyzed via Welch's t-tests.

Results: As expected, among rats that consumed plain (control) gelatin, aged rats performed worse than young on the working memory task ($F(1,30)=10.38$, $p = .003$). More importantly, there was an age x drug condition x delay interaction, such that THC had distinct effects in young adult vs. aged rats, particularly at long delays ($F(6,180)=2.45$, $p = .03$). Focused comparisons of the effects of THC in each age group showed that among young adults, rats that consumed THC gelatin performed comparably to rats that consumed plain gelatin (no main effects or interactions involving drug condition). In contrast, aged rats that consumed THC gelatin performed significantly more accurately than rats that consumed plain gelatin ($F(1,14)=5.01$, $p = .04$), particularly at long retention delays ($F(6,84)=2.76$, $p = .02$). There were no sex differences in the effects of THC consumption in either young adult or aged rats (no interactions involving sex and drug condition). In the second experiment, rats that consumed THC gelatin for 3 weeks had reduced levels of some pro-inflammatory cytokines (e.g., TNFα, $t(19.99 = 16.61$, $p < .001$) and increased levels of some anti-inflammatory cytokines (e.g., IL-10, $t(19.99) = 6.55$, $p < .001$).

Conclusions: These findings suggest that at advanced age, chronic cannabis/cannabinoids may have beneficial effects on at least one aspect of executive function (working memory). In addition, the oral route of administration (which is used frequently by older adults) and the fact that consumption was voluntary indicate that the THC dose employed here is within a tolerable range that does not produce significant adverse effects. Finally, given that alterations in the peripheral inflammatory environment have been associated with age-related cognitive impairments, the fact that chronic THC appears to attenuate some aspects of inflammatory signaling suggests one mechanism by which it may exert beneficial effects on working memory.

Keywords: Cannabis, Aging, Working Memory, Rat, THC

Disclosure: Nothing to disclose.

P19. Ketogenic Diet Interacts With Estrogen to Restore Memory in Aging Females

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Background: Women typically live to older ages than men and are more likely to be afflicted by Alzheimer's disease (AD). Indeed, the influence of biological sex and sex hormones is the focus of ongoing study to determine the mechanisms that contribute to worse than-expected cognitive outcomes in aging women and to identify interventions that are appropriate to protect cognition in a sex-dependent manner. The ketogenic diet has emerged as a potential intervention to treat central and peripheral signs of AD, which include defective brain glucose metabolism and elevated resting blood glucose. However, it is not clear the degree to which normally aging males and females experience the same cognitive benefits of the ketogenic diet and whether age-related reductions in circulating estrogens, which typifies menopause, interacts with effects of this diet. Consequently, we investigated the effects of a ketogenic diet on cognition in normally aging male and female rats (Study 1) or the effects of diet in a surgical model of menopause with and without hormone replacement (Study 2).

Methods: In Study 1, male and female Fisher 344/Brown Norway F1 hybrid rats were obtained from the National Institute of Aging at 6 or 24 months of age. Rats were assigned to consume sex-adjusted, calorie-matched diets that provide most calories from carbohydrates (control diet) or medium-chain triglycerides (ketogenic diet). A subset of rats was included as ad libitum-fed controls, with unrestricted access to normal rat chow, to differentiate effects of calorie-restriction from diet composition. ($n = 7$-10/sex/age/diet). After 6 weeks on diet, during which time body weight and blood levels of glucose and β-hydroxybutyrate were monitored continuously to confirm nutritional status, all rats were characterized in the Morris water maze (MWM) using an 8-day place-learning, spatial reference memory protocol to evaluate hippocampus-dependent cognition. For Study 2, 18-months-old female rats were ovariectomized (OVX) and implanted subcutaneously with silastic implants to release estrogen (E2) or inert vehicle (OIL), before assignment to specialized diets ($n = 10$-12/hormone/diet) and behavioral testing as described in Study 1.

Results: In Study 1, sex, age, and diet all significantly influenced spatial memory; males were overall better than females, aged performed worse than young, and calorie-restriction and

ketogenic diet improved memory relative to ad libitum controls. There was also a trend towards a sex-by-age-by-diet interaction. Follow-up comparisons revealed that young males exhibited better spatial memory following calorie restriction but in older males, only ketogenic diet improved memory compared to ad libitum-fed controls. In females, calorie-restriction improved spatial memory, but only young females, not aged females, showed better memory after consuming ketogenic diet. The loss of benefit conferred by consumption of ketogenic diet could relate to changes in circulating E2 as we determined in Study 2 that replenishing E2 to middle-aged rats after OVX led to ketogenic diet-related enhancement of memory that was not evident in OIL-treated controls. Further, replenishing E2 to animals consuming the calorie-matched control diet did not improve performance relative to OIL.

Conclusions: We conclude that dietary interventions, including calorie restriction and ketogenic diet, are practical and effective interventions that protect against age-associated cognitive decline. However, the efficacy of certain diets to preserve memory varies between males and females over the lifespan. Aging females do not show the same cognitive benefits of ketogenic diet that was observed in younger females or aged males. Positive effects of ketogenic diet in aging females can be observed after administration of E2, suggesting that this diet might confer cognitive advantages in the context of hormone replacement therapy initiated close to the onset of menopause in middle-aged women. Collectively, this insight could be used to optimize and personalize dietary guidelines for subpopulations of older individuals at risk for memory loss and AD.

Keywords: Ketogenic Diet, Declarative Memory, Sex Differences, Brain Aging, Alzheimer's Disease

Disclosure: Nothing to disclose.

P20. Neuroadaptive Acetylcholinesterase Regulation in Stress and Cognitive Aging

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Background: Synaptic release of the neurotransmitter "acetylcholine (ACh)" has been implicated in the neuromodulation of cognitive capacity. Our previous research has shown that a reduction in ACh transmission in cortical networks increases cognitive vulnerability in aging. However, the mechanisms that regulate age-related changes in cholinergic transmission and individual differences in cognitive aging remain unknown. Acetylcholinesterase (AChE) is a hydrolytic enzyme which promotes ultra-fast cholinergic signaling in brain circuits. Here we sought to identify the role of synaptic (AChE-S) and readthrough (AChE-R) variants in AChE regulation and cognition in aging. These variants are reported to be altered in age-related neuropathologies. Because stress exposure has previously been shown to alter AChE expression and psychological stress is a risk factor for age-related cognitive decline, we also examined the effects of chronic variable stress (VS) on AChE expression and catalytic activity.

Methods: Young (3 mo) and aged (22 mo) rats of both sexes were trained in an operant attention task that requires the animals to discriminate between signaled and non-signaled cues for subsequent reward. After attaining performance criterion, rats were subjected to a distractor test session following which brain tissues from the prefrontal cortex (PFC) and hippocampus (HPC) were microdissected for AChE mRNA and protein isolation. The impact of VS on AChE biochemistry was investigated in another cohort of adult male and female rats. For these studies, the AChE activity was assessed in both salt-soluble and detergent soluble fractions that contain different proportions of AChE-R encoding monomeric (G1) and AChE-S encoding tetrameric (G4) subunits, respectively.

Results: In general, aged rats performed poorly on the distractor test session as compared to their young counterparts ($F(1,28)=15.17$; $p < .001$). However, their performance remained highly variable with some animals exhibiting significant impairments ($p < .001$ vs age-unimpaired) while others performed at par with the young animals ($p > .43$). Quantification of mRNA expression using RT-qPCR revealed an age-dependent reduction in total AChE expression in the PFC ($p < .05$) that was primarily driven by the S variant. Interestingly, the average ratio of R/S variants was found to be higher in aged rats in both brain regions (PFC: 1.27 vs.1.01 in young; HPC: 1.93 vs. 1.02) illustrating a transcriptional shift. Our stress experiments revealed sex-specific differences with higher AChE activity in subcellular fractions rich in G1 and G4 subunits in females from the PFC ($p < .04$). However, VS reduced AChE activity in detergent-soluble fraction regardless of sex ($F(1,19)=30.65$; $p < .001$). Moreover, a sex x VS interaction was observed ($F(1,18)>3.72$; $p < .05$) depicting higher AChE-R subunits in males and lower AChE-S subunits in females.

Conclusions: Age-related reduction in AChE-S (more prominent synaptic variant) may possibly reflect a neuroadaptive role in preserving cholinergic function by reducing ACh hydrolysis. This interpretation also parallels reduced catalytic activity in the detergent soluble fraction mostly rich in the G4 isoform in VS-exposed rats. However, an increase in AChE-R variant during stress or aging may offset this protective effect. Collectively, these data indicate that an imbalance in AChE-R/S variants may underlie cognitive vulnerability in aging and accelerated cognitive decline with stress. Moreover, stress-induced compensatory changes in cholinergic signaling and its impact on age-related changes in cognitive processes may differ between sexes.

Keywords: Aging, Cognition, Stress, Acetylcholinesterase, Splice Variants

Disclosure: Nothing to disclose.

P21. In Vivo Calcium Imaging Reveals Sex Differences in Ventral Hippocampal Activity in Alzheimer's Disease Mice

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Background: Neuropsychiatric disturbances, such as depression and anxiety, are observed in 90% of Alzheimer's disease (AD) patients and are frequent in those at risk for AD. We've previously shown that anxiety is prevalent at an earlier age in female APP/PS1 AD mice and that women with anxiety and amyloid burden transition more quickly to dementia than men in the ADNI human cohort. We've also shown that there are sex-specific circuit and brain-wide network changes in AD mice at 6 months of age. Additionally, early-stage AD patients often exhibit hyperexcitability throughout the hippocampus which correlates to memory impairment and eventually worsening of the disease. Here, we aimed to determine how and when this neuronal activity is changed in aging and AD mice.

Methods: Male and female control and APP/PS1 mice at 2 and 6 months of age were injected with a AAV-syn-GCaMP8f virus and a GRIN lens was implanted targeting the ventral hippocampus ($n = 4$-6). After 3 weeks of recovery, mice were run through open field, elevated plus maze, and contextual fear conditioning to

assess anxiety-like behaviors and cognition. During each task, we recorded calcium activity using Inscopix software and analysis programs. Data were analyzed using ANOVA, with repeated measures when appropriate. Tukey was used for all post-hoc comparisons. Alpha was set to 0.05 for all analyses. Data are expressed as means + /- SEM.

Results: We found sex-specific changes in neuronal activity and behavior. Females exhibited earlier anxiety and cognitive decline as previously reported ($p < 0.05$). Male AD mice did not exhibit increased anxiety-like behavior in these tasks relative to controls. However, male AD mice exhibited changes in neuronal activity compared to controls depending on time of day in the elevated plus maze ($p < 0.001$). Additionally, this increase in calcium transients correlated with increased anxiety behavior in males ($p < 0.05$). We are actively continuing these experiments to add a 6-month age group to determine how aging impacts the neuronal excitation/inhibition balance.

Conclusions: Both men and women with an AD diagnosis will develop cognitive decline, but the mechanisms driving this decline might be different. Therefore, it is essential to understand the differences in disease progression in order to create personalized therapeutics. Our initial studies provided a snapshot of the sex-specific mood and circuit changes throughout the brain, but with our in vivo calcium imaging system we are now able to view dynamic brain changes while the animal ages and track individual cellular activity across different behavioral paradigms. These results provide evidence that brain hyperexcitability starts earlier in males while females show more behavior changes early in the disease.

Keywords: Alzheimer's Disease, Anxiety, Sex Differences, In Vivo Calcium Imaging, Hippocampus

Disclosure: Nothing to disclose.

P22. Prelimbic Correlates of Fear Memory Generalization in Endocannabinoid 2-Arachidonoylglycerol Deficient States

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Background: Post-traumatic stress disorder (PTSD) is a psychiatric disorder that develops following exposure to a traumatic event. Its lifetime prevalence is estimated to be 6.8%. Fear responses to stimuli that were previously present during a traumatic experience enables survival. However, in PTSD, patients may experience generalization of their fear responses even to otherwise safe stimuli. The endocannabinoid (eCB) system is a retrograde neurotransmitter system that has been implicated in regulating fear and anxiety. 2-arachidonoylglycerol (2-AG), one of the major centrally active eCB lipids, is thought to mediate resiliency to traumatic experiences. Here we aim to explore if 2-AG is involved in regulating fear generalization and characterize how prelimbic neurons are involved in fear discrimination and how 2-AG mediates this process.

Methods: We used a mice model of fear-conditioning in combination with systemic injections of a drug (DO34) that blocks synthesis of 2-AG. Male mice were exposed to 8 tone presentations (CS+), each co-terminating with a brief electric foot-shock. The following day (Day2), mice were injected with DO34 (50 mg/kg) intraperitoneally 2 hours prior a memory recall test conducted by exposing mice to alternating presentations of 2 novel tones (NT) and 2 CS + tones. Fear responses were measured by quantifying the percentage of time during tone presentations that the mice exhibit freezing (immobility except movement required for breathing). For our in vivo recording experiments, mice were injected with a viral vector expressing the calcium indicator GCaMP7f in the prelimbic cortex (PL) and a miniature GRIN lens above PL. Mice were allowed to recover and were habituated to having a miniaturized microscope attached to a baseplate sitting over the lens. Following habituation, mice were conditioned to tones as described above while simultaneously recording calcium activity using the miniaturized microscope. The following day, mice were injected with either vehicle or DO34 and were exposed to NT and CS + tones as described above. The recorded imaging data was processed and individual calcium traces were extracted using CNMFe segmentation algorithm. Peritone histograms were generated from the Z-scored data. Neurons with post-shock responses exceeding ± 3 Z-scores in any 2 consecutive 1s bins during the 10s post-shock period were considered to have significant excitatory or inhibitory responses ((+) responsive and (-) responsive, respectively). SVM decoder analysis was performed on peri-event extracted traces to assess if NT and CS + were decodable from each other.

Results: To address if 2-AG is involved in regulating fear generalization, mice were injected with DO34 prior to fear recall ($n = 19$ and 16, vehicle and DO34 respectively). Mice injected with DO34 showed a significant increase in freezing only to novel tone presentations (all p's<0.0338), but not to the CS + , suggesting that blocking 2-AG signaling enhances fear generalization to novel tones.

To address how fear generalization is represented at the neural level and how this representation is modified by reductions in 2-AG, we repeated our fear conditioning experiment while recording changes in single cell calcium activity within PL. Our imaging data shows that DO34 increased average neuronal activity to novel tones ($n = 636$ and 670 neurons, vehicle and DO34 respectively). Focusing on neurons that significantly change their activity to the tone did not reveal any differences in magnitude between groups for (+) responsive ($n = 114$ and 204 neurons, vehicle and DO34 respectively). Interestingly, the proportion of neurons that respond to both CS + and novel tones was significantly larger for the DO34 group (10.62 % vs 18.36 %; chi square statistic= 7.3072, $p = 0.00687$) while the proportion of neurons that respond to the CS + was smaller (32.23 % vs 20.82 %; chi square statistic= 12.771, $p = 0.000352$. Thus reducing 2-AG signaling leads to increased generalization and this in turn is associated with an increased proportion of PL neurons that signal equally to both CS + and novel tones. We hypothesized that the increase in fear generalization may be due to a loss in the ability to discriminate between the NT and CS + by PL neurons. Surprisingly, using an SVM decoder we found that NT and CS + can be decoded from each other and in fact the decoder accuracy increases in the DO34 group (96.0% for Veh, 99.5% DO34, 43.3% shuffled control; all p's< 0.0002) .

Conclusions: Our present data suggests that 2-AG signaling may be required for maintaining the specificity of fear memories. Furthermore, reducing 2-AG leads to a larger proportion of PL neurons that fail to discriminate between CS + and novel tones. Interestingly, while there is an increase in the proportion of PL neurons that respond to both tones, the coding characteristics for NT and CS + are distinct enough that they can be decoded from each other. Thus, while the proportion of PL tone responsive neurons reflect generalization/discrimination the characteristic of the neuronal responses to NT and CS + are decodable from each other and independent of generalization state. All this may suggest that generalization may be driven by inputs to PL that are sensitive to 2-AG deficient states rather than arising in PL itself.

Keywords: Fear Conditioning, Prelimbic, Miniscope, in Vivo Calcium Imaging, Endocannabinoids

Disclosure: Nothing to disclose.

P23. Stress Cues Exposure Induced Excitatory Plasticity in the Pentapartite Synapse

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Background: Converging epidemiological studies indicate that a history of acute life-threatening events increases the incidence of post-traumatic stress disorder (PTSD), and a diagnosis for PTSD carries 30-50% comorbidity with substance use disorders (SUDs). Thus, patients with comorbid PTSD/SUDs have greater drug use severity and show poorer treatment outcomes than patients diagnosed with either alone. Using a rodent model, we found that a single stressful event experienced 3 weeks prior can enhance drug intake and trigger a number of enduring adaptations within corticostriatal synapses of the nucleus accumbens core (NAcore), which resemble drug-induced adaptations. We recently found that pairing the stressful event with a novel odor (stress conditioned stimulus, stress CS) elicits a conditioned stress response and recapitulates some of the behavioral and physiological aspects of PTSD. Previous publications have shown that drug-associated cue presentation evokes transient increases in the pentapartite synapse, including the pre and postsynaptic neurons, astrocytes, microglia, and the extracellular matrix (ECM). Given the overlap between the enduring adaptations produced by acute restraint stress and withdrawal from drug use, we hypothesize that the exposure to a stress CS elicits synaptic plasticity in the pentapartite synapse in NAcore. Moreover, medium spine neurons (MSNs) constitute 90-95% of the neurons in the NAcore and are chemically coded into two subtypes that selectively express D1 or D2 dopamine receptors. These two populations appear to subserve distinct behavioral functions, with D1 activation generally promoting behaviors and D2 activation inhibiting behaviors. However, the effect on stress CS exposure on accumbal population remains elusive.

Methods: Male and female Sprague Dawley and Long Evan rats were restrained for 2 hours in plexiglas cylinders and exposed to an odor that became a stress CS, or sham animals were left in new home-cage boxes exposed to an odor (neutral stimulus - NS). Three weeks after the stressful experience animals were exposed to a cage that contained bedding in one corner and stress CS or NS on the opposite corner (noxious object which to be buried). After completing the defensive burying task, animals were then euthanized, perfused and tissue was processed for the following analysis: 1) spine morphology studies using DiI lipophilic colorant, 2) matrix metalloproteinase (MMP) activity was quantified using zymography gel microinjections, 3) astrocyte morphology studies using GFAP-hM3dq-mCherry virus, and 4) microglia/macrophage populations expressing IBA-1 were semi-quantitatively analyzed by immunohistochemistry. Additionally, we recorded single cell Ca2+ dynamics in D1- and D2-MSNs from freely moving rats (D1 or D2-cre rats) using a miniature microscope (nVista) and virally expressed Cre-dependent Ca2+ indicator (GCaMP8M) during defensive burying task and extinction to stress CS.

Results: In all, we have observed key features of stress CS-induced changes in pentapartite synaptic plasticity. Stress CS exposure is associated with synaptic potentiation in NAcore, quantified by an increase in dendritic spine head diameter and spine density, and increased matrix metalloprotease-9 activity that catalyzes proteins from the extracellular matrix (ECM). Stress CS exposure also induced down-regulation of astroglial glutamate transporters (GLT-1) and retraction of astrocyte synaptic coverage compared to control and stress NS animals. Also, stress CS animals had induced changes in microglia morphology, as well as the number and volume of IBA-1+ cells compared to control and stress NS animals. Furthermore, we observed differential changes in Ca2+ dynamics in D1 vs D2-MSN during the defensive burying task and extinction to stress CS.

Conclusions: These data suggest that the neuroadaptations and calcium dynamics that coincide with 15 min defensive burying task may be correlated with the synaptic plasticity in NAcore that lead to differential stress coping responses. Previous data from the lab has shown that stress CS exposure did not induce sucrose-seeking in sucrose-trained rats, thus we hypothesize that the plasticity described here is potentially indicative of pathological features of stress disorder.

Keywords: Post Traumatic Stress Disorder, Nucleus Accumbens, Calcium Imaging

Disclosure: Nothing to disclose.

P24. Cell-Type Specific Induction of Cyclo-Oxygenase-2 in Layer II/III Prefrontal Cortical Neurons Mediates Stress-Induced Anxiety Phenotypes in Mice

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Background: The ability of the medial prefrontal cortex (mPFC) to exert top-down control of behavior is affected by stress. Stress is also known to produce transcriptional responses that influence long-term behavioral adaptations. The molecular response of mPFC to stress is incompletely understood, however, in part because of the region's cellular heterogeneity. There is great need for novel therapeutic targets for stress-induced behavioral disorders.

Methods: We used single nuclear sequencing (InDrops) to sequence over 100,000 nuclei from mPFC after a stress-inducing Pavlovian fear conditioning assay ($n = 8$ groups of $n = 2$-3 male mice) and identified cell-type specific differentially expressed genes after fear conditioning and fear expression. We have used fluorescent in situ hybridization (FISH) and immunohistochemical techniques to confirm markers for cell clusters found with single nuclear sequencing ($n = 4$-5 male mice). We have used a combination of pharmacology, cre/lox recombination, and slice electrophysiology with behavior to characterize candidates functionally.

Results: We identified Ptgs2, encoding cyclo-oxygenase 2, as an important candidate that is upregulated in layer II/III excitatory neurons after stress. Specifically, Ptgs2 was transiently upregulated with shock-induced fear learning and fear expression, along with Bdnf, Nptx2, and Lingo1, in a layer II/III neuronal population marked by the neuronal excitatory gene Slc17a7 and cell-type specific neuropeptide Penk. These dynamic cell-type specific expression patterns identified with snRNAseq were validated with quantitative fluorescent in situ hybridization. Using a pharmacological approach, we found that systemic lumiracoxib, a selective Ptgs2-inhibitor, led to a significant reduction in fear expression (2-way ANOVA, Drug effect, $p = 0.03$). Furthermore, genetic ablation of Ptgs2 in mPFC led to reduced stress-induced anxiety-like behaviors in the elevated plus maze (Unpaired student's t-test, $p = 0.02$). Layer II/III neurons expressing virally-induced Cre in a floxed Ptgs2 background exhibit baseline depolarization compared with non-transduced neighboring cells (Resting membrane potential, unpaired student's t-test, $p = 0.002$).

Conclusions: Together these findings suggest that Ptgs2 is expressed in a dynamic, cell-type specific way in Layer II/III Penk+

neurons in mPFC, and that its role in prostaglandin and /or endocannabinoid regulation within these neurons may be an important mediator of stress-related anxiety behavior.

Keywords: COX-1 and COX-2, Anxiety and PTSD, Medial Prefrontal Cortex, Mouse Models, Single-nucleus RNA Sequencing

Disclosure: Nothing to disclose.

P25. A Link Between Social Buffering of Acute Stress and Tumor Necrosis Factor

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Background: The social buffering of stress occurs when an animal displays a reduced stress response in the presence of a conspecific. Few studies have investigated the physiological changes that underlie and accompany this phenomenon, but the immune system has emerged as a common denominator in the social buffering of stress. In rodents, socializing has yielded positive outcomes for wound healing and stroke recovery. Indeed, it is well studied that tumor necrosis factor (TNF), a key cytokine, plays an important synaptic role as a neuromodulator. We have recently demonstrated mechanistically that TNF mediates stress-induced plasticity (Kemp et. al., In Press - Molecular Psychiatry, 2022). In this original poster, I present emerging evidence for links between socializing and TNF in the context of acute stress.

Methods: All described experiments were conducted according to the Canadian Council for Animal Care as reviewed and approved by the Montreal General Hospital Facility Animal Care Committee. C57BL/6J mice between 8 and 16-weeks-old were exposed to two stress paradigms: forced swim stress and restraint stress. After both paradigms, animals were either single housed, or group housed in new cages with their stressed littermates. Unstressed control groups were age matched and were subjected to the same housing and handling conditions without the exposure to stress paradigms. After 24 hours, the behavioral output of the acute-stress response was tested using the light-dark box test (10 min total) (Crawley and Goodwin, 1980). Time spent in the light box inversely correlates with anxiety-like behavior. The biochemical output of acute-stress response was also measured 24 hours post-stress: mice were euthanized by isoflurane, blood samples were collected by cardiac puncture, and bilateral ventral hippocampal tissue (vHPC) was excised to measure the biochemical output of acute stress.

TNF levels were measured using the mouse TNF ELISA kit (eBioscience, Mouse TNF alpha ELISA Ready-SET-Go! Kit #88-7324). Samples were standardized to total protein-input measured by a bicinchoninic acid assay (BCA) kit from Thermo Fisher Scientific (#23227). Samples also were analyzed for other cytokines using a Milliplex panel (Mouse High Sensitivity T-Cell, Millipore, St. Charles, MO, USA) and analyzed using the Bio-Plex™ 200 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Serum corticosterone (CORT) was measured by ELISA kit (Cayman Chemical, cat. #501320).

Results: Anxiety-like behavior was increased in single-housed males subjected to forced swim stress (FST) (student t-test; $p = 0.0115$, $N = 9$-10 / group), but there was no comparable phenotype detected in females (student t-test; $p = 0.5976$, $N = 7$-8 / group). The described phenotype in males had already been shown to be accompanied by a significant increase in TNF (Kemp, In Press). Furthermore, single housing on its own in this context does not increase anxiety-like behavior nor TNF levels (Kemp, In Press).

Intriguingly, unlike single-housed mice, group-housed mice post-FST did not show an anxiety-like phenotype (student t-test, $p = 0.5066$, $N = 10$-11 / group). This observation was also replicated using restraint stress (one-way ANOVA $F(2, 31) = 7.440$, $p = 0.0023$, Tukey's post hoc analysis of control versus single-housing post-RS $p = 0.0047$, control versus group-housing post-RS $p = 0.9680$, single-housing post-RS versus group-housing post-RS $p = 0.0073$, $N = 11$-12 / group). Additionally, group-housed animals post-stress showed a reduction in vHPC TNF (student t-test $p = 0.0439$). The levels of four other cytokines, namely IL-1b, IL-10, IL-12 and MIP-2, were also significantly increased in socialized animals post-stress when probed using a cytokine multiplex (all one-way ANOVAs were significant; Tukey's post hoc analysis of control versus group-housing post-stress for IL-1b $p = 0.0356$, IL-10 $p = 0.0432$, IL-12 $p = 0.0331$, and MIP-2 $p = 0.0403$, $N = 4$-5 / group)

When we tested the levels of corticosterone (CORT), preliminary data showed that TNF levels better correlate with anxiety-like behavior than CORT. Indeed, there was a main effect of prior exposure to stress on serum CORT levels (24 hours post-stress; two-way ANOVA $F(1, 10) = 8.551$, $p = 0.0152$, $N = 3$-4 / group) and there was no difference between group-stress and single-stress animals ($p = 0.9952$).

Conclusions: We report a moderating effect of housing conditions post-stress on anxiety-like behavior and TNF cytokine levels in the vHPC. Indeed, socially housed animals post-stress do not display an anxiety-like phenotype and show an active suppression of vHPC TNF levels compared to single-housed animals post-stress. Furthermore, we show an induction of other cytokines in the absence of anxiety-like behavior post-stress. Therefore, these findings contribute to an ongoing scholarly discussion whether resilience and susceptibility are two sides of the same coin or if are they two different phenomena with distinct active mechanisms for each. Indeed, our results point to active molecular mechanisms involved in the social buffering of stress as a form of resilience. Further investigation of these mechanisms could provide us with a path to characterize the molecular underpinning of resilience.

Keywords: Social Stress Buffering, Cytokines, Corticosterone, Anxiety-Like Behavior, Tumor Necrosis Factor

Disclosure: Nothing to disclose.

P26. Traumatic Brain Injury Increases Contextual Fear Generalization, Which is Paralleled by Hippocampal Memory Trace Dysfunction and Can Be Decreased by (R,S)-Ketamine Administration

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Background: Traumatic brain injury (TBI) is a debilitating neurological disorder caused by an impact to the head by an outside force. TBI results in persistent cognitive impairments, including fear generalization, which is defined as the inability to distinguish between aversive and neutral stimuli. Fear generalization can be assayed in mice using a contextual fear discrimination (CFD) paradigm, which tests fear behavior in learned aversive versus neutral contexts.

Methods: To identify the fear generalization neural ensembles altered by TBI, we utilized ArcCreERT2 x enhanced yellow fluorescent protein (eYFP) mice, which allow for activity-dependent labeling and quantification of memory traces. ArcCreERT2 x eYFP mice were administered either a sham surgery or the controlled cortical impact (CCI) model of TBI. One month

following surgery, mice were injected with 4-OHT to open up a tagging window and administered a 1-shock contextual fear conditioning (CFC) paradigm. Mice were then administered an additional 5 days of CFD and euthanized following exposure to the aversive (A) or neutral (B) contexts. Dentate gyrus (DG) neural activity was quantified. (n = 13-15 mice / group). In a separate set of studies, we determined if administering (R,S)-ketamine 1 hour after a CCI would decrease fear generalization in TBI mice. (n = 6-8 mice / group).

Results: TBI mice exhibited increased fear generalization (e.g., lack of discrimination between the aversive and neutral context) in the CFD paradigm (ANOVA: Context x Time *p = 0.0464; Context p = 0.6064; Time p < 0.0001). This is in contract to the sham mice, who discriminated between the aversive and neutral context (ANOVA: Context x Time ***p < 0.0001; Context ***p < 0.0001; Time ***p < 0.0001). TBI did not alter the number of eYFP+ DG cells activated during fear encoding (ANOVA: Treatment x Context p = 0.7645; Treatment p = 0.7926; Context p = 0.9476) or the number of c-fos+ DG cells during memory retrieval (ANOVA: Treatment x Context p = 0.3028; Treatment p = 0.3138; Context p = 0.3166). Sham mice exhibited a significant increase in the percentage of co-labeled DG eYFP + /c-fos+ cells in the aversive context when compared with the neural context. However, TBI mice exhibited comparable percentages of co-labeled DG eYFP + /c-fos+ cells in both contexts (ANOVA – Treatment x Context p = 0.4171; Treatment p = 0.2120; Context **p = 0.0030).

Finally, previous data in our lab have shown that a single administration of (R,S)-ketamine (30 mg/kg) prior to a stress can buffer against learned fear and facilitate CFD. Here, we report that administering (R,S)-ketamine (30 mg/kg) 1 hour after CCI decreased TBI-induced fear generalization mice (ANOVA – Context x Drug p = 0.4111; Context **p = 0.0030; Drug p = 0.1670).

Conclusions: Our data show that 1) TBI results in increased fear generalization; 2) this behavior is paralleled by altering fear memory traces in the DG; and 3) this behavioral deficit that can be reversed with post-impact subanesthetic administration of (R,S)-ketamine. This work enhances our understanding of the neural basis of fear generalization in individuals with TBI and reveals potential avenues for further translational research.

Keywords: TBI, (R,S)-Ketamine, Fear Generalization, Memory Engram Cell, Hippocampus

Disclosure: Nothing to disclose.

P27. Projection-Targeted Photopharmacology Reveals Anxiolytic Function of Presynaptic mGluR2 in Cortical-Amygdala Circuits

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Background: Anxiety disorders are the most common psychiatric disorders, affecting about 30% of US adults during their lifetime. Anxiolytic medications primarily work through serotoninergic or GABAergic signaling, while there are no approved treatments that work via a principally glutamatergic mechanism. Metabotropic glutamate receptor 2 (mGluR2) is mainly localized presynaptically and together with mGluR3 mediates feedback inhibition of glutamate release. mGluR2/3 mixed agonists have been shown to mediate anxiolytic effects in humans through an unknown mechanism. In this study, we describe distinct anxiolytic roles of mGluR2 at medial prefrontal (mPFC) and insular (IC) projections to the basolateral amygdala (BLA).

Methods: Using adeno-associated viruses in a transgenic Grm2-Cre mice, mGluR2+ projectors to the BLA were labeled. The specific roles of mPFC and IC projections in anxiety-related behavior were characterized using optogenetic techniques and fiber photometry. The specific function of presynaptic mGluR2 was probed using a novel photopharmacological tool, photoswitchable orthogonally and remotely tethered ligands, to specifically activate mGluR2 in prefrontal or insular terminals. Conventional and photopharmacological manipulations were performed during in vitro electrophysiology recordings to characterize its function in synaptic transmission and in vivo during behavioral assays that elicit various forms of anxiety-related avoidance reactions. N = 5-20 male mice per group.

Results: Systemic administration of a mGluR2/3 mixed agonist (LY37) decreased spatial avoidance, while a mixed antagonist (LY34) increased avoidance. LY37 infused into the BLA also mediated anxiolysis. mGluR2 was found to be enriched in projectors to the BLA from the mPFC and IC. Neuronal activation of both cell populations was observed during anxiety-related assays using fiber photometry. In vitro, 89% (n = 19) and 100% (n = 14) of BLA principal neurons were activated by mGluR2+ projections from the mPFC and IC, respectively. This activation was acutely dampened by mGluR2 photoactivation, while long-term depression was induced by prolonged photoactivation. Acute photoactivation of mGluR2 in mPFC, but not IC, terminals decreased spatial avoidance, and prolonged photoactivation led to anxiolysis at 4 hours but not 7 days post-light. In contrast, acute photoactivation of mGluR2 in IC, but not mPFC, led to diminished social avoidance and avoidance of predator urine. Photoactivation of mGluR2 in either projection decreased the latency during novelty-suppressed feeding.

Conclusions: We describe both distinct and overlapping anxiolytic functions of mGluR2 in mPFC and IC projections to the BLA. mGluR2 represents a promising therapeutic target for the treatment of anxiety.

Keywords: Photopharmacology, GPCR, Metabotropic Glutamate Receptor 2 (mGluR2), Medial Prefrontal Cortex, Basolateral Amygdala

Disclosure: Nothing to disclose.

P28. Development of an Improved Oral Tablet Formulation of BNC210, a Negative Allosteric Modulator of the Alpha 7 Nicotinic Acetylcholine Receptor, Suitable for Evaluation as an Acute Treatment for Social Anxiety Disorder

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Background: The DSM-5 describes Social Anxiety Disorder (SAD) as a marked fear or anxiety about one or more social situations in which the individual is exposed to possible scrutiny by others. Examples include social interactions (e.g., having a conversation, meeting unfamiliar people), being observed (e.g., eating or drinking), and performing in front of others (e.g., giving a speech). SAD is a chronic disorder which may also include severe episodes of acute anxiety such as those precipitated by performance-type events. A safe and effective, fast-acting therapy would be of benefit in situations like these. The only FDA-approved drugs for SAD are select antidepressants (fluvoxamine, sertraline, paroxetine, venlafaxine) which are not suitable for acute treatment due to their slow (2-4 weeks) onset of action. While no drugs have been approved to treat acute SAD, beta blockers and benzodiazepines (BZDs) are used off label. However, beta blockers mainly address physical symptoms (flushing, increased heart rate,

trembling), while the serious safety concerns associated with BZDs have restricted their use.

BNC210 is a novel drug acting through negative allosteric modulation of the alpha 7 nicotinic acetylcholine receptor (α7 nAChR) and has received FDA Fast Track designation for acute treatment of SAD and other anxiety disorders. It has been administered to more than 400 participants in Phase 1 and 2 clinical studies and lacks the severe side effects associated with the drugs that many patients with SAD are currently taking. BNC210 has demonstrated anxiety-lowering effects on human behavior and on neural correlates of anxiety in acute dosing clinical trials (using a liquid suspension formulation of BNC210 given with food to facilitate absorption). In a CCK-4 challenge model of panic attack in healthy volunteers, single doses of BNC210 significantly reduced panic symptom number and intensity using the Panic Symptom Scale (PSS). In Generalized Anxiety Disorder (GAD) patients performing the Emotional Faces Task during fMRI, single doses of BNC210 significantly reduced two neural correlates of anxiety; amygdala activation caused by viewing emotional faces and connectivity between the anterior cingulate cortex and the amygdala (this connection is strong in pathological anxiety). In the same study, BNC210 significantly reduced threat avoidance in a behavioral task and reduced state anxiety (measured using the State-Trait Anxiety Scale) in the GAD patients.

BNC210 is currently being evaluated in a Phase 2 study for the acute treatment of SAD (PREVAIL Study, NCT05193409). Using a new oral tablet formulation of BNC210 with improved absorption characteristics over the previously used suspension formulation, single doses of BNC210 (225 mg or 675 mg) are being compared to placebo (n = 50 per group) on reducing anxiety provoked by a speaking challenge as measured using the Subjective Units of Distress Scale (SUDS) in patients with SAD.

Methods: A novel solid tablet formulation of BNC210 was developed and contains a spray dried dispersion of BNC210 to improve the solubility of the compound. During the development process, several prototype formulations were compared in in vitro dissolution assays and the lead formulations were compared in dog pharmacokinetic (PK) studies. The final selected formulation was then evaluated in two single oral dose human PK studies to compare the PK properties of the tablet with the suspension formulation (300 mg BNC210, fed vs fasted), and then to determine PK parameters and dose linearity with ascending doses of the new tablet (300 to 1,200 mg BNC210, fasted).

Results: The first human PK study showed that the final formulation developed for the BNC210 tablet overcame the food effect of the liquid suspension. At a 300 mg dose, the liquid suspension showed a marked increase in mean Cmax (2.3-fold) and mean AUC (3.5-fold) values following a high fat meal compared to fasted, whereas at the same dose, there was little impact on these tablet PK parameters whether given with or without food (mean Cmax was marginally reduced by 12% and mean AUC was increased by only 27% in the presence of a high fat meal). Furthermore, with the tablet, the median time to reach maximal plasma concentration (Tmax) was reduced from 4 hours with a high fat meal to 1.75 hours in the fasted state. The PK parameters for single dose administration of the tablet showed dose linear exposure over the range of 300 mg to 1,200 mg BNC210 in the fasted condition, and the median Tmax was 0.75 to 1.75 hours at the doses evaluated.

Conclusions: BNC210 has been reformulated into a tablet with improved PK properties, potentially making it suitable for PRN use in an outpatient setting for the treatment of SAD. Drug exposure is no longer dependent on concomitant food intake, allowing for maximal plasma concentrations to be reached after approximately 1 to 1.5 hours after dose administration. This means that BNC210 could be taken just prior to an anticipated anxiety-provoking event such as a performance or social event. Additionally, plasma concentrations remain at reasonable levels for several hours after maximum concentration is reached, suggesting that repeat dosing may not be needed. Topline data from the ongoing BNC210 Phase 2 study for the acute treatment of SAD (PREVAIL Study, NCT05193409) are expected in late 2022/early 2023.

Keywords: Social Anxiety Disorder, Acute Treatment, Phase II Clinical Trial, BNC210

Disclosure: Nothing to disclose.

P29. Pharmacometrics Analysis and Drug Reformulation of BNC210 to Optimize Its Evaluation in a Phase 2 Trial in PTSD Patients

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Background: Currently approved therapeutics for Post-Traumatic Stress Disorder (PTSD) have adverse effects and limitations that signify an unmet medical need for more effective and better-tolerated therapies. BNC210 is a first-in-class negative allosteric modulator of the alpha 7 nicotinic acetylcholine receptor (α7 nAChR) in development for the treatment of trauma- and stressor-related disorders and anxiety disorders. Extensive characterization in nonclinical and clinical studies has revealed diverse properties with potential to treat PTSD symptoms and some of the known pathophysiology underlying PTSD. BNC210 has received FDA Fast Track designation for the treatment of PTSD and other trauma- and stressor-related disorders and is currently being evaluated in a monotherapy Phase 2b trial in ~200 patients with PTSD (ATTUNE Study, NCT04951076).

BNC210 was previously evaluated in a Phase 2 PTSD trial (RESTORE Study, NCT02933606) where participants received BNC210 (150, 300 or 600 mg) or placebo b.i.d., in a liquid suspension formulation that needed to be taken with food for optimal absorption. No significant separation from placebo was measured on the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5) Total Symptom Severity score over the 12-week treatment period. However, positive information from the trial data included: statistically significant effects on depression symptoms (CAPS-5 Criterion D) at early time points, and no trend for increased adverse events, cognitive impairment, nor suicidal ideation or worsening behavior, with treatment. Population pharmacokinetics (PK) estimated drug plasma exposures (AUC) in the participants and revealed that the liquid suspension gave lower than projected exposure in the outpatient setting, a finding which triggered the development of a new, oral tablet formulation of BNC210. Pharmacometrics modelling was performed to explore an exposure-response (PK/PD) relationship for BNC210 based on the estimated AUCs and observed CAPS-5 scores.

Methods: Population PK model: Plasma samples were obtained from participants on the RESTORE study at 3 timepoints over the 12-week period and BNC210 concentrations were measured. Using these data, a population PK analysis was performed, and AUC values were estimated from a one compartment PK model that allowed for time varying bioavailability.

PK/PD model: A PK/PD analysis was performed to evaluate the exposure-response relationship for BNC210 estimated AUC values and CAPS-5 Total Symptom Severity scores as a continuous direct effect. The effect was modelled on the logit scale which bound it not to be larger than the highest possible score or lower than the smallest possible score. Effects were evaluated using three models: linear, Emax and power function.

Reformulation: Spray dried dispersion technology was applied to develop an oral solid dose tablet formulation of BNC210 with

improved solubility that could overcome the limitations of the liquid suspension, specifically, a strong food effect and non-linear absorption. Three human PK studies were conducted to compare tablet PK parameters to the suspension, establish dose linearity with single ascending doses, and to determine a dosing regimen that could be used in future PTSD clinical trials.

Results: Population PK model: The model indicated that the estimated BNC210 AUC values in the RESTORE Study outpatient trial were lower than projected, and the higher BNC210 dose levels were associated with decreasing bioavailability over time. After 12 weeks of treatment the estimated achieved exposure for the 600 mg b.i.d. dose group was ~50% lower than previously measured in an in-clinic 7-day PK study.

PK/PD model: An inhibitory Emax model, including estimates of inter-individual variability produced the best PD model fit and predicted an exposure-response relationship for CAPS-5 Total Symptom Severity scores (p-value<0.01), where higher AUC values were related to a larger effect. The model predicted that the AUC90 (90% of the maximum drug effect) was ~25,000 ng.hr/mL which corresponded to a predicted difference from placebo on the CAPS-5 scale in the range of a potentially clinically meaningful result.

Reformulation: In single dose human PK studies, the BNC210 tablet overcame the food effect of the suspension formulation e.g., a 300 mg dose (fed and fasted) achieved respective mean values for Cmax of 1,528 and 1,727 ng/mL, and AUC values of 14,000 and 11,000 ng.hr/mL, and exposure was dose linear over the range of 300 to 1,200 mg. A multiple dosing PK study (900 mg of the tablet formulation b.i.d.) achieved more than the target exposure of 25,000 ng.hr/mL for further evaluation of BNC210 in a PTSD study.

Conclusions: The population PK model showed that exposure from the BNC210 liquid suspension formulation was insufficient to achieve efficacy in the RESTORE PTSD study where estimated mean AUC at steady state for the 600 mg b.i.d dose group was only 10,900 ng.hr/mL at Week 12. However, the pharmacometrics model predicted the potential for BNC210 to have benefit in PTSD provided that adequate blood levels could be achieved. These analyses, as well as the development of the new tablet formulation, justified further evaluation of BNC210 in PTSD patients and provided a basis for optimal design of the ongoing Phase 2b trial to demonstrate efficacy in PTSD (ATTUNE Study, NCT04951076); top line data are expected in mid-2023.

Keywords: PTSD, Phase II Clinical Trial, Exposure-Response Model, Reformulation, BNC210

Disclosure: Nothing to disclose.

P30. Altered Heartbeat-Evoked Neural Responses During Peripheral Adrenergic Stimulation in Generalized Anxiety Disorder

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Background: Altered perceptual processing of signals originating from within the body (i.e., interoceptive dysfunction) has been linked to anxious psychopathology. In a recent pharmaco-fMRI study we showed that individuals with generalized anxiety disorder (GAD) exhibit autonomic hypersensitivity evidenced by elevated heart rates, heightened interoceptive awareness of cardiorespiratory sensations, increased anxiety, and a blunted neural response localized to the ventromedial prefrontal cortex during low level beta-adrenergic stimulation (Teed et al (2022)). Here, we examine whether the peripheral adrenergic modulation of cardiac signals with isoproterenol differentially affected the heartbeat evoked potential (HEP), an electroencephalogram (EEG) marker of neural activity in response to heartbeats, in females with GAD versus healthy comparison females (HC).

Methods: Simultaneous EEG-fMRI data from this crossover randomized clinical trial were collected in 52 adult females (26 GAD, 26 HC matched on age and body mass index (BMI), the same study population as in Teed et al (2022)) during administration of intravenous bolus infusions of isoproterenol (0.5 and 2.0 micrograms, μg) and saline, each administered twice in a double-blind fashion within a single scanning session. Participants continuously rated the perceived intensity of their cardiorespiratory sensations by rotating an MRI-compatible dial throughout each infusion. EEG and electrocardiogram (ECG) signals were recorded during each 240-second infusion using an MRI-compatible 32-channel scalp EEG system including simultaneous single lead ECG. During offline processing, data were band-pass filtered between 0.3 and 30 Hertz, and the EEG data was referenced to the common average signal. Cardiac R-wave peaks were detected from the ECG signal for every heartbeat using a semi-automated approach. EEG data were epoched in an event-related manner, from -100 milliseconds (ms) to 650 ms after the ECG R-peak, with the cardiac R-wave serving as the temporal reference. We minimized several cardiac-related artifacts in the EEG signal, including the cardiac field artifact and the ballistocardiogram artifact, using the optimal basis set approach followed by application of independent component analysis to remove residuals of the ballistocardiogram artifact. Baseline correction was performed by subtracting the mean of the 100 ms window preceding the epoch of statistical analysis from the entire grand-averaged HEP signal. We implemented a data-driven statistical analysis approach, cluster-based permutation, to compare HEP amplitudes during isoproterenol and saline infusions in individuals with GAD vs HC in the time range [100-600 ms] after the R peak.

Results: The GAD group (mean age: 26 ± 7 years, BMI: 25.8 ± 4.8) and HC group (mean age: 24 ± 5 years, BMI: 24.1 ± 3.2) did not differ on age or BMI. During the peak response period of the 0.5-μg isoproterenol dose, the GAD group showed changes in HEP amplitude that significantly differed from the HCs at right centro-parietal electrodes; specifically, the HEP amplitude during the peak response period was more positive in GAD (Cohen $d = 1.18$; $P < 0.001$) within a latency from 216–272 ms after the R-peak. During the early recovery period, HEP amplitudes were more negative within a latency from 528-592 ms after the R-peak (Cohen $d = 0.90$; $P < 0.01$). In addition, relative to the HC group, the GAD group reported significantly greater increases in cardiorespiratory sensation intensity during the 0.5-μg dose of isoproterenol (Cohen $d = 0.80$; $P < 0.01$), that were non-significantly correlated with changes in HEP amplitude at a trend level across all participants (peak response: $r = -0.25$, $P = 0.08$; early recovery: $r = -0.27$, $P = 0.05$). During saline infusion, the GAD group also exhibited a larger HEP amplitude in right fronto-central electrodes (Cohen $d = 0.97$; $P < 0.001$) within a 92-ms window starting 224 ms after the R-peak. Control analyses showed that the altered HEP amplitudes observed in GAD vs HC were not due to cardiac-related confounding factors, such as differences in heart rate or T-wave amplitude. At the transdiagnostic sample level, correlational analyses of physiological and psychological indices revealed that HEP amplitudes were significantly correlated in opposing directions with the anxiety subscale of the PROMIS Negative Affect questionnaire (0.5 μg isoproterenol: $r = 0.46$, $P < 0.001$; saline: $r = -0.42$, $P < 0.01$) and with the physical concerns subscale of the Anxiety Sensitivity Index questionnaire (0.5 μg isoproterenol: $r = 0.47$, $P < 0.001$; saline: $r = -0.34$, $P < 0.01$). No significant HEP changes or multilevel associations were identified at the 2 μg dose.

Conclusions: In this study GAD individuals showed altered HEPs and heightened cardiac interoceptive awareness, which is

consistent with autonomic hypersensitivity during low levels of peripheral adrenergic stimulation. This and previous results support the hypothesis of increased bottom-up sensitivity to adrenergic arousal signals in the disorder. In addition, the increased HEP responses observed during saline infusion suggest that GAD individuals may also exhibit a top-down sensitivity toward heartbeat signals, perhaps associated with abnormal cognitive processing of interoceptive signals. Taken together, our findings further support the notion that GAD individuals exhibit a peripheral autonomic hypersensitivity which contributes to dysfunctional cardiac interoception in this condition.

Keywords: Generalized Anxiety Disorder, Interoception, Heartbeat Evoked Potential, Cardiac Sensation

Disclosure: Nothing to disclose.

P31. Darigabat Reduces Acute Psychological and Physiological Panic and Fear Symptoms Induced by CO2 Inhalation in Healthy Participants

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Background: Panic disorder (PD) is a serious condition that may cause significant psychological and physical distress. PD is associated with dysregulation in fear and anxiety neurocircuitry involving serotonin, norepinephrine, and gamma-amino-butyric-acid (GABA) pathways, with current pharmacotherapy consisting mainly of drugs that target these systems, such as selective serotonin reuptake inhibitors and benzodiazepines (BZDs). However, no one class of drugs adequately addresses both the subjective (e.g., fear, dread) and somatic (e.g., tachycardia) symptoms of PD. As such, a significant proportion of patients with PD have incomplete or partial response to available treatments with a low likelihood of remission and a significant probability of relapse. Given there have been no new approved drugs for the treatment of PD since 2005 there is a need for innovative novel treatment options.

There is substantial nonclinical evidence that α2/3 subunit–containing GABAA receptors are responsible for the anxiolytic effects of benzodiazepines, and that the α1 subtype is associated with the sedative properties. Darigabat was rationally designed to have non-sedative anxiolytic potential by selectively enhancing the effect of GABA at α2/3/5 GABAA receptor subtypes, while sparing activity at α1, and is in development for the treatment of neurological and psychiatric disorders. To characterize the potential panicolytic effect of darigabat, CO2 inhalation was applied as an experimental model to reliably induce panic and fear in healthy participants. The model is sensitive to pharmacological manipulation by anxiolytics, allowing investigation of pharmacodynamic effects of drugs in early clinical development.

Methods: This randomized, double-blind, placebo- and active-controlled trial assessed the panicolytic effect of multiple doses of darigabat on panic and fear symptoms evoked by CO2 inhalation in healthy participants (NCT04592536). Only individuals sensitive to the anxiogenic effects of the 35% CO2 double-breath inhalation at screening were eligible for randomization. In this two-period, two-sequence partial crossover design, each eligible participant was randomized to receive either placebo and one of three active treatments in 3 separate cohorts (n = 18-20/cohort) for 8 days: cohort 1 darigabat 25 mg BID, cohort 2 alprazolam 1 mg BID, and cohort 3 darigabat 7.5 mg BID. Darigabat was titrated to achieve the target maintenance dose on Day 5. On Day 8 of each crossover period, a CO2 challenge was performed at 3 hours after dosing. Alprazolam was used as a positive control to establish assay sensitivity. With each participant's placebo period serving as their own control, the change in panic and fear symptoms measured before and immediately after CO2 inhalation using the Panic Symptom List-IV total score (PSL-IV; primary endpoint) and fear visual analog scale (VAS Fear; secondary endpoint) were assessed. The PSL-IV contains 13 items derived from those listed for panic disorder in the Diagnostic and Statistical Manual of Mental Disorders, version 4 (DSM-4) covers aspects of the PD experience including the psychological (such as fear of dying) and the physiological (such as palpitations and gasping for breath. Cardiovascular parameters (blood pressure, and heart rate) were objectively measured continuously using a non-invasive finger-cuff-based hemodynamic monitoring system (Finapres) for approximately 15 mins prior to and following CO2 challenge to determine the effects of drug treatment on the physiological panic and fear response (secondary endpoint). While the trial was not prospectively designed for formal hypothesis testing with statistical power, nominal P values for each comparison are presented. Steady state pharmacokinetic samples were obtained at 2 and 4 hours after dosing on Day 8.

Results: In the primary outcome measure PSL-IV total score on Day 8, the darigabat 7.5 mg and 25 mg BID treatment groups demonstrated a 3.9-point ($P = 0.036$) and 4.5-point ($P = 0.008$) improvement versus placebo, respectively. In the secondary outcome measure VAS Fear, the 7.5-mg and 25-mg BID treatment groups demonstrated a 12.8-point ($P = 0.026$) and 7.8-point ($P = 0.282$) improvement versus placebo, respectively. Compared with placebo, alprazolam 1 mg BID exhibited outcomes in line with expectations, with placebo-adjusted improvements of 1.6-points ($P = 0.286$) and 0.9-points ($P = 0.876$) on PSL-IV total score and VAS Fear on Day 8, respectively. The results on the individual PSL-IV items indicated a broad reduction of both the psychological and physiological symptoms experienced as a result of CO2 inhalation.

Both doses of darigabat attenuated the transient increase in blood pressure induced by CO2 inhalation on Day 8, suggesting darigabat reduces the physiological response associated with CO2 challenge. Alprazolam did not attenuate the physiological response associated with CO2 challenge.

Plasma concentrations of darigabat were consistent with previous trials and estimated to achieve approximately 50% and 80% receptor occupancy at α2-containing GABAA receptors at 7.5 mg and 25 mg BID, respectively. Darigabat was generally well tolerated.

Conclusions: This trial demonstrated the panicolytic potential of darigabat, based on reduction of acute panic and fear symptoms in a validated, experimental clinical panic model in healthy participants. These data warrant the further evaluation of darigabat in patients with anxiety disorders.

Keywords: GABA-A, Positive Allosteric Modulators, Panic, Novel Therapeutics, Experimental Methods

Disclosures: Cerevel Therapeutics: Employee(Self) Eliem Therapeutics: Employee (Spouse), vZenium: Founder (Spouse)

P32. Insights From Rodent Work: Can Environmental Contaminants Contribute to the Development of Anxiety Disorders?

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Background: The increase in the diagnosis of mental health disorders such as anxiety has created a need to evaluate for external factors that might contribute to the development of these disorders. Recently, epidemiological studies have begun to show links between exposure to environmental contaminants and anxiety disorders. One possible contaminant is the extensively used herbicide, glyphosate, which has been identified in food and water sources. Glyphosate was initially considered safe for mammals because it acts by inhibiting a metabolic route almost exclusive to plants. However, a correlation has been seen between the increased diagnosis of anxiety and the use of glyphosate. Glyphosate, as other possible contaminants, is regulated by the Environmental Protection Agency (EPA). The EPA has established a chronic reference dose of 2.0 mg/kg of glyphosate to have no detrimental effects on health. However, the effect of glyphosate at this dose on the development of anxiety has not been studied. Therefore, we aim to evaluate the effect of glyphosate on anxiety-like and exploratory behaviors. Moreover, given that the amygdala and prefrontal cortex have both been shown to play an important role in the expression and modulation of anxiety and exploration, we aim to evaluate these brain regions for neuronal activity to identify a mechanism for any behavioral changes.

Methods: Male rats ($n = 13$) were given water containing glyphosate ad libitum for 16 weeks. Water consumption was measured weekly, and water was prepared for a target dose of 2.0mg/kg daily of glyphosate. Control rats ($n = 12$) received filtered drinking water. Anxiety-like behaviors were assessed after 10 weeks of exposure in an elevated plus maze (EPM). Exploratory response to novelty was measured using an open field with a novel object in the center (NOET) after 14 weeks, as well as an auditory startle response test (ASR, 5 repetitions of a novel tone in a familiar context) after 16 weeks. Rats were then sacrificed, and brain tissue was fixed and collected. Immunohistochemsitry for c-Fos was performed in brain slices containing amygdala, as well as the prelimbic (PL) and infralimbic (IL) cortices of the prefrontal cortex to evaluate for neuronal activity. Student's t-test was used for analysis of behavioral results and immunohistochemistry analysis.

Results: In the EPM, glyphosate decreased the time spent in the open arms (glyphosate: 51.4; control: 108.9; $t22 = 2.98$; $p = 0.0069$), indicating increased anxiety. In the NOET, glyphosate decreased the time spent interacting with the novel object (glyphosate: 45.34; control: 97.9; $t22 = 2.73$; $p = 0.0122$), consistent with decreased exploration. Moreover, glyphosate increased the percent time spent freezing in the presence of a novel tone (glyphosate: 14.38; control: 5.94; $t23 = 2.094$; $p = 0.0475$), suggesting increased startle to a novel, neutral stimulus. Brain tissue analysis showed that glyphosate did not affect neuronal activity in PL (glyphosate: 270.5; control: 382.0; $t10 = 1.365$; $p = 0.2023$), IL (glyphosate: 201.4; control: 253.3; $t10 = 1.059$; $p = 0.3147$) nor amygdala (glyphosate: 98.83; control: 100.3; $t10 = 0.1318$; $p = 0.8978$).

Conclusions: Contaminants, such as glyphosate, even at doses considered safe by the EPA, can increase anxiety-like behaviors and decrease exploration in rats. These finding support the idea that environmental contaminants should be evaluated for detrimental effects on mental health. Further exploration of the mechanism by which contaminants, such as glyphosate, causes behavioral changes is needed. Future directions include performing immunohistochemistry on brain regions involved in anxiety-like behaviors such as the bed nucleus of the stria terminalis and ventral hippocampus.

Keywords: Anxiety, Mental health Disorders, Animal Models, Environmental Risk Factors

Disclosure: Nothing to disclose.

P33. Relationship Between Emotion Regulation Styles and Fear Conditioning in Trauma Exposed Veterans With and Without PTSD

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Background: Effortful emotion regulation strategies such as cognitive reappraisal and expressive suppression share common neural mechanisms with fear learning and extinction (Birthe Macdonald, 2020). Disrupted fear learning and extinction processes are thought to support severity of symptoms of post-traumatic stress disorder (PTSD). In healthy populations, use of cognitive reappraisal is associated with reduced acquisition of fear learning and greater extinction learning and recall (Andrea Hermann, 2014). Other studies have not demonstrated a relationship between explicit ER strategies and fear conditioning/ extinction learning (Haruka Kitamura, 2022), suggesting that further consideration of individual factors is needed. PTSD has been associated with increased use of expressive suppression, as well as alterations in fear conditioning, extinction, and extinction retention (A J Khan, 2021; Inslicht et al., 2013; Orr et al., 2000). Further characterization of the relationship between ER processes and fear learning processes in trauma exposure individuals may help us target skills to increase the effectiveness of PTSD interventions.

Methods: A laboratory study was conducted in trauma-exposed men and women with and without PTSD to examine the impact of emotion regulation styles on fear conditioning and extinction. Tendencies towards using cognitive reappraisal and expressive suppression were assessed via the Emotion Regulation Questionnaire. Participants underwent a fear conditioning, extinction, and retention procedure adapted from Orr et al. (Orr et al., 2000) and previously described (Inslicht et al., 2013), which took place over the course of three sessions. All psychophysiology testing sessions took place in a dedicated psychophysiology laboratory. The UCS was a 500 ms electric pulse, which ranged from .5 to 5.0 mA, as previously determined by the participant to be "highly annoying but not painful". During the first session, participants underwent a habituation phase during which participants were presented with 5 of each of the colored circles (to be CS + and to be CS-) in the absence of the shock (UCS). This was followed by the fear acquisition phase, in which each presentation of the CS + was followed by a 500 ms shock and the CS– was not. The fear extinction session took place 72 hours following the first session. During this session, participants were presented with 10 non-reinforced presentations each of the CS + and CS − . One week following extinction, extinction retention session took place. Participants were shown 4 non-reinforced presentations each of the CS + and CS − .

Results: PTSD diagnosis was found to moderate the relationship between cognitive reappraisal and differential SCR response to CS + and CS- during acquisition ($z = 2.23$, $P = 0.02$). Individuals without PTSD showed a greater negative relationship between cognitive reappraisal and differential SCR, such that greater cognitive reappraisal predicted reduced differential SCR during fear acquisition ($z = -2.00$, $P = 0.05$). Individuals with PTSD did not demonstrate a significant relationship between cognitive reappraisal and differential SCR ($z = 0.49$, $P = 0.62$). PTSD did not moderate the relationship between expressive suppression and differential SCR response during extinction learning, as predicted ($P = 0.33$). However, there was a main effect of expressive suppression on differential SCR during extinction learning, such that greater expressive suppression predicted greater differential

SCR during extinction retention ($z = 2.06$, $P = 0.04$). Group differences in use of cognitive reappraisal and expressive suppression were not observed between individuals with and without PTSD diagnosis.

Conclusions: The current analysis adds to evidence that cognitive reappraisal predicts reduced fear acquisition. Further, our data suggest that expressive suppression predicts maintenance of fear responding in the presence of safety cues, suggesting a failure to encode the inhibitory association between CS + and shock during extinction learning is related. This suggests that common mechanisms are being impacted by these two, conceptually distinct, facets of emotion – explicit emotion regulation and fear conditioning. Contrary to hypothesis, differences in ER strategies between PTSD groups were not observed in our sample and PTSD diagnosis did not moderate the relationship between expressive suppression and extinction retention. Several methodological considerations may explain this finding, including that our control group consisted of individuals who had been previously trauma exposed but had never met criteria for PTSD. Future work should include inclusion of a non-trauma exposed control group, to further clarify these relationships.

Keywords: Fear Conditioning and Extinction, Emotion Regulation, PTSD

Disclosure: Nothing to disclose.

P34. Parallel Dopamine Circuit Dynamics in Chronic Stress-Induced Behavioral Outcomes

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Background: Comorbidity is more the norm than the exception, where more than 93% of Medicare dollars are used for patients who suffer from comorbidities in the United States. Anxiety, anhedonia, and depression are highly prevalent comorbid symptoms in human neuropsychiatric profiles. This co-occurrence is associated with complex symptomatology, greater treatment resistance, chronicity, disability, and higher suicide attempt rates in comorbid patients. However, the shared or segregated mechanisms remain largely unknown. Clinical and preclinical studies implicate the ventral tegmental area (VTA) dopamine circuits in the emergence of anxiety, anhedonia and depressive phenotypes. The VTA dopamine neurons project widely throughout the brain, including to emotion-related brain regions, such as the cortex, the nucleus accumbens (NAc), and the amygdala (AMG). Here we aim to identify the parallel alterations of the dopaminergic circuits and how they control singular anxiety or co-occurring anxiety, anhedonia and depressive-like behaviors following chronic stress exposure.

Methods: We use a chronic social defeat stress paradigm (CSDS), known to induce individual and complex behavioral stress-related outcomes, to capture singular or co-occurring measures of anxiety, anhedonia and depressive-like behaviors in mice. Following CSDS, we assess social behaviors, reward sensitivity and processing, and approach/avoidance behaviors. We employ viral strategies and transgenic mice to isolate VTA dopamine circuits and isolate VTA dopamine neurons projecting to the AMG from VTA dopamine neurons targeting the NAc. We then combine neural circuit-probing techniques with in vivo fiber photometry and electrophysiological approaches to define the physiological characteristics and dynamics of the VTA dopamine circuits emerging in response to CSDS exposure. We also use optogenetics to selectively link VTA circuit activity with the distinct stress-induced behavioral outcomes.

Results: We first observe that CSDS induces depressive-like behaviors (ANOVA, $P < 0.01$, $n = 40$), alterations in cognitive function (ANOVA, $P < 0.05$, $n = 28$), also and anxiety-like behaviors (Kruskal-Wallis, $P < 0.01$, $n = 40$) that emerge independently from the depressive-like phenotype (Spearman $r = 0.07$; $r = 0.06$, $P > 0.05$, $n = 25$). Our circuit-probing approaches showed that VTA-AMG and VTA-NAc dopamine circuits emerge from two distinct neuronal populations. In line with our previous studies revealing that VTA-NAc dopamine neuron hyperactivity encodes social avoidance behaviors, while VTA-AMG dopamine neuron hypoactivity is linked with anxiety-like behaviors, here we identify that CSDS alters the VTA-AMG and VTA-NAc circuit dynamics differently. We define that the activity of the VTA-AMG circuit selectively regulates anxiety-like behaviors following social stress exposure but not social avoidance behaviors, while the VTA-NAc circuit dynamics regulate reward processing and social behaviors (ANOVA, $P < 0.05$, $n = 11$). We further observed distinct VTA-NAc and VTA-AMG extrinsic and intrinsic physiological alterations when compared to control mice (ANOVA, $P < 0.01$, $n = 10$-12). Finally, our physiological studies establish that anxiety-like behaviors are associated with reduced VTA-AMG circuit activity and increased AMG neuron excitability (Kruskal-Wallis, $P < 0.01$, $n = 8$-10; ANOVA, $P < 0.05$, $n = 10$-12).

Conclusions: Over 50% of patients suffering from anxiety or major depressive disorders report a history of other mental illnesses. The complex and variable symptomatology that emerges following chronic stress exposure -a major risk factor for triggering psychiatric disorders- challenges the single brain circuit/ single phenotype preclinical framework. Here, our results show that chronic social stress exposure induces opposite cellular and physiological alterations in parallel VTA circuits. Our studies identify VTA-AMG circuit reduced activity as a common biomarker and treatment target for anxiety-like behaviors across complex symptomatology such as singular or co-occurring anxiety with depressive-like phenotypes. Our studies thus provide insight for future clinical studies exploiting the distinct dopamine circuits as pharmacological targets for anxiety, anhedonia, and depressive disorders.

Keywords: Anxiety, Depression, Dopamine Circuits, In Vivo Fiber Photometry, Electrophysiological Approaches

Disclosure: Nothing to disclose.

P35. Ventral Hippocampal Input to the Infralimbic Cortex is Necessary for the Effects of Extinction on Set Shifting After Chronic Stress

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Background: Psychiatric disorders such as post-traumatic stress disorder and major depressive disorder are characterized by deficits in cognitive flexibility. Exposure therapy can be effective in reversing cognitive deficits in these patients. Fear extinction in rodents bears similarity to exposure therapy. Extinction reverses chronic stress-induced deficits in cognitive flexibility on the attentional set-shifting test (AST), a medial prefrontal cortically-mediated executive process. Extinction requires the activity of pyramidal neurons in the infralimbic cortex, and BDNF-initiated signaling cascades to reverse stress-induced impairments in set shifting. However, the circuit mechanisms governing the extinction-mediated BDNF plasticity in the infralimbic are

unknown. The ventral hippocampus plays a key role in regulating infralimbic activity during extinction learning, and plasticity in the ventral hippocampus is necessary for extinction memory consolidation. In these experiments, we investigated the role of ventral hippocampal (vHipp) input to the infralimbic cortex (IL) for the effects of extinction after chronic stress in reversing cognitive deficits in male and female rats.

Methods: To selectively inhibit or activate ventral hippocampal input to the infralimbic, we injected either AAV- CaMKIIa-hM4D(Gi)-mCherry or AAV-CaMKIIa-EGFP into the ventral hippocampus. Immediately prior to extinction, rats received bilateral microinjections of CNO into the IL to silence ventral hippocampal terminals in the IL. In separate experiments, stressed rats received AAV-CaMKIIa-Gq in the ventral hippocampus (and CNO in the IL) to activate ventral hippocampal terminals in the IL in lieu of extinction. 24 hours after treatment, rats were tested on the AST to assess cognitive flexibility.

Results: Our results demonstrate that chemogenetically silencing pyramidal cell input from the vHipp to the IL blocks the phosphorylation of the BDNF receptor TrkB, and prevents the effects of extinction in reversing stress-induced cognitive deficits ($n = 10$-12/group, $p < 0.01$). Further, we demonstrate that activating vHipp input in the IL, in the absence of extinction, is sufficient to reverse stress-induced deficits in set shifting ($n = 6$-12/group, $p < 0.01$). Importantly, the effects of activating vHipp terminals in the IL are dependent on BDNF signaling, as local infusion in the IL of a neutralizing antibody prevented these beneficial effects.

Conclusions: These findings suggest vHipp-driven BDNF signaling in the IL is critical for extinction to counteract the deleterious cognitive effects of chronic stress.

Keywords: Extinction, Ventral Hippocampus, Infralimbic Cortex, BDNF

Disclosure: Nothing to disclose.

P36. A Prospective Evaluation of Fear Conditioning During COVID-19 in Persons With Trauma History

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Background: The fear conditioning model has been widely used to explain hallmark features of PTSD, including intrusive recollections and hyperarousal symptoms that underlie chronic physiological alterations in sympathetic and neuroendocrine activity. Enhanced fear conditioning and impaired extinction have been associated with PTSD in cross sectional laboratory studies. While it has been suggested that "conditionability" is trait-like and reflects learning mechanisms that occur during or soon after threatening traumatic experiences, little work has validated this prominent explanatory model of PTSD during real-life ongoing traumatic stress. The theoretical model would predict that individuals who are more conditionable or have impaired extinction would be more stress reactive to the real-world threat that many have experienced during the COVID-19 pandemic. Our overall objective was to test the ecological validity of the fear conditioning model by examining whether fear conditioning responses prior to the pandemic predict stress reactivity during COVID-19. We predicted that greater fear conditioning (higher differential skin conductance responses to CS + vs CS- stimuli during acquisition) and decreased extinction (higher differential skin conductance responses to CS + vs CS- stimuli during extinction) would be associated with greater stress related to COVID-19.

Methods: We conducted a longitudinal follow up study of a pre-existing cohort of PTSD and trauma-exposed non-PTSD participants who previously completed fear conditioning experiments. Extensive data were previously collected from 208 men and women (50% female); ages 18-65. PTSD status was previously determined by DSM-IV criteria using the Clinician Administered PTSD Scale (CAPS) administered prior to the pandemic. Participants previously underwent a fear conditioning and extinction procedure (involving shock as the unconditioned stimulus) which took place over two study visits, separated by 72 hours. Follow up measures examined COVID-19 exposure, including self-report of illness in self, family, close contacts, severity of illness, and stress reactivity related to COVID-19 with the Impact of Events Scale, Ruminative Responses Scale, Yale Brown Obsessive-Compulsive Scale, and Perceived Stress Scale. Hierarchical linear regressions at each phase (fear conditioning, fear extinction) were performed separately with differential skin conductance (CS + vs CS-) predicting stress symptoms during COVID-19.

Results: Smaller differential responses during fear conditioning were associated with greater obsessive and compulsive symptom severity scores (Beta = -.17, $p < .05$). Smaller differential responses during fear extinction were associated with greater COVID-19-related intrusions (Beta = -.29, $p = .07$), ruminations, Beta = -.35, $p < .05$) and perceived stress (Beta = -.41, $p < .05$). The coefficients for all other variables showed effects in the same direction, although were not significant.

Conclusions: Our findings suggest that pre-pandemic responses during fear conditioning and extinction in the lab prospectively predict stress reactivity during the pandemic. Contrary to initial predictions, we found that a smaller differential skin conductance response to CS + and CS- cues, primarily during fear extinction, measured prior to the pandemic were broadly associated with greater stress reactivity during the pandemic across multiple measures. One possible explanation is that decreased stimulus discrimination, or the awareness to adequately differentiate between danger and safety cues, may lead to increased generalized threat and heightened contextual anxiety. Further evaluation of whether the fear-based model predicts the persistence of stress responses over time and as threat changes will provide valuable information on mechanisms underlying recovery. This information will provide the basis for the development of strategies to support and protect populations that are vulnerable to the ongoing and future similar crises.

Keywords: Anxiety and PTSD, Fear Conditioning and Extinction, COVID-19

Disclosure: Nothing to disclose.

P37. Neural Correlates of Implicit Regulation of Emotional Conflict in Post-Traumatic Stress Disorder and Mild Traumatic Brain Injury

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Background: Functional neuroimaging studies have previously reported dysfunctional activation and connectivity within cortical and subcortical structures in post-traumatic stress disorder (PTSD) and mild traumatic brain injury (mTBI). The aberrant neural activations within these circuits is likely to contribute to emotion dysregulation, a key pathology in both disorders. Implicit regulation of emotional conflict is an important component of emotion regulation that has not been fully studied in these

disorders. Using functional MRI data from 305 participants that underwent the emotional conflict task, we examined the neural correlates of emotional conflict detection and its implicit regulation in patients with PTSD, mTBI and their comorbidity.

Methods: The 305 participants analyzed in this study included 50 with full or subthreshold PTSD, 93 with mTBI, 29 with full or subthreshold PTSD + mTBI, and 133 healthy comparison. In the emotional conflict task, participants were asked to name the emotion expressed on a face while ignoring an overlaying emotional word. Congruent trials were defined as trials where the emotional expression on the face (either fear or happy) and word (FEAR and HAPPY) match. Incongruent trials were defined as trials where the face and word do not match. Two primary contrasts were considered. One represented the detection of presence or absence of conflict between the face and the word (incongruent vs. congruent trials). Another contrast represented the evaluation and regulation of conflict (incongruent preceded by an incongruent trial vs. incongruent preceded by a congruent trial). Three analytic strategies were used. The first tested activations induced by the task pooling across all participants. For this analysis, we used FWE correction at the voxel level across the brain. In the second set of analyses, we compared healthy controls versus all diagnoses combined. For the third set, we compared healthy controls versus separate diagnoses (HC vs. PTSD, HC vs. TBI, HC vs. PTSD and PTSD + TBI, and HC vs. PTSD + TBI). We used a whole-brain voxel level threshold of $p < 0.001$ and FWE correction at the cluster level for the second and third analyses.

Results: Across all participants, conflict detection induced robust activations within the dorsal anterior cingulate (dACC), thalamus, and bilateral insular cortices (IC). Absence of conflict induced activations in the ventromedial prefrontal cortex, middle frontal cortex and precuneus. Regulation of conflict induced activations within the sensorimotor area, middle frontal gyrus, right IC, and precentral gyrus. For the second set of analyses, during the detection of conflict, compared to HC the diagnosed group exhibited significantly higher activation in the left hippocampus. The HC group showed higher activations within the dACC, precuneus, and thalamus during the evaluation of conflict. No significant between-group differences were observed during the regulation of conflict. For the last set of analyses, we did not detect any statistically significant between-group differences. While no significant differences in activation were noted within the amygdala and rostral anterior cingulate in any of the contrasts analyzed, functional connectivity analyses seeding the hippocampus and dACC revealed significant connectivity with the amygdala and the rACC across all participants.

Conclusions: When encountering emotionally conflicting cues, our data suggest that multiple cortical and subcortical neural nodes are critical for processing sensory and emotional information to maintain emotional homeostasis. These regions include the thalamus, hippocampus, amygdala, precuneus, anterior cingulate, insular, ventral and medial prefrontal cortices. A subset of these neural nodes showed dysregulated activations in participants with PTSD, mTBI and PTSD + mTBI, suggesting aberrant detection and evaluation of conflict. A surprising finding in in the last set of analyses is the absence of significant results from the comparison of the separate diagnoses to the healthy participants. This might be attributed to one of three possibilities: 1) patient populations examined maintain intact neural circuits to detect and regulate emotional conflict 2) that the task and/or current neuroimaging tools are unable to detect subtle neural differences pertinent to the detection and/or processing of emotional conflict, or 3) insufficient power. Given that we did observe significant findings when combining all diagnosed participants, and given recent neuroimaging studies suggesting the need for ~100 subjects per group, we speculate that insufficient power is the most likely explanation. Collectively, however, our results replicate and strongly affirm the role of several neural nodes in the implicit regulation of emotional conflict.

Keywords: PTSD, Conflict Monitoring, TBI, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P38. Neuroticism and Cortisol Levels in Response to the COVID-19 Pandemic Predict Post-Traumatic Stress Symptoms in Children: A Biopsychosocial Approach to Identify At-Risk Children

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Background: Although a majority of individuals will be exposed to a potentially traumatic event at some point in their life, only a significant minority will develop post-traumatic stress disorder (PTSD). The lifetime prevalence is estimated to be 9%, affecting twice as many women as men. In addition, this effect is particularly pronounced starting from puberty onwards. To optimize prevention, detection, and treatment plans, a wealth of research has been conducted to identify the psychological and biological factors that may render an individual more vulnerable to developing PTSD when confronted with a traumatic event. On a psychological level, it is well established that various personality traits can modulate one's risk to develop the pathology, such as anxiety and neuroticism. At a biological level, various systems have been examined, notably the hypothalamic-pituitary-adrenal (HPA) axis, for which the main end product is cortisol (a major stress hormone in humans). Most studies have shown lower cortisol levels in patients suffering from PTSD. For a long time, low HPA axis functioning was thought to result from the pathology, but more recent evidence suggests that dysregulated patterns of cortisol prior to trauma exposure is a risk factor for the development of the pathology. In children, very few studies have assessed the relationship between HPA axis functioning prior to trauma and the development of PTSD. Further, the few studies conducted on the subject suggest a hyperactivation of the HPA axis. Due to its unprecedented nature, the recent COVID-19 pandemic has been a highly stressful event that has induced significant psychological distress in the population. Moreover, important individual differences have been observed. Using a longitudinal design, we took advantage of the pandemic context to assess whether cortisol levels assessed before and during the first few months of the first lockdown could predict post-traumatic stress symptoms in children and whether psychological traits could moderate this effect.

Methods: 92 children who previously took part in one of our laboratory-based experiments between 2017-2019 and were free of physical and mental health problems were re-contacted in May 2020 to participate in this longitudinal study on psychological distress during the pandemic. In June 2020 (T1), 68 children (39 girls, 29 boys) aged between 9 and 14 provided a 6 cm hair sample, which was then divided into two segments of 3 cm. These two hair segments allowed us to capture cumulative cortisol levels that were secreted 1) 3 months before the onset of the first lockdown in Quebec, Canada (December 2019 to March 2020) and 2) during the first 3 months of the first lockdown (March 2020 to June 2020). From these two segments, we calculated the percentage change in cumulative cortisol levels between the beginning of and prior to the pandemic. At T1, children also filled out the Big Five Questionnaire for Children (BFQ-C) to assess neuroticism. Post-traumatic stress symptoms were assessed four times, every three months (June 2020-T1; September 2020-T2;

December 2020-T3, and March 2021-T4), using the Children's Revised Impact of Event Scale (CRIES). Given that anxiety symptoms are highly correlated with post-traumatic stress symptoms, anxiety symptoms were also measured at each timepoint (T1 to T4) using the State-Trait Anxiety Inventory for Children (STAI-C; state scale). Thereafter, state anxiety scores were added as covariates to the model.

Results: We conducted a linear mixed-effects model including time, neuroticism, and cumulative cortisol percent change, as well as their interaction as fixed effects. Sex and trait anxiety were used as covariates in the model. The analyses revealed a significant interaction between time, neuroticism, and cumulative cortisol percent change. Specifically, a decrease in cortisol levels early in the pandemic was associated with increased post-traumatic stress symptoms for children with heightened levels of neuroticism at T1 and T2 ($n = 67$, $F(3, 172) = 3.51$, $p = .017$, $R2m = .24$, $R2c = .59$).

Conclusions: These results suggest that insufficient activation of the HPA axis when faced with a stressful event, combined with high levels of neuroticism, may represent a risk factor for the short-term development of post-traumatic stress symptoms in healthy children. This highlights the importance of considering both psychological and biological factors when striving for a better understanding of the pathology. Gaining insight into the predictors of psychological distress in healthy children when confronted with a highly stressful enduring stressor might be informative for the early identification of at-risk children during future crises.

Keywords: Cortisol, COVID-19, Children, Post-Traumatic Stress Symptoms, Neuroticism

Disclosure: Nothing to disclose.

P39. Gray Matter Association With Extinction-Induced Neural Activation in Patients With Anxiety Disorders

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Background: The failure to appropriately retain extinguished fear is a key feature of clinical anxiety. Thus, exploring the neural correlates that underlie the extinction of conditioned fear is critical to advance our understanding of the psychopathology of anxiety disorders. Initial neuroimaging data reveal some connections between clinical symptoms and structural measures of several sub/cortical regions. However, the specific impact of structural size and the correspondence between cortical thickness and changes in brain activation remain a space for greater exploration. Here, we performed an analysis using voxel-based morphometry (VBM) to assess differences in gray matter volume (GMV) and its associated brain activations during fear extinction memory recall between healthy controls and patients with anxiety disorders.

Methods: We analyzed structural images from 170 participants, including 75 controls and 95 with anxiety disorders, who underwent a two-day threat conditioning and extinction paradigm. The VBM analysis was performed using the computational anatomy toolbox (CAT12). We first assessed the main effect of group (HC vs. ANX) in GMV using a voxel-wise linear regression analysis, with total intracranial volume, age, and sex as covariates. For regions that showed significant group difference in GMV, we further examined whether the abnormal GMV of anxiety disorders correlates with brain activation during extinction memory recall. We examined GMV and brain activations of several region-of-interests (ROI) that were thought to be critical in threat acquisition and its extinction, including the amygdala, hippocampus, insular cortex, dorsal anterior cingulate cortex (dACC), and ventromedial prefrontal cortex (vmPFC). An initial criterion of voxel-level $p < 0.005$, and a small volume family-wise error (FWE) correction pFWE<0.05 were used to detect significant clusters. A whole-brain analysis was also conducted with a criterion of voxel-level $p < 0.001$ and cluster-level pFWE < 0.05 to identify significant brain regions.

Results: Relative to the healthy controls, decreased GMV in the anterior hippocampus (small-volume correction pFWE<0.05), and increased GMV in the right dorsolateral prefrontal cortex (dlPFC, whole-brain cluster-level pFWE<0.05) were noted in patients with anxiety disorders. The GMV differences between control and anxiety disorders were associated with differing brain activations during extinction memory recall. Specifically, in healthy controls, hippocampus volume positively correlated with ventromedial prefrontal cortex (vmPFC) activation (small-volume correction pFWE<0.05), while in patients with anxiety disorders, hippocampus volume negatively correlated with dACC activation (small-volume correction pFWE<0.05). Additionally, dlPFC volume positively correlated with activations of dACC, pre- and post-central gyrus, and supramarginal gyrus (whole-brain cluster-level, all pFWE<0.05) in healthy controls but not in patients with anxiety disorders.

Conclusions: The data presented suggest that difference in functional activations during extinction recall may be associated with differences in GMV. There lies a correspondence between GMV of one region and activation of a region it subserves or interacts with. As such, disruptions to the functioning of regions corresponding with extinction retention (i.e., vmPFC) may be due to both, decreased cortical volumes of that region and decreased activations of the region due to GMV-dependent associations of another region (i.e., hippocampus). The associations between GMV and extinction-related activation represent an additional level of nuance to the impact of GMV in psychiatric psychopathology and therapeutic responses.

Keywords: Gray Matter Volumes, fMRI, Anxiety, Threat, Anxiety Disorders, Functional and Structural MRI

Disclosure: Nothing to disclose.

P40. Hippocampal Resting-State Functional Connectivity in Posttraumatic Stress Disorder: Preliminary Results From the PGC-ENIGMA PTSD Working Group

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Background: Alterations in the neurobiology of individuals with PTSD compared to controls have been found in the threat neurocircuitry, including the amygdala and hippocampus. Studies have observed abnormal resting-state functional connectivity (rs-FC) between regions underlying the threat neurocircuitry compared to controls, such as a weaker anticorrelation between the amygdala and rostral anterior cingulate cortex (rACC) and weaker connectivity between the hippocampus and regions of the prefrontal cortex. However, these studies were very small ($n < 25$), and larger sample sizes are needed for more unbiased analyses.

Methods: As part of the PGC-ENIGMA PTSD Working Group, seed-based resting-state fMRI data were centrally preprocessed using a standardized pipeline (HALFpipe) for $N = 3007$. Next, data were visually inspected for quality using the Linux command slicesdir by three independent raters (CAH, CCH, SVR). Data were excluded from the analysis for artifacts or low signal ($N = 387$). Across 22 collection sites, resting-state fMRI data was available for $N = 794$ PTSD and $N = 1092$ Control participants for the right amygdala, $N = 797$ PTSD and $N = 1097$ Control participants for the left amygdala, and $N = 796$ PTSD and $N = 1096$ Control participants for the right and left hippocampus. Group-level whole-brain

seed-based analyses were conducted on the right and left amygdala and hippocampus using SPM12 using a $p < 0.005$ and FWE-corrected cluster threshold.

Results: PTSD participants compared to Controls showed more positive rs-FC between the right amygdala and subgenual ACC (sgACC; peak, MNI: x = -8, y = 14, z = -22; $p < 0.001$, k = 625, FWE cluster-level corrected, $p = 0.001$) and similarly, the left amygdala and sgACC (peak, MNI: x = -8, y = 18, z = -22; $p < 0.001$, k = 286, FWE cluster-level corrected, $p = 0.109$).

PTSD patients compared to Controls showed more positive rs-FC between the right hippocampus with thalamus/brainstem (peak, MNI: x = 6, y = -18, z = -14; $p < 0.001$, k = 555, FWE cluster-level corrected, $p = 0.002$), and similarly, between the left hippocampus and thalamus/brainstem (peak, MNI: x = 0, y = -24, z = -18; $p < 0.001$, k = 573, FWE cluster-level corrected, $p = 0.002$).

Conclusions: Using the largest centrally analyzed resting-state dataset for PTSD to date, our results illustrate greater rs-FC between the amygdala and sgACC and between the hippocampus and thalamus in PTSD patients compared to controls. Previous studies using smaller samples, and both rs-FC and threat-related task-based designs, have suggested an aberrant functional relationship in PTSD participants within the amygdala and regions of the ventromedial prefrontal cortex (vmPFC). Our preliminary findings provide further evidence of the inadequate regulatory relationship between these two regions using the largest study to utilize resting-state data. Regarding our hippocampal findings, increased rs-FC between the hippocampus and thalamus could contribute to altered threat processing in PTSD. These findings are important as they can help guide treatments targeting these aberrant connections, such as neuromodulation.

Keywords: Resting-State Functional Connectivity, Posttraumatic Stress Disorder, Amygdala, Hippocampus

Disclosure: Nothing to disclose.

P41. Greater Social Cognition-Related Right Temporal Pole Activation in World Trade Center Responders With PTSD - Preliminary Evidence

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Background: The ability to accurately 'read' and respond to others is key in developing and maintaining a supportive social network. People with posttraumatic stress disorder (PTSD) often report low access to social support and more difficulty in interpersonal relationships. Further, recent neuroimaging findings suggest that social cognition deficits could affect how people with PTSD perceive threat cues. However, few neuroimaging studies have investigated social cognition in trauma-exposed adults across dimensions of resilience (i.e., presence or absence of psychopathology) and degree of exposure to the event. In this study, we sought to examine social cognition-related brain activation during a mentalizing task in symptomatic individuals with PTSD, compared both to highly resilient and lower-exposed controls. We hypothesized that the PTSD group would show lower social cognition-related BOLD activation in hypothesized regions of interest relative to both lower-exposed and resilient control groups.

Methods: As part of a larger, multimodal study of PTSD and resilience in World Trade Center (WTC) responders, we recruited 74 adults who experienced the 9/11/2001 attacks on the WTC, and/or were involved in post-9/11 recovery work in New York City 2001-2002. Stratified sampling aimed to recruit participants in three groups: (1) PTSD (any number of WTC -related exposures; met full criteria for lifetime WTC-related PTSD based on the Clinician-Administered PTSD Scale for DSM-5 [CAPS-5], with persistent clinically significant PTSD symptoms in the past month); (2) highly resilient (four or more WTC-related exposures linked to risk for PTSD; no current or lifetime psychopathology); and (3) lower-exposed (three or fewer WTC exposures; no current or lifetime psychopathology). The target sample size in this ongoing study is N = 105 (CDC/NIOSH # U01OH011473).

Participants attended a neuroimaging session in which they completed an in-scanner version of the Reading the Mind in the Eyes social cognition task. On each trial, participants had to decide which one of two words shown corresponded to a cropped photograph of a person's face. Word pairs were either social (e.g., "apprehensive", "pensive") or non-social (e.g., "dark hair", "looking left"). The Social > NonSocial contrast represented the effect of social cognition, rather than general perceptual and decisional functions.

fMRI data preprocessing used fMRIPrep 21.0.2 to generate individual motion-, susceptibility distortion, and slice timing-corrected echoes, which were subsequently denoised using TE-dependent independent components analysis (TEDANA 0.0.12), and finally smoothed with a 4-mm FWHM Gaussian kernel. Four of the 74 participants were excluded due to excessive motion (n = 3) or technical error (n = 1), leaving a final n = 22 in the PTSD group, n = 31 in the resilient group, and n = 17 in the lower-exposed group.

Results: Linear models identified a main effect of trial type (Social vs. NonSocial) on task performance: mean reaction time was slower ($F = 36.3$, $p < .0001$) and participants made more errors ($F = 248.2$, $p < .0001$) on Social trials. There was no main effect of group on mean reaction time ($F = 1.49$, $p = .22$) or accuracy ($F = 2.85$, $p = .064$) and no interaction with trial type. For the fMRI data, Social > NonSocial contrast images at the single-subject level were aggregated for the group-level analysis. A whole brain 1-sample t test for the Social > NonSocial effect identified clusters matching a priori ROIs related to social cognition, including medial prefrontal cortex, temporal poles, inferior frontal gyrus pars orbicularis, and precuneus/posterior cingulate cortex, using a threshold of pFWE = .05 and k = 50. There was a main effect of group for the right temporal pole cluster (50 16 -32), $F(2,67) = 3.98$, $r2 = .08$, $p = .023$.

Contrary to hypothesis, Social > NonSocial BOLD signal in the right temporal pole cluster was significantly greater in the PTSD group compared to the resilient ($p = .027$) and lower-exposed control ($p = .011$) groups, while control groups did not differ significantly ($p = .491$). The continuous measure of PTSD severity (CAPS5-Past Month scores) also predicted higher Social > NonSocial right temporal pole activation, $F(1,68) = 6.92$, $p = .01$).

Conclusions: Our findings are consistent with prior research implicating structural and functional differences in the right temporal pole in individuals with PTSD. They also provide additional support for social cognition as an emerging but important area for future investigations of the neural processes involved in psychological factors linked to risk or resilience after traumatic stressors.

Keywords: PTSD, Post Traumatic Stress Disorder, Social Cognition, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P42. Open-Label Accelerated 1-Hz rTMS Pilot Targeting Intraparietal Sulcus in Generalized Anxiety Disorder

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Background: Repetitive transcranial magnetic stimulation (rTMS) is a promising avenue to develop novel treatments for anxiety.

Despite this promise, the primary development pathway for anxiety has been to adapt protocols designed for depression treatment. Although these disorders are highly comorbid, the underlying neural mechanisms may differ, leading to mixed efficacy among anxiety patients. Accordingly, there is a critical need for novel neuromodulatory protocols that specifically target the neural circuits affected by anxiety. Additionally, such protocols should be developed using dimensional measures that reliably capture behavioral phenotypes associated with network dysfunction.

Methods: The purpose of this study is to provide preliminary evidence to determine the safety and efficacy of parietal stimulation for anxiety. The current results are from an ongoing open label pilot targeting the right IPS in Generalized Anxiety Disorder (GAD) patients. Patients received week of accelerated 1 Hz rTMS (5 days, 8 sessions/day, 600 pulses/session) at 100% of resting motor threshold. Anxiety was measured at study start and ~76 hours post rTMS using threat of unpredictable shock. Anxiety was quantified as the increase in startle magnitude during the threat periods compared to the safe periods (anxiety potentiated startle; APS).

Results: At the time of writing, this ongoing study has 5 enrolled patients with 3 completers. Using an intent-to-treat analysis, APS was reduced from pre to post rTMS with a moderate effect size (cohen's $d = 0.51$).

Conclusions: Although preliminary and qualitative, these results offer promising support for future studies aimed at treating GAD with parietal rTMS. These results are consistent with our previous work showing that the IPS exhibits hyperexcitability and hyperconnectivity during unpredictable threat, and that reducing this hyperexcitability with 1 Hz stimulation can reduce anxiety, as indexed by APS. Together with this previous work, the current results suggest that the IPS may be a key region for anxiety expression and a prime target for inhibitory neuromodulation. Future clinical trials should replicate this work using appropriate blinding, adequate control conditions, and a large enough sample size to ensure adequate power ($N > 70$/group).

Keywords: rTMS, Generalized Anxiety Disorder, Startle

Disclosure: Nothing to disclose.

P43. Impact of Trauma Type on Neural Mechanisms of Threat Conditioning and its Extinction

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Background: Associative learning theories suggest that psychopathology following trauma exposure might arise from dysfunctions in the neural circuits underlying threat conditioning and extinction learning. Within the context of threat and fear extinction, prior studies in posttraumatic stress disorder (PTSD) have consistently reported impaired activations within regions of the medial prefrontal cortex, insular cortex, hippocampus, amygdala, and various parietal and temporal cortical regions. To our knowledge, none of the studies to-date have been able to evaluate the impact of different types of trauma exposure on the neurobiology of threat and fear extinction due to limited power. In this study, we combined data from 3 studies to examine whether trauma type (violent vs nonviolent) differentially impacts the neurobiology of threat conditioning and its subsequent extinction mechanisms.

Methods: We analyzed data from 207 trauma-exposed individuals, with or without PTSD diagnosis, who underwent an established 2-day threat conditioning, extinction learning, and extinction recall paradigm. We analyzed skin conductance responses (SCR) and functional magnetic resonance imaging (fMRI) data. Reported traumatic events were categorized as either violent or nonviolent. The violent category included experiencing or witnessing: combat, physical/sexual assault or abuse, resulting in total sample of $n = 126$ in this category—we refer to this group as the violent trauma-exposed (VTE) group. The other 81 participants fell into the nonviolent trauma-exposed (NVTE) group. This category included experiencing or witnessing: accidental injury, natural disaster, unexpected death. To further refine our analyses within the VTE group, we subdivided this cohort into sexual ($n = 55$) vs nonsexual trauma ($n = 71$) categories. For the fMRI data analyses, we first examined regions that were thought to be critical for conditioning and extinction, including amygdala, hippocampus, insular cortex, ventromedial prefrontal cortex, and dorsal anterior cingulate cortex (small-volume correction, family-wise error [FWE] corrected). For whole-brain analysis, we used a voxel-level threshold of $p < 0.005$ and cluster-level pFWE<0.05.

Results: For the SCR analyses, the VTE group showed significantly higher SCR to the extinguished conditioned stimuli relative to the NVTE group ($p < 0.01$), suggesting impaired extinction memory recall in the VTE group. No other statistically significant SCR differences were noted between these two groups or within the sub-groups analyses (all ps>0.10). In the fMRI analyses, we observed significantly increased activations within the posterior insular cortex and visual cortices (cluster-level pFWE<0.05) during threat conditioning and significantly decreased activations in the dorsal anterior cingulate cortex, inferior parietal cortex, and posterior insular cortex during late extinction learning (cluster-level pFWE<0.05). The dysfunctional activations during late extinction in the VTE group are consistent with impaired extinction. As for the sub analyses focusing on the sexual vs. nonsexual VTE, the group in the nonsexual VTE exhibited higher activation in the rostral anterior cingulate cortex during early conditioning (cluster-level pFWE<0.05), and higher activations in the inferior parietal insular, and temporal cortices during extinction memory recall (cluster-level pFWE<0.05).

Conclusions: We report findings showing that participants who experienced violent types of trauma demonstrated impaired fear extinction at the SCR level and dysfunctional activations within neural nodes important for attention and sensory processing of threat cues during threat acquisition and during the extinction learning. We did not observe any significant dysfunctional activations within neural circuits involved in threat detection or emotion regulation (i.e., amygdala, hippocampus) in any of our analyses, including sexual vs. nonsexual trauma groups. These null findings suggest that, for the most part, violent trauma, regardless of it being sexual or nonsexual, appears to have comparable impact on the neural circuits of threat detection and emotion regulation. Violent vs. nonviolent trauma, however, seems to have a more differential impact on brain circuits related to attention and perception.

Keywords: PTSD, fMRI, Trauma

Disclosure: Nothing to disclose.

P44. Neural Correlates of Treatment Response Differ Between CBT and Active Therapy Control in Adolescents With Anxiety Disorders: Evidence From an Approach-Avoidance Conflict Paradigm

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Background: Cognitive-behavioral therapy (CBT) is a gold-standard intervention for pediatric anxiety disorders. Part of its efficacy derives from exposure therapy, in which children approach feared stimuli and learn to decrease their anxiety in a graded fashion. Despite its clinical track record, 20-40% of patients fail to respond to CBT (Kendall and Peterman, 2015), which could be explained in part by reduced approach motivation needed to engage with feared stimuli. The purpose of this study was to investigate neural correlates of treatment success for CBT using data from a previously-published RCT (Silk et al., 2018), which compared the treatment of youth with anxiety disorders with CBT to an active therapy control, child-centered therapy (CCT), including comparisons with youth without psychiatric diagnoses (NPD). We examined data from an Approach-Avoid Conflict fMRI task (Schlund et al., 2010), hypothesizing that compared to NPD, anxious youth would have greater avoid-related activity, but those who responded to treatment would have greater approach- and approach-avoid conflict activity at baseline. Regions of interest included ventral and dorsal striatum, amygdala, dorsal ACC and anterior insula.

Methods: A total of 97 youth with an anxiety disorder (generalized, social, separation) (ANX) age 9-14 randomized 2:1 to CBT (N = 67) or CCT (N = 30) and had usable imaging data; 38 age- and sex-matched NPD youth also had usable data. Youth underwent a baseline assessment using a standardized DSM-IV diagnostic interview (K-SADS-PL) and a measure of anxiety severity (the Pediatric Anxiety Rating Scale (PARS), then a baseline MRI, then had 16 sessions of CBT or CCT therapy prior to a second assessment and MRI. Treatment response was defined as a reduction of 35% or more in PARS score. MRI data were preprocessed using fmriprep (https://fmriprep.org/en/stable/) and analyzed using FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki). Multiple comparison correction was done using cluster-based random field theory with a cluster forming threshold of z = 3.1 and a corrected cluster p-value threshold of 0.05. All analyses controlled for age and sex.

Results: The majority of ANX youth (N = 67) responded to treatment. Contrary to our hypotheses, there were no differences in approach-, avoid- or approach-avoid conflict-related brain activity between anxious and NPD youth, nor did activity predict treatment response either at baseline or in pre-post brain activity changes. However, when we examined the interaction between therapy group and treatment response, there were significant activations for the approach condition in bilateral dorsolateral and medial PFC, right dorsal striatum, bilateral precuneus/posterior cingulate, bilateral posterior insula, inferior parietal, superior temporal and occipital cortices. For the avoid contrast, the interaction term produced activations in bilateral dlPFC, bilateral vlPFC, left amygdala, left ventral and dorsal striatum, bilateral superior and inferior parietal cortices, bilateral temporal cortices, and occipital cortex. Visualization of these interactions revealed that treatment non-response in the CBT group corresponded to decreases in approach and avoid-related activity, whereas treatment response corresponded to either increases or no change in activity. The CCT group demonstrated the opposite pattern, such that treatment non-response corresponded to increases in activity, while treatment response corresponded to decreases or no change in activity.

Conclusions: Although the majority of ANX youth responded to treatment, patterns of change in neural activity differed between treatment types, suggesting that mechanisms of treatment efficacy may be unique to CBT. Change in brain activity related to both approach and avoidance motivation, both within and outside of defined regions of interest, indicates that both neural systems are relevant to treatment of anxiety in youth but activate differentially in exposure-based relative to supportive modes of treatment.

Keywords: Adolescent Anxiety, Task fMRI, Approach/Avoidance, Cognitive Behavior Therapy, RCT

Disclosure: Nothing to disclose.

P45. Real-Time fMRI Neurofeedback of Left Dorsolateral Prefrontal Cortex During Emotional Cognitive Control: A Randomized Controlled Trial

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Background: Real-time fMRI neurofeedback (rt-fMRI-nf) is an endogenous neuromodulation approach whereby individuals self-regulate their own neural activity in the moment using strategies related to a particular construct of interest. Cognitive control deficits in an emotional context negatively impact people with mental health disorders, including those with depression, anxiety, and PTSD. The left dorsolateral prefrontal cortex (dlPFC) has been shown to be a centrally important region for facilitating emotional cognitive control. Using a double-blind randomized controlled trial design, this study tested a novel rt-fMRI-nf protocol that sought to increase left dlPFC blood-oxygen level dependent activity during emotional cognitive control and improve self-report and behavioral measures of emotional cognitive control.

Methods: Healthy adult participants (N = 70; 42 females; mean age = 31.2 years) enrolled in the study and were randomized to either active (n = 37; left dlPFC) or sham (n = 33; left postcentral gyrus) single-session rtfMRI-nf (clintrials.gov identifier: NCT04543513). Feedback was given from a 7-mm sphere region-of-interest centered on coordinates in left dlPFC or left postcentral gyrus, respectively. During neurofeedback, participants focused on executive functioning tasks (e.g., mental math, word list recall, rehearsing to-do list) while distracting negative emotional words were presented, which engaged emotional cognitive control. Single-session neurofeedback consisted of five rt-fMRI-nf runs, starting with practice, followed by three training runs, and lastly a transfer run without feedback. Participants also completed self-report [Patient-Reported Outcomes Measurement Information System (PROMIS): Self-Efficacy for Managing Emotions] and behavioral measures [emotional Stroop task (EST) interference effect] of emotional cognitive control prior to rt-fMRI-nf and at one-day follow-up. The primary outcome was left dlPFC activity during neurofeedback, and secondary outcomes were self-report/behavioral measures of emotional cognitive control. A series of linear mixed-effects models (LMEs) were conducted to examine if active rt-fMRI-nf, compared to sham, led to greater changes for each outcome measures [fixed effects: group, time, group-by-time interaction; random effect: subject id]. We also examined protocol tolerability and subjective emotional distress associated with the negative words using a post-neurofeedback survey with 1-10 ratings.

Results: LMEs showed a significant group-by-time interaction on left dlPFC activity ($p = .031$; $\eta 2 = .021$), and this interaction was such that active neurofeedback led to greater left dlPFC activity in run 1 ($p = .030$; $d = .66$) and run 2 ($p = .042$; $d = .62$), but not for run 3 ($p = .82$) nor the transfer run ($p = .30$). LMEs showed no significant effects on either self-report or behavioral measures of emotional cognitive control ($p's > .46$). Note that these self-report/behavioral measures were significantly inversely correlated with each other ($p < .001$; $r = -.41$), indicating that those with higher PROMIS: Self-Efficacy for Managing Emotions scores had lower emotional interference on the EST. Participants across conditions described the protocol as being tolerable with minimal distress, and they were unable to identify their rtfMRI-nf condition ($p = .83$). While the overall level of emotional distress reported in reaction to the negative words shown during neurofeedback was quite low across both groups ($M = 1.57$ on a 1-10 scale), participants in the active group reported significantly lower distress ($M = 1.32$) compared to the sham group ($M = 1.84$; $p = .039$; $d = -.51$).

Conclusions: These findings support the feasibility of left dlPFC rt-fMRI-nf and identify potentially acute effects on left dlPFC activity and subjective emotional distress. However, overall results indicate that rt-fMRI-nf had an initial but not a sustained effect on left dlPFC activity, and thus, they present complexity and nuance to consider when using rt-fMRI-nf to modulate neural activity and improve psychological function. The sustained impact of the current dlPFC neurofeedback protocol may have been limited by participant fatigue associated with a long protocol, individual variability in left dlPFC architecture, and diminishing challenge of the cognitive tasks over time. Future studies are needed to optimize the cognitive strategies used, the dlPFC target, and the timing and duration of the neurofeedback protocol, as well as to determine whether dlPFC neurofeedback may have differential impacts for clinical populations.

Keywords: Non-invasive Neuromodulation, Functional MRI (fMRI), fMRI Negative Affective Stimuli, Cognitive Enhancement, Real-Time fMRI Neurofeedback

Disclosure: Nothing to disclose.

P46. Behavioral Activation and Exposure Therapy Improve Anxiety Symptoms and Alter Neural Processing of Approach-Avoidance Conflict

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Background: Individuals with anxiety disorders often engage in maladaptive decision-making when faced with approach-avoidance conflict (AAC), a behavioral pattern that is addressed in exposure-based therapy (EXP) and behavioral activation (BA). Identifying mechanisms by which these treatments promote adaptive behavior may guide the development of process-specific treatment approaches. In the present study, we examined behavioral and neural responses to AAC before and after BA and EXP in a sample of adults with generalized anxiety disorder (GAD).

Methods: Participants were randomized to complete 10 weekly sessions of either BA or EXP. Symptoms were assessed pre-treatment, at each treatment session, and post-treatment with the GAD-7, PROMIS Anxiety and PROMIS Depression scales. Individuals who completed at least 7 out of the 10 therapy sessions were considered treatment completers and included in clinical outcome analyses. Treatment completion and treatment response, defined as at least 30% reduction in GAD-7 scores from pre- to post-treatment, were compared across treatment arms using logistic regressions. Linear mixed-effects regressions were conducted for symptom scores with time (all timepoints) and treatment arm (BA, EXP) entered as predictors.

Before and after treatment, participants completed an AAC task during fMRI. In the task, participants chose how much to approach or avoid based on explicit cues indicating the most likely outcomes associated with each position along a horizontal runway onscreen. On each trial, an approach decision could result in an aversive outcome (negatively-valenced images and sounds), an appetitive outcome (earning 2, 4, or 6 cents), or both (i.e., an AAC trial). Treatment completers who completed the AAC task pre- and post-treatment, and had useable fMRI data, were included in analyses of behavior and brain activity during the AAC task. We conducted linear mixed-effects regressions for the effects of time (pre-, post-treatment), treatment arm, and trial-type (conflict with 2, 4, or 6 cents) on runway position and response time across AAC trials. Computational parameters reflecting emotional conflict (i.e., aversiveness of threats relative to rewards) and decision uncertainty, both of which have been previously found to be elevated in anxious individuals, were extracted from approach and avoidance behavior, and we conducted linear mixed-effects regressions for the effects of time and treatment arm on these parameters. Next, neural responses (blood-oxygen-level-dependent [BOLD] signal) were extracted for a priori regions of interest, including dorsolateral prefrontal cortex (dlPFC), amygdala, anterior cingulate, anterior insula, and striatum responses to the decision phase; and striatum responses to picture presentation and reward receipt. We conducted linear mixed-effects regressions for each region of interest, with time, treatment arm, and trial-type (aversive; appetitive; conflict with 2, 4, or 6 cents) as predictors, and neural responses as the outcome variable. Age, sex, and education were included as covariates in all group-level regressions.

Results: Of the 102 participants enrolled in the study, 70 completed treatment (38 BA, 32 EXP), reflecting comparable completion across treatment arms ($p = 0.127$). A majority of treatment completers responded to treatment in both BA (66%) and EXP (59%) with comparable response across treatment arms ($p = .761$). Effect sizes on GAD-7 scores pre- to post-treatment were large for both BA ($d = -1.14$) and EXP ($d = -0.97$). Pre- to post-treatment contrasts did not differ across treatments for GAD-7 scores ($p = .540$), but did differ across treatments for PROMIS Anxiety ($p = .039$, $d = .13$) and PROMIS Depression scores ($p = .029$, $d = .14$), in both cases reflecting greater symptom improvement in BA compared to EXP.

49 participants (29 BA, 20 EXP) were included in behavioral and fMRI analyses. Following both treatments, reaction times decreased ($p = .008$, $\eta p2 = .03$) and, unexpectedly, approach behavior also decreased ($p = .048$; $\eta p2 = .02$) on AAC trials. The computational parameter reflecting emotional conflict increased following treatments ($p = .017$, $\eta p2 = .12$); the decision uncertainty parameter did not significantly change following treatments ($p = .162$). Analyses of neural responses revealed that BA, more than EXP, was associated with increased left dlPFC engagement during decision-making (time by treatment-arm interaction: $p = .020$; $\eta p2 = .01$), and both treatments were associated with increases in striatal response to image outcomes ($p = .037$, $\eta p2 = .03$) and monetary reward receipt ($p = .015$, $\eta p2 = .04$).

Conclusions: BA and EXP are shown to be effective treatments for GAD. Increased activation in left dlPFC during decision-making and increased striatal engagement during emotionally salient outcomes may reflect the neural basis of a process by which BA and EXP influence approach-avoidance conflict decision-making. One interpretation is that, with treatment, individuals increase dlPFC-mediated effortful deliberation during AAC decision-making, and increase striatally-mediated encoding of salient outcomes, both in the task and in real-world scenarios. Increases in left dlPFC activity were particularly robust in BA, potentially due to BA's focus on deliberately engaging in values-driven behavior. Further research is warranted to investigate whether psychological treatments can be optimized by targeting specific decisional processes.

Keywords: Anxiety, Generalized Anxiety Disorder, fMRI, Decision Making, Approach-Avoidance Conflict

Disclosure: Nothing to disclose.

P47. Sex-Specific White Matter Microarchitectural Alterations in Preadolescent Youth With Anxiety Disorders

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Background: Anxiety disorders (ADs) emerge in childhood and early adolescence and are among the most common psychiatric illnesses. With the onset of puberty, females are twice as likely as males to have ADs. Considerable research has implicated alterations in the white matter (WM) microstructure of prefrontal-limbic tracts, as well as other WM regions, in the pathophysiology of ADs. These findings are of interest because WM consists primarily of myelinated axons, and myelin plays an important role in mediating optimal neuronal communication. However, few studies have examined anxiety-related WM alterations in youth and in relation to sex. To further examine associations between childhood ADs, WM microstructural integrity, and sex, we analyzed diffusion tensor imaging (DTI) data from 295 preadolescent youth with and without ADs.

Methods: Preadolescent youth with ADs (social, generalized, and/or separation AD) and healthy controls were enrolled between ages 8-12 at 2 study sites (UW-Madison, NIMH) and completed a DTI scan on a 3T-scanner. The final sample ($n = 295$; 201 females) included 163 children with ADs and 132 controls. Tractography- and voxel-based analyses examined the main effect of ADs, as well as the AD by sex interaction, for WM metrics [fractional anisotropy (FA), radial diffusivity (RD), mean diffusivity (MD), and axial diffusivity (XD)] in seven bilateral tracts of interest and in WM across the whole brain.

Results: Results demonstrated widespread anxiety-related alterations in DTI metrics across multiple WM regions. Critically, anxiety-related effects demonstrated a significant interaction with sex, such that AD-related FA reductions and RD increases were observed exclusively in boys and not in girls. Preadolescent boys with ADs exhibited decreased FA and elevated RD relative to healthy control boys (P-FWE < 0.05) across various WM regions throughout the brain, in association (UF, EC, IFO, SLF, ST, ILF), commissural (CC), projection (CR, IC), and brainstem (CST, CP, ML) tracts, while no group differences were seen in girls.

Conclusions: This study constitutes the largest cross-sectional DTI study of childhood anxiety to date. The results show that childhood ADs are associated with broadly distributed alterations in WM microarchitecture across the brain, and, importantly, this relationship is evident only in boys. These findings – particularly the combination of reduced FA and increased RD – point to the possibility that WM microstructure and myelination processes may be related to the underlying pathophysiology of childhood ADs, laying the foundation for future work exploring the viability of WM microstructure as a treatment target for novel therapies.

Keywords: Childhood Anxiety, White Matter Microstructure, Diffusion Tensor Imaging (DTI), Myelination, Sex Differences

Disclosure: Nothing to disclose.

P48. Brain Regions Conferring Psychological Resilience: A Meta-Analysis

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Background: Neuroimaging investigations in psychiatry have historically sought to identify neurobiological mechanisms placing individuals at risk for developing psychiatric disorders. But a growing interest has emerged for identifying neurobiological mechanisms that promote psychological resilience, a MeSH term (D055500) defined in 2009 as "The human ability to adapt in the face of tragedy, trauma, adversity, hardship, and ongoing significant life stressors." To address this growing interest, we conducted a systematic review and neuroimaging meta-analysis of brain regions which may promote psychological resilience.

Methods: We conducted a systematic review of 4 bibliographic databases (PubMed, Embase, PsychInfo, and Web of Science) for journal articles including keyword "resilience" and a functional neuroimaging technique. (See poster for exact, database-specific search queries.) These searches yielded 498 unique articles. 429 articles were excluded for reasons including a non-psychological definition of resilience (e.g., cognitive resilience following neurosurgery), not recruiting a resilient sample, and not providing neuroimaging data or coordinates, yielding 69 articles included for analysis. This search also yielded 42 review articles whose bibliographies were searched for relevant articles: these articles encompassed an additional 2205 articles of which 78 met inclusion criteria, for $69 + 78 = 147$ unique articles. All elements of the systematic review – including reason for exclusion, sample size, and brain coordinates – were memorialized in the non-proprietary reference manager Zotero for rigor and reproducibility. Custom python code then extracted articles' ROI coordinates in Talairach or MNI atlas space for activation likelihood estimation (ALE) meta-analysis by GingerALE. The 147 studies yielded 499 foci more significantly associated with the resilient groups than with the healthy control and/or patient groups. GingerALE was performed with uncorrected $p = 0.001$ and 100 permutations for FWE $p = 0.01$. All code available by request.

Results: The ALE meta-analysis associated three regions with psychological resilience: right amygdala (ALE = 0.060, Z = 7.76), right amygdala (ALE = 0.056, Z = 7.41), and dorsal anterior cingulate (ALE = 0.032, Z = 5.04). Post-hoc examination of studies contributing to each region found that the amygdalae were largely comprised from studies of PTSD (~45%) and depression (~30%), while the anterior cingulate was predominantly comprised from studies of PTSD (38%) and schizophrenia (38%).

Conclusions: The amygdalae and anterior cingulate play critical roles in diverse cognitions including threat detection, error detection, and emotion regulation. Notably, amygdala and anterior cingulate hyperactivity has been associated with both PTSD and MDD. Our findings suggest these regions play more complex roles in promoting risk for and resiliency against psychiatric disorders than previously thought. Future work will seek to identify disorder-specific predictors of risk and/or resilience.

Keywords: Risk and Resilience, Human Neuroimaging, Emotional Regulation

Disclosure: Nothing to disclose.

P49. Altered Default-Mode (DMN)-Frontoparietal (FPN) Network Connectivity Following Mindfulness-Based Cognitive Therapy (MBCT) in PTSD Patients Linked to Treatment Response - A Randomized Controlled Trial With Active Comparator

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Background: Posttraumatic stress disorder (PTSD) is common, can be chronic and debilitating, and has high human and economic costs to individuals, families, and society. While existing trauma exposure-based psychotherapies show high efficacy, many PTSD patients decline trauma-focused therapy, and meta-analyses report 30-50% of PTSD patients treated with trauma-focused therapy do not show clinically meaningful improvement, suggesting "one size may not fit all". Accumulating evidence finds clinically significant efficacy of 8 wk group mindfulness-based approaches like Mindfulness-based Cognitive Therapy (MBCT) for acute depression, anxiety, and PTSD, in addition to original target of relapse prevention for depression and substance use disorders,

warranting more research on efficacy for PTSD and potential neural mechanisms. We reported in 2016 that a mindfulness-based intervention for PTSD led to increased resting state functional connectivity (rsFC) between posterior cingulate cortex (PCC, key node of Default Mode Network, DMN) and dorsolaterasl prefrontal cortex (dlPFC, key node of frontoparietal network, FPN); and mindfulness-associated increased PCC-dlPFC rsFC has since been independently replicated by 3 laboratories. In 2017 we registered hypotheses with NCCIH (R61 "Go-criteria") that MBCT - compared to an active Mind-body comparator, progressive muscular relaxation (PMR) – would lead to increased PCC-dlPFC rsFC, and both MBCT and PMR would lead to decreased PCC-insula rsFC (which is increased in PTSD).

Methods: We conducted a randomized controlled trial (RCT) with PTSD patients comparing MBCT to an active mind-body control (PMR, designed to be structurally similar to MBCT but no mindfulness instruction) using cluster randomization (blinded to assignment during recruitment). Functional MRI (3T GE scanner) scans were collected at intake and within 3 weeks post-treatment. We consented 161 persons from the community, and enrolled 77 with PTSD diagnosis by CAPS-5 and intake fMRI scan. Due to COVID pandemic, 22 patients were lost to follow-up, and interventions were shifted to remote delivery ("Zoom"), and 41 participants completed the RCT with both pre- and post-therapy fMRI scans. Assessments (CAPS-5 and HAM-D) were conducted by blinded assessors, and CGIs obtained by consensus of clinical team, and the PI and analysts were blinded. Self-report measures (PCL-5, decentering, PTSD cognitions, rumination, etc) were conducted using web-based REDCap. fMRI paradigms including resting state (8 min), a previously validated self-referential task, and a contextual processing task. We assessed rsFC using a PCC seed in CONN toolbox, and used a flexible ANOVA in SPM12 testing a time (pre- vs post-therapy) by treatment (MBCT vs PMR) interaction term.

Results: We report results from our rsFC data testing our registered hypotheses that MBCT leads to increased PCC-dlPFC and decreased PCC-insula rsFC. Both MBCT and PMR led to clinically meaningful improvements in PTSD (MBCT mean 8.9 point CAPS-5 reduction, Hedge's $g = 1.0$, $p < .001$, PMR 10.1 point CAPS-5 reduction, $g = 1.0$, $p < .001$), no significant between treatment effect. Both interventions were well-tolerated and had completion rates (5+ sessions) >80%, Rates of "responders" (CGI-I 1–very much or 2-much improved) were 48% MBCT and 50% PMR. In the PCC-seed rsFC ANOVA interaction Z-map we found a cluster in right dlPFC/ BA 10 MINI:(30, 48, 12), $F = 20.91$, $Z = 3.90$, $k = 94$ voxels, SVC pFWE=0.021. Test of the change score (pre- vs post delta) in the extracted dlPFC (5 mm sphere) beta values found significantly greater ($p = .002$) increase in rsFC in the MBCT group compared to the PMR group (Hedges $g = 1.03$). Furthermore, change in PCC-dlPFC rsFC was greater in MBCT responders vs non-responders ($p < .05$, $g = 0.57$). Both MBCT and PMR led to decreased PCC-insula rsFC (pre vs post extracted aal insula $g = -0.35$ in MBCT, $g = -0.53$ in PMR, $p < .05$).

Conclusions: We found evidence supporting our registered hypothesis (R61 "Go-criteria") that MBCT but not PMR lead to an increase in PCC-dlPFC rsFC (altered DMN-FPN cross-network connectivity). This is also consistent with recently reported findings from independent research groups. Furthermore, the increase in PCC-dlPFC rsFC was significantly related to clinical improvement (blinded CGI-I scores for PTSD) in the MBCT group only. These data further support PCC-dlPFC FC as a potential treatment target mechanism of mindfulness interventions, potentially related to increased volitional meta-cognitive attention and emotional regulation of spontaneous distressing thoughts. Both MBCT and PMR decreased PCC-insula rsFC, suggesting a general mechanism related to improvement of PTSD symptoms.

Keywords: Mindfulness, PTSD, Default Mode Network (DMN), Frontoparietal Network, Insula Connectivity

Disclosure: Nothing to disclose.

P50. Validation of an Updated Brain Circuit to Decode the Neural Signature of Threat Conditioning and Fear Homeostasis

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Background: Threat conditioning and extinction is one of the primary translational models for understanding anxiety and trauma-related psychopathology. The 'threat circuit', which mainly includes the medial prefrontal cortex, insular cortex, hippocampus, and amygdala, is thought to play a key role in the threat acquisition and its extinction. Recent evidence, however, suggests that the processing of threating and fearful stimuli is highly distributed across multiple neural systems. In this study, we combined functional MRI data and machine-learning methods to identify and validate distributed neural representations of stimuli coding threat or safety. We then used external datasets from multiple paradigms to test the specificity of the newly identified circuit.

Methods: Task-based functional MRI data from a total of 1455 participants were analyzed in the present study. We used the neural activations within and beyond the 'threat network' to distinguish conditioned stimuli associated with threat (CS+) or safety (CS-) across experimental phases of threat conditioning and extinction. We evaluated the performance of the decoding models using cross-validation on a discovery dataset ($n = 420$) and tested the generalizability of the models using two external datasets ($n = 126$ and 94). We then estimated predictive weight of each voxel and identified brain regions that significantly contributed to the decoding of the CS + and CS-. We validated a new circuit by applying the decoding models trained with its neural activations to multiple external datasets using other paradigms ($n = 815$ across 7 datasets). Permutation tests were used to assess the significance of the decoding accuracies.

Results: Neural signal within the 'threat network' successfully decoded conditioned stimuli-induced representations on the discovery dataset (accuracies 62.6%~75.2%) and generalized to the two external datasets (dataset 1: 58.3%~70.8%, dataset 2: 58.7%~82.7%). Importantly, classification performances were substantially improved using activations from distributed networks (discovery dataset: 72.6%-88.6%, external dataset 1: 66.7%-80.2%, external dataset 2: 60.3%-90.6%). The results from the distributed network that significantly contributed to the decoder included somatomotor regions, prefrontal and parietal regions, thalamus, cerebellum, and caudate. All regions that significantly contributed to the classification of the CS + and CS- (including the threat circuit) composed an updated circuit which we refer to as the 'fear homeostasis circuit'. These regions either consistently coded the CS + /CS- across the experimental phases, or dynamically switched its preference to CS + /CS- depending on the experimental phase. Classifiers trained by neural patterns from the newly defined circuit successfully applied to three visual threat conditioning datasets that used different paradigms ($n = 299$, 94 and 48, highest accuracy: 91.0%, 91.0%, 87.5%), one auditory threat conditioning dataset ($n = 68$, highest accuracy: 83.8%), and one dataset examined subjective reported fear ($n = 65$, highest accuracy: 81.5%). Applying the classifiers to brain activations pertaining to neural representations not related to associative learning and memory (yet still related to emotional stimuli) led to reduced accuracies: picture-evoked negative affect ($n = 182$, highest accuracy: 75.3%), physical pain ($n = 59$, highest accuracy: 71.2%), and social rejection ($n = 59$, highest accuracy: 61.0%).

Conclusions: Optimizing classifiers using a large fMRI dataset enabled us to show that conditioned stimuli can be classified

using neural activations of the 'threat network', and that better performance can be achieved when neural activations from broadly distributed neural networks were used. Based on these results, we updated the 'fear homeostasis circuit' by extending the 'threat circuit' to include other brain regions that are important to threat and fear processing. Analyses on the external datasets showed robust generalizability of the decoder across different experimental settings, sites, and scanners with different acquisition parameters. And lastly, the sensitivity and specificity of the 'fear homeostasis circuit' in representing threat conditioning, fear, and emotionally salient cues were verified.

Keywords: Multivariate Pattern Analysis, Fear Conditioning and Extinction, Classification, Generalizability

Disclosure: Nothing to disclose.

P51. Computational Mechanisms Underlying Cognitive Processing During Threat Exposure Reflect a History of Panic Attacks in Anxiety Patients

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Background: Patients diagnosed with clinical anxiety disorders exhibit heightened sensitivity to threatening information and altered cognitive processing. However, it is currently unclear whether threatening information impacts similar or distinct computational processes in healthy participants and patients with clinical anxiety disorders. Previous research has suggested that experimental models of anxiety (e.g. induced via unpredictable threat of shock), function to facilitate performance during on the Go/No-Go paradigm. We set out to determine how clinical anxiety disorders and a history of panic attacks impact Drift Diffusion model parameters underlying cognitive processing during experimental anxiety.

Methods: 39 healthy participants and 42 anxiety patients, 14 of which had a history of panic attacks, performed the Go/No-Go paradigm during periods of safety and periods of induced anxiety. Induced anxiety facilitated performance during on the No-Go trials irrespective of clinical diagnosis.

Results: Our computational analysis demonstrated that induced anxiety significantly reduced the "boundary separation" parameter. Within the group of anxiety patients, the impact of induced anxiety on "boundary separation" was significantly weakened by a history of panic attacks.

Conclusions: Boundary separation corresponds to the amount of information required in order to trigger a decision and our results may reflect more cautious decision-making during threat exposure in anxiety patients with a history of panic attacks. Collectively, our results suggest that computational models can help us understand the mechanisms through which experimental models of anxiety influence cognitive processes in clinical patients.

Keywords: Anxiety, Computational Cognitive Neuroscience, Panic Attacks

Disclosure: Nothing to disclose.

P52. Visualizing Functional Connectivity in Posttraumatic Stress Disorder: A Novel Use of Multidimensional Scaling

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Background: Posttraumatic stress disorder (PTSD) is debilitating, difficult to diagnose, and often treatment resistant. Functional imaging has the potential to provide a deeper understanding of PTSD neurobiology. However, such data is inherently complex, and finding a tractable approach is challenging. Here, we have adopted a network visualization algorithm called multidimensional scaling (MDS), which reduces high-dimensional data while preserving pairwise distances, in order to visualize functional connectivity networks and evaluate connectivity changes associated with PTSD severity.

Methods: We collected resting-state fMRI on a sample of $n = 50$ PTSD subjects. Using 100 relevant regions of interest, maps of pairwise functional connectivity were calculated. Participants were divided into two groups: those with high versus low PTSD symptom severity using a median split of total scores on the PTSD Checklist for DSM-5 (PCL-5). The Pearson cross-correlation values for regional pairs comprising the maps were transformed to dissimilarities and a range of data sparsity was generated using a negative exponential transformation. MDS was then performed, embedding the 4,950 regional connectivity pairs in a two-dimensional space. Permutation testing (500 MDS iterations on randomly shuffled data) was used to determine which regions' connectivity differed significantly between groups. The experiment was repeated for PTSD symptom clusters of intrusion, avoidance, cognition/mood, and arousal based on median splits of the respective PCL-5 subscale scores.

Results: The median total PCL-5 score for our sample fell in the moderate severity range (median=46.5; 95% CI 24-71), and PCL-5 total and subscale scores were highly and significantly correlated with each other (Pearson correlation coefficients ranging from 0.38 to 0.81). For total PTSD severity, three regions demonstrated significant group connectivity differences: the left dorsolateral prefrontal cortex (DLPFC) and the right ventrolateral prefrontal cortex (both frontoparietal network nodes) as well as the left anterior paracingulate (salience network node) ($p = 0.021$, 0.044, and <0.002 respectively). Analyses on PTSD symptom clusters similarly showed significant connectivity differences between high and low severity groups. For the intrusion cluster, 9 regions were significant: the left thalamus, the DLPFC bilaterally, the ventromedial prefrontal cortex bilaterally, the right orbital cortex, the right mid cingulate/paracingulate of the frontoparietal network, as well as the left striatum and the right anterior cingulate/paracingulate in the default mode network ($p = 0.009$, 0.040 [left], 0.025 [right], 0.005 [left], 0.003 [right], 0.033, 0.035, 0.042, and 0.034 respectively). The avoidance cluster demonstrated differences in the right basolateral amygdala as well as the left orbital cortex and the left medial prefrontal cortex in the default mode network ($p = 0.029$, 0.041, and 0.035 respectively). Connections to several other relevant network nodes also showed significant differences for the cognition/mood and arousal symptom clusters.

Conclusions: We have successfully adopted a network analysis tool for novel application to functional imaging data, revealing associations of PTSD total and cluster severity with connectivity to and from many relevant network nodes, with the most robust findings in the intrusion cluster. This provides new insight into computational and functional modeling of PTSD severity which can help to guide future objective diagnostics and treatments.

Keywords: Post Traumatic Stress Disorder, Resting State Functional Connectivity, Computational Psychiatry

Disclosure: Nothing to disclose.

P53. Computational Measures of Altered Uncertainty Learning in Transdiagnostic Anxiety

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Background: Difficulty learning and responding to uncertain threat is a core impairment in anxiety (Grupe and Nitschke, 2013). Neurocomputational modeling can disambiguate competing hypotheses about sources of uncertainty learning disruptions in anxiety (Brown et al., 2021; Montague et al., 2012; Pulcu and Browning, 2019). Specifically, different computationally-derived forms of uncertainty (from noise versus changepoints in stimulus-outcome contingencies) have different neural substrates and effects on learning (Mackintosh, 1975; Nassar et al., 2018; Pearce and Hall, 1980; Piray and Daw, 2021; Yu and Dayan, 2005).

Even with the aid of modeling, these types of uncertainty can be methodologically challenging to measure simultaneously; as a result, how clinically anxious people are impaired in learning about uncertainty is unknown. Whether impaired uncertainty learning is related to somatic and hyperarousal symptoms present in fear-based disorders (Craske et al., 2009; McTeague and Lang, 2012; Watson, 2005), or shared across internalizing distress-based disorders more generally, is also unclear.

In the current study, we developed a novel task and computational learning model to measure learning about uncertainty. We assessed relationships between model-derived behavioral indices of uncertainty learning and measures of anxiety and distress to understand how uncertainty learning is disrupted in anxiety.

Methods: Data collection is ongoing; preliminary results are reported from 40 participants (age mean [SD]: 32.3 [9.7]; gender # [%]: 29 [73%] female, 10 [25%] male, 1 [2.5%] non-binary; race # [%]: 29 [73%] White, 7 [17.5%] Black, 1 [2.5%] Asian, 3 [7.5%] multiracial) oversampled for high anxiety symptoms. Participants completed modules from the DIAMOND and MINI semi-structured interviews (Lecrubier et al., 1997; Tolin et al., 2018) to assess DSM-5 diagnoses as well as a dimensional self-report measure of depression and anxiety (IDAS-II; (Watson et al., 2012).

Uncertainty learning was measured with an aversive learning task where participants chose among options that differed in two types of uncertainty: noise and changepoint probabilities. Participants' choices were first fit to an established uncertainty learning model (Nassar et al., 2010, 2016), which assumes participants learn near-optimally about uncertainty. This model estimates values for different sources of uncertainty (noise and changepoint probability) for each option on each trial assuming normative learning. Next, a novel version of this model allowing for individual differences in each type of uncertainty learning was developed. Participants' choices were also fit to this individual differences model to estimate trial-level uncertainty values accounting for individual differences in uncertainty learning.

Relationships among trial-by-trial noise and changepoint probability estimates of uncertainty (from the normative and individual differences models) and switching behavior were assessed for relationships with anxiety and distress using multi-level regression models.

Results: Participants showed a range of psychiatric symptoms: most (30/40) met criteria for an anxiety, trauma-related, obsessive-compulsive, and/or mood disorder, and of those, most (25/30) met criteria for multiple disorders.

In the normative model, trial-by-trial measures of noise decreased, and changepoint probability increased, the likelihood of switching options (main effect of noise: $z = -7.39$, $p < 0.001$; changepoint probability: $z = 5.02$, $p < .0.001$). A factor measuring anxiety-specific symptoms on the IDAS-II, but not a factor measuring distress symptoms, was related to a reduced effect of noise on switching behavior (anxiety*noise interaction: $z = 3.08$, $p = 0.002$; distress*noise: $z = -1.052$, $p = .29$).

The model allowing individual differences in learning showed excellent parameter recovery (correlation between simulated and recovered parameters: rs from 0.89 to 0.99; correlation between recovered parameters: all rs < 0.35), suggesting that the model could independently measure different uncertainty-related changes in learning with low error. The relationships between trial-by-trial measures of true noise and changepoint probability versus estimates from the individual differences model had negative interactions with anxiety symptoms (true noise*anxiety on estimated noise: $t = -2.38$, $p = 0.017$; true change point probability*anxiety on estimated changepoint probability: $t = -3.58$, $p < 0.001$), indicating a weaker relationship between true and learned uncertainty measures with greater anxiety.

Conclusions: We successfully measured learning from different types of uncertainty in participants with a range of anxiety and distress symptoms. Anxiety symptoms were related to reduced effects of noise, assessed using a normative model, on behavior. Using a model that could represent individual differences in learning, participants' estimated values of uncertainty (both noise and changepoint probability) diverged more from the true values as anxiety symptoms increased. Some of these relationships were specific to anxiety and not distress symptoms. These findings support difficulties with uncertainty learning in anxiety; specifically, that uncertainty learning differences result in perceptions of uncertainty diverging from true values and in a way that affects choices in anxiety.

Keywords: Anxiety, Aversive Learning, Computational Modeling, Uncertainty

Disclosure: Aya Technologies: Consultant(Self)

P54. Large-Scale Neural Network Computations and Multivariate Representations During Approach-Avoidance Conflict Decision-Making

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Background: Many real-world situations require navigating decisions for both reward and threat. While there has been significant progress in understanding mechanisms of decision-making and mediating neurocircuitry separately for reward and threat, there is limited understanding of situations where reward and threat contingencies compete to create approach-avoidance conflict (AAC).

Methods: Here, we leverage computational learning models, independent component analysis (ICA), and multivariate pattern analysis (MVPA) approaches to understand decision-making during a novel task that embeds concurrent reward and threat learning and manipulates congruency between reward and threat probabilities. 30 adult participants recruited from the general community completed the task during fMRI.

Results: Computational modeling supported a modified reinforcement learning model where participants integrated reward and threat value into a combined total value according to an individually varying policy parameter, which was highly predictive of decisions to approach reward vs avoid threat during trials where the highest reward option was also the highest threat option (i.e., approach-avoidance conflict). ICA analyses demonstrated unique roles for salience, frontoparietal, medial prefrontal, and inferior frontal networks in differential encoding of reward vs threat prediction error and value signals. The left frontoparietal network uniquely encoded degree of conflict between reward and threat value at the time of choice. MVPA demonstrated that delivery of reward and threat could accurately be decoded within salience and inferior frontal networks, respectively, and that decisions to approach reward vs avoid threat were predicted by the relative degree to which these reward vs threat representations were active at the time of choice.

Conclusions: These data shed light on computational and neurocircuitry mechanisms supporting approach-avoidance conflict decision-making. Computational modeling suggested that participants integrate reward and threat according to an individually varying preference for approaching reward vs avoiding threat. Reward, threat, and reward vs threat conflict were separately encoded in unique large-scale neural networks. The MVPA results suggest that navigating AAC decisions involves generating mental representations for possible decision outcomes, and relative activation of these representations may bias subsequent decision-making towards approaching reward or avoiding threat accordingly.

Keywords: Approach-Avoidance Conflict, Computational Modeling, Independent Component Analysis, Multivariate Pattern Analysis

Disclosure: Nothing to disclose.

P55. Prenatal Citalopram Exposure Promotes Resilience in Male Offspring Exposed to Maternal Stress

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Background: Mood and anxiety disorders are highly prevalent during pregnancy and can lead to adverse maternal and offspring outcomes. Selective serotonin reuptake inhibitors are the most common medications used to treat mood and anxiety disorders. Both human and animal studies suggest that serotonin signaling plays an important role in the vulnerability to and the manifestation of stress-associated affective disorders. Moreover, the serotonin system is an early orchestrator of brain development.

Methods: In this study, timed-pregnant mice underwent chronic, unpredictable stress during the latter half of their pregnancies using ethologically relevant and/or mild stressors. Some of these mice received the antidepressant citalopram concomitantly in their drinking water. After birth, brain tissue serotonin levels at three postnatal developmentally relevant timepoints for serotonin system maturation were assayed in the offspring. A subset of the adult offspring was tested in behavioral assays to assess the long-term effects of in utero exposures. Finally, male adult offspring underwent microdialysis in the ventral hippocampus to investigate long-term neurochemical changes.

Results: Offspring of stressed mothers had higher serotonin tissue levels and protein concentrations in the forebrain at postnatal day seven compared to control animals. Male adult offspring displayed greater anxiety-like behavior and stress responsiveness than sex-matched control animals. These effects were rescued in male mice whose mothers were exposed to concomitant citalopram. No changes were observed in basal or stimulated hippocampal serotonin levels during adulthood. However, male adults exposed to in utero stress had increased kappa opioid receptor agonist-induced serotonin release in the presence of serotonin transporter inhibition, which was attenuated by in utero exposure to citalopram.

Conclusions: These findings suggest intergenerational benefits of treating maternal depression or anxiety during pregnancy particularly for imparting stress resilience in adult male offspring.

Keywords: Maternal Stress, Prenatal Antidepressant Exposure, Ventral Hippocampus, Intergenerational Transmission of Stress, Stress Resilience and Susceptibility

Disclosure: Nothing to disclose.

P56. Aberrant Brain Function Reconfiguration During Naturalistic Fear Induction in Pediatric Anxiety Disorders: Novel Approach With Implications for Pathophysiological Mechanisms

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Background: Anxiety disorders typically emerge during childhood and adolescence, are prevalent and impairing, and predict negative outcomes. As such, early intervention is of great clinical importance. While anxiety disorders consistently manifest as excessive fear responses in anticipation of threat, understanding of pathophysiological mechanisms is lacking, hindering treatment development for affected youth. Limited mechanistic insight is primarily due to challenges in experimentally evoking fear in children and the distributed nature of brain networks subserving fear responses. Naturalistic paradigms evoking fear states during functional imaging may provide an ecological way to examine fear mechanisms which is particularly suitable for children with anxiety. Graph theory is a computational approach generating metrics that can quantify distributed functional interactions across the brain during naturalistic paradigms. Combining naturalistic fear induction with graph theory approaches could constitute a novel method to identify pathophysiological mechanisms underlying excessive fear in children with anxiety disorders.

Methods: 30 youths with anxiety disorders (AD; treatment-seeking, medication-free) and 34 healthy comparisons (HC) (aged 9-17 years, 38 females) completed two functional neuroimaging (fMRI) scans: resting-state scan (baseline) and one with an animated "scary" movie (fear induction). We recorded cognitive (nervousness ratings) and physiological (skin conductance) fear responses, and used a graph-theory approach based on functional connectivity to identify links between brain networks reconfiguration and pathological anxiety.

Results: Increase in nervousness in anticipation of the movie, $t(60)=3.06$, $p = 0.003$, and decrease after it, $t(60)=2.74$, $p = 0.008$, confirmed fear induction. AD reported greater nervousness than HC before the movie, $t(62)=4.19$, $p < 0.001$, and demonstrated greater physiological responses during the movie, $t(57)=2.25$, $p = 0.029$, indicating successful elicitation of excessive fear responses. Imaging analyses indicated changes in brain network configuration from baseline to movie, $p = 0.001$. Importantly, a reduction in global between-network connectivity during the movie was noted in HC, $V = 122$, $p = 0.02$, but was absent in AD. This effect also manifested dimensionally with anxiety symptom severity, $r(62)=-0.39$, $p = 0.002$. Follow-up analyses indicated a reconfigured control-salience-default-mode network that was more strongly connected to attention, visual, and reward networks in AD relative to HC, all pFDRs<0.05.

Conclusions: The movie successfully and robustly induced excessive cognitive and physiological fear responses in youth with anxiety disorders. The induced fear state was associated with aberrant reconfiguration of distributed circuitry that related to pathological anxiety, pointing to a putative pathophysiological mechanism. This encourages continued research on pathological mechanisms using naturalistic paradigms and a network perspective that captures aberrant patterns of distributed brain function.

Keywords: Anxiety, Functional MRI (fMRI), Developmental Psychopathology, Graph-based Analysis

Disclosure: Nothing to disclose.

P57. PTSD Independently Associates With Major Adverse Cardiovascular Events: Analysis of a Large Biobank Sample

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Background: Posttraumatic stress disorder (PTSD) has repeatedly been observed to associate with an increased risk of major adverse cardiovascular events (MACE; e.g., myocardial infarction, stroke, heart failure), as well as MACE risk factors (e.g., elevated blood pressure). However, controversy exists as to whether this association survives adjustment for potential confounders, including socioeconomic and lifestyle factors. A National Institutes of Health-led working group identified numerous gaps in this literature and emphasized a need to evaluate the PTSD-MACE link further in large biobanks, as well as to characterize sex differences. Accordingly, the current study utilized a large healthcare biobank to determine whether PTSD diagnosis was associated with increased risk for MACE while controlling for important covariates. Further, we examined potential sex differences in this association.

Methods: Participants included N = 118,995 adults who consented to participate in the Mass General Brigham Biobank. A subset of N = 49,795 completed a health behavior questionnaire. PTSD and MACE diagnoses, as well as covariates, were determined by the presence of corresponding ICD-10 codes. All analyses were conducted in SPSS version 24 using a significance level of $p < .05$. Covariates included socioeconomic (education, employment, income), lifestyle (exercise, smoking, obesity, alcohol use), and cardiovascular disease risk factors (hypertension, hyperlipidemia, diabetes).

Results: PTSD diagnosis significantly associated with MACE (Odds ratio [95% confidence interval] = 2.04 [1.91, 2.18], $p < .001$, N = 102,395) after adjustment for cardiovascular risk factors. This effect remained significant after also controlling for socioeconomic factors (1.90 [1.68, 2.13], $p < .001$, N = 50,881), as well as lifestyle factors (1.81 [1.60, 2.04], $p < .001$, N = 49,795). A univariate ANOVA was used to test the main effects of PTSD and sex on MACE while controlling for cardiovascular risk factors. A significant main effect was observed for PTSD ($F[1,102,477] = 23.74$, $p < .001$), such that MACE risk was higher among those with versus without PTSD. A significant main effect was also observed for sex, such that MACE risk was higher in men compared to women ($F[1,102,477] = 37.32$, $p < .001$). The PTSD by sex interaction was not significant.

Conclusions: Our results from a large healthcare biobank extend those of prior studies by showing that PTSD is a significant risk factor for MACE after adjusting for key confounders, including cardiovascular disease risk factors, lifestyle factors, and socioeconomic factors. We also demonstrated that MACE risk is greatest among men with PTSD, which is consistent with prior studies demonstrating that premenopausal women experience lower MACE risk than men (likely due to higher estradiol levels). Mechanistic studies are critical to determine the pathways by which men versus women with PTSD develop MACE risk and how sex hormones affect these pathways.

Keywords: PTSD, Cardiovascular Disorders, Sex and Gender

Disclosure: Nothing to disclose.

P58. Mapping the Cell Type-Specific Regulome of PTSD in the Human Cortex

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Background: Post-traumatic stress disorder is a multigenic disorder occurring in the aftermath of severe trauma exposure. Recent studies have begun to detail the molecular biology of the postmortem PTSD brain using bulk-tissue transcriptomic and epigenetic analyses. However, given the array of PTSD-perturbed molecular pathways identified thus far (e.g. glucocorticoid signaling, GABAergic transmission, and inflammatory signaling) it is unlikely that a single cell type is responsible. It is therefore necessary to uncover the individual cell type contributions to the molecular pathology of PTSD.

Methods: We isolated ~1M nuclei from human postmortem dorsolateral prefrontal cortex (BA 9/46) from cases and controls for single nucleus RNA sequencing across three diagnostic cohorts: PTSD, MDD (Psychiatric control), and neurotypical controls to identify neuronal and non-neuronal cell type clusters and cell type-specific gene expression changes. We then performed paired sequencing of the same samples for ATAC-sequencing, to measure differential chromatin accessibility. We identified open genomic regions harboring risk alleles for PTSD and through integration of snRNA and snATAC we identified disease specific cis-regulatory elements. We used the most current and largest PTSD and MDD GWAS from the Million Veteran Program to identify cell types enriched for risk variants. We performed spatial transcriptomics on a subset of our cohorts to identify specific cortical lamina where PTSD gene expression changes are aggregating. Finally, we confirmed our findings using a combination of small molecule fluorescent in situ (FISH) and digital quantitative PCR.

Results: We identified 67 distinct cell type clusters including neuronal and non-neuronal cell types. We identified over 800 FDR significant differentially expressed genes across many cell types and confirmed expression changes of several genes implicated in PTSD pathophysiology by FISH and digital PCR. We found PTSD specific cis-regulatory elements for several genes including ELFN1, FKBP5, and SGK1. Integration of large GWAS data with our snATAC dataset showed enrichment of variants for PTSD, MDD and other quantitative clinical phenotypes in excitatory and inhibitory neurons and genome-wide spatial transcriptomics confirmed alterations of GABAergic signaling transcripts in specific layers of the DLPFC.

Conclusions: Taken together, this work is the first step in the creation of a cell type-specific atlas of stress disorders. Applying functional genomic approaches to characterize risk alleles within specific cell types will help determine which processes are most impacted by stress. These findings provide a global picture of the cell type-specific molecular regulatory mechanisms that govern stress effects on the human cortex and provides a blueprint for integrating single cell type genomic data to characterize the molecular landscape of other brain regions implicated in traumatic stress.

Keywords: PTSD, MDD, Genomics, Single Cell Omics

Disclosure: Nothing to disclose.

P59. An Integrated Multi-Omics Analysis of PTSD Across Brain Regions and Cell Types

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Background: The gene-regulatory landscape of the brain is highly dynamic in health and disease, coordinating many biological processes across distinct cell types. Complex psychiatric disorders such as PTSD result from differences at various levels of regulation

(epigenomic, transcriptomic, and proteomic) and converge on specific biological pathways with clinical significance. We are beginning to understand more about the neurobiology of PTSD and the molecular mechanisms associated with it, however, many critical questions still remain.

Methods: Here we generated a large multi-omic, postmortem brain dataset of PTSD donors compared to neurotypical controls and a psychiatric control group (major depressive disorder) that includes RNA expression, DNA methylation, and protein expression from the dorsolateral prefrontal cortex, amygdala, and the hippocampus. Single nucleus RNA-seq data was generated to infer cell-type-specific expression.

Results: Mutli-omics integration identified pathways, including glucocorticoid signaling, GABAergic transmission, and inflammation as differentially enriched in PTSD and MDD. At the RNA and methylation level there was an aggregation of differential expression in specific PTSD risk loci including CRHR1, ELFN1, and MAD1L1.

Conclusions: This approach highlights unique genomic characteristics of both disorders and demonstrates how convergent pathways across molecular modalities contribute to stress-associated etiology.

Keywords: PTSD, GWAS, ATAC-seq, RNAseq

Disclosure: Nothing to disclose.

P60. Epigenome-Wide Markers and Mechanisms of Disease Risk and Psychotherapy Response in Anxiety Disorders

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Background: Within the vulnerability-stress pathogenetic model of mental disorders, epigenetic mechanisms such as DNA methylation have been suggested to play a dynamic role at the interface between the genetic and the environmental level. Here, genome-wide DNA methylation patterns were investigated as disease markers of anxiety disorders and as potential predictors and/or mechanisms of response to psychotherapy.

Methods: A longitudinal epigenome-wide association study (Illumina MethylationEPIC BeadChip) was performed at baseline (BL), post-treatment (POST) and 6-month follow-up (FU) in the so far largest sample of patients with anxiety disorders (N = 415, f = 238; mean age±SD: 33.29 ± 11.3 years; panic disorder with or without agoraphobia: N = 257; social anxiety disorder: N = 129; multiple specific phobias: N = 29) treated with a standardized exposure-based cognitive behavioral therapy (CBT), and in 317 healthy controls.

Results: In the panic disorder subsample as compared to healthy controls, significant epigenome-wide association was discerned for altered DNA methylation at 256 CpGs. In the overall sample of patients with anxiety disorders, suggestive evidence was found for altered DNA methylation at ten and two CpG sites, respectively, to predict treatment response at POST and FU. Suggestive evidence for epigenome-wide DNA methylation changes along with CBT response in anxiety disorders was observed at 13 CpGs from BL to POST and at nine CpGs from BL to FU. Several hits map to genes previously implicated in anxiety-, learning- or plasticity-related processes.

Conclusions: The identification of epigenetic biomarkers of anxiety disorders may aid in developing resilience-increasing preventive measures. The definition of epigenetic signatures as predictors or core mechanisms of action of exposure-based interventions is hoped to contribute to the development of more targeted, personalized treatments of anxiety disorders.

Keywords: EWAS, Epigenetic biomarkers, DNA Methylation, Psychotherapy, Precision Medicine for Mood and Anxiety Disorders

Disclosure: Janssen Inc.: Advisory Board (Self)

P61. Changes in Basolateral Amygdala Activity Correlate With Distinct Innate Defensive Behaviors During Predator Odor Exposure in Male and Female Mice

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Background: Life-threatening traumatic experiences can have lasting negative consequences, many of which involve the development of maladaptive pathologies. A complex relationship exists between traumatic stress events and chronic neuropsychiatric conditions, including PTSD, anxiety, and depression. One translational model of life-threatening trauma in rodents uses exposure to a predator odor (2MT) to elicit fear behavior in mice. In both rodents and humans, the neural substrates underlying traumatic experiences are multifaceted, with the basolateral amygdala (BLA) heavily involved in trauma and stress processing.

Methods: We examined in vivo BLA neural activity during predator odor exposure by measuring calcium transients with fiber photometry in male and female mice.

Results: We have previously shown 2MT increases amygdalar endocannabinoid levels (2-AG), and here we report systemic pharmacological manipulation of endocannabinoid modulates BLA activity during predator odor exposure.

Conclusions: Different ways of responding to trauma correlate directly with different patterns of activity in the BLA, and these effects are mediated by the endocannabinoid system.

Keywords: Amygdala, Acute Traumatic Stress, Endocannabinoids

Disclosure: Nothing to disclose.

P62. Using the Genetically Encoded Sensor iSeroSnFR2.0 to Measure Serotonin Release During Fear and Stress Behaviors in Free Moving Mice

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Background: We developed a novel serotonin biosensor, iSeroSnFR2.0, which has improved sensitivity and a lower dissociation constant (Kd) compared to iSeroSnFR1.0, thus granting increased ability to measure serotonin concentrations on a subsecond basis across multiple trials using fiber-photometry. Here we used this powerful tool to record serotonin release dynamics during two anxiogenic conditions. We recorded from bed nucleus of the stria terminalis (BNST) and orbitofrontal cortex (OFC) during fear learning, because these regions are innervated by distinct populations of serotonergic neurons in the dorsal raphe.

We also investigate the impact of chronic social defeat on serotonergic transients. By measuring serotonin release across chronic stress conditions as well as acute, we aim to gain a more complete picture of how exposure to adverse stimulus modulates serotonin release.

Methods: Experiment 1: All recording was done via fiber photometry using either iSeroSnFR1.0 or iSeroSnFR2.0 expressed in BNST or OFC of adult male mice. Mice then underwent a 3-day fear conditioning paradigm. During the first day of testing mice were exposed to 5 tone trials, followed by 10 tone/shock trials. On the second day of testing mice were exposed to 15 tone/shock trials. On the third day of testing mice were exposed to 5 tone/shock trials, followed by 10 tone trials. n = 7-9 per group.

Experiment 2: All recording was done via fiber photometry using iSeroSnFR2.0 expressed in BNST of adult male mice. Mice underwent 10 consecutive days of chronic social defeat (or control handling) with 10 different CD1 stressor mice. 24 hours after the last day of defeat mice were tested in a caged social interaction test. During this test mice spent 3 minutes in an empty neutral arena, 3 minutes with an empty wire cage placed into the arena, and 3 minutes with a caged, novel CD1 aggressor placed into the arena. Two days later mice were tested again, this time in an uncaged social interaction test. During this test mice spent 3 minutes in an empty neutral arena, 3 minutes with a novel same strain male, and 3 minutes with a novel CD1 stressor male. Control n = 2, stressed n = 5.

Statistical analysis: Raw photometry data was down sampled 100x. The isosbestic channel was then fitted to a biexponential curve and that curve was subtracted from the signal to correct for bleaching. ΔF/F% was calculated as [100∗(465 signal - fitted signal) / fitted signal)] and those results were then z-scored. Data was then averaged across trials and animals. Area under the curve analyses (AUC) were conducted from the start of tone to immediately prior to the onset of shock. The AUC was calculated as the sum of the area below the mean trace and above y = 0. Parametric t-tests were used to assess for significant differences between AUC values.

I also include preliminary data of machine learning tracking of mice during caged and uncaged social interaction testing using deeplabcut. Use of the position estimation data to track animal behavior and correlate with photometry data is in-progress.

Results: Experiment 1: AUC analysis shows significantly less response to tone-only trials in trials before shock exposure than in trials comprised of a tone + shock, or in trial-only after previous shock exposure with both iSeroSnFR1.0 BNST ($p < 0.01$), iSeroSnFR1.0 OFC ($p < 0.01$), iSeroSnFR2.0 BNST ($p < 0.01$), and iSeroSnFR2.0 OFC ($p < 0.01$). In the beginning tone/shock trials during day 1, we found that serotonin is released into BNST/OFC after the mouse has received the foot shock, but as the trials progress the serotonin influx becomes cued by the onset of the tone instead. This pattern is most strongly apparent in mice injected with iSeroSNFR2.0.

Experiment 2: For caged social interaction: All test mice had no significant response to time spent in the empty arena or placement of an empty cage. All test mice had increased serotonin release into the BNST in response to the placement of the caged CD1 aggressor into the arena, with no significant difference between groups. For uncaged social interaction: There were no statistical difference in significant response for time spent in the empty arena or at the start of an uncaged interaction with a novel C57 male. Stressed males showed an increase of serotonin into BNST at the start of an uncaged interaction with a novel CD1 stressor male that is not seen in unstressed males (AUC $p < 0.05$).

Conclusions: While the firing patterns of serotonergic neurons have been described, other neuroimaging techniques did not allow for measurement serotonin release from spatially displaced terminals with high degree of temporal specificy. Here my work demonstrates that iSeroSnFR2.0 is able to track trial-specific subsecond changes of endogenous serotonin release. These results indicate that the increased sensitivity to lower volumes of serotonin that is achieved by iSeroSNFR2.0 allows for the collection of a completer and more accurate picture of serotonin release dynamics than is possible with iSeroSNFR1.0.

Results from both fear conditioning and chronic social stress show subsecond serotonin release temporarily and rapidly tracks fear state and anticipates punishment. Indicating serotonin may play a critical role in preventing risky behavior or promote rapid withdrawal from an adverse situation. Future directions will include acute vs. chronic SSRI administration prior to fear conditioning or chronic social stress.

Keywords: Serotonin, Fiber Photometry, Optical Biosensors, Acute and Chronic Stress

Disclosure: Nothing to disclose.

P63. Fatty Acid Amide Hydrolase Levels in Social Anxiety Disorder: Preliminary Findings From a PET Study Using [11C] CURB

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

Methods: Sixteen participants meeting DSM-5 diagnostic criteria for SAD completed PET imaging with arterial blood sampling. [11C]CURB λk3, an index of brain FAAH levels, was investigated in while brain and 9 regions of interest (ROI) using a repeated-measures ANCOVA, controlling for genetic variability known to affect [11C]CURB binding (FAAH rs324420 C > A). SAD symptom severity was assessed using the Liebowitz Social Anxiety Scale (LSAS).

Results: Individuals with SAD (n = 16; F/M = 13/3; 26.88 ± 5.78 years; FAAH rs324420: 11 CC, 4 AC, 1 AA) demonstrated 8.78% higher whole brain [11C]CURB λk3 ($F(1,59)=5.190$, $p = 0.026$) compared to HC (n = 46; F/M = 24/22; 27.17 ± 8.47 years old; FAAH rs324420: 32 CC, 12 AC, 2 AA). A significant Group[2] x ROIs[9] interaction was also observed ($F(5.023,296.36)=2.52$; $p = 0.029$). Post-hoc LSD pairwise comparisons revealed that [11C]CURB λk3 was significantly elevated in 3 of 9 ROIs ($p < 0.05$), (hippocampus, ventral striatum, dorsal striatum). A partial correlation, controlling for FAAH genotype revealed that whole brain [11C]CURB λk3 was not correlated with total LSAS score or any LSAS sub-scores.

Conclusions: In line with our hypothesis and preclinical evidence, our preliminary findings suggest that whole brain FAAH levels are significantly elevated in individuals with SAD compared to HCs, as inferred from [11C]CURB binding. The mechanism behind enzymatic activity of FAAH in SAD is unknown. However, initial results from this pilot study support up-regulated FAAH activity as a potential neurobiological mechanism in SAD and may inform future development and research of FAAH-targeted interventions.

Keywords: Social Anxiety Disorder, Endocannabinoid System, PET Imaging, FAAH

Disclosure: Empowerpharm: Consultant (Self)

P64. 7T Functional Magnetic Resonance Spectroscopy of Glutamate Variations During Working Memory in PTSD

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Background: People with PTSD commonly report difficulties with working memory, yet the neural basis of its dysfunction is not well understood. Previous fMRI studies of PTSD have shown reduced activation in the dorsolateral prefrontal cortex (DLPFC), a crucial brain region for working memory. Converging evidence from animal models and human studies points to glutamatergic dysfunction in key brain regions in PTSD. It is possible that glutamatergic abnormalities could underlie the differential activation patterns observed in the DLPFC; however, this has not been directly tested. Functional magnetic resonance spectroscopy (fMRS) can potentially address this issue by measuring glutamate changes associated with neural activity in response to stimuli. Moreover, there is a lack of MRS studies of the DLPFC in PTSD. Therefore, in this study, we used 7T resting-state MRS as well as fMRS during the N-back working memory task to measure glutamatergic changes in the DLPFC of people with PTSD, trauma-exposed people without PTSD, and people without trauma. We hypothesized that (1) glutamate would be reduced in people with PTSD, (2) glutamate would increase with working memory load, and (3) the glutamate-working memory relationship would be altered in PTSD.

Methods: Participants completed the Life Events Checklist (LEC-5 extended version) and the PTSD Checklist for DSM-5 (PCL-5). This study included 26 people with PTSD (age: 28.2 + /- 8.3; sex: 20 F / 6 M), 21 trauma-exposed people without PTSD (TE; age: 29.6 + /- 9.0; sex: 10 F / 11 M), and 24 people without trauma (NT; age: 29.6 + /- 11.2; sex: 15 F / 9 M). Imaging was performed at the Auburn University MRI Research Center on a Siemens MAGNETOM 7T MRI scanner equipped with a 32-channel head coil. Structural images were obtained for voxel placement. Spectra were acquired from the left DLPFC (25 x 25 x 25 mm) using an ultra-short TE STEAM sequence (TR/TE/TM = 10,000/5/45 ms), FASTESTMAP shimming, and VAPOR water suppression. For resting-state MRS, 32 averages with water suppression and 4 averages without water suppression were acquired while participants fixated on a crosshair. During fMRS, participants completed the N-back task. Stimuli were single letters presented one at a time. The task included 9 alternating blocks of 0-back, 1-back, and 2-back conditions (3 blocks of each working memory load). For fMRS, 8 water-suppressed averages were acquired during each block, and the spectra were combined in MATLAB. MRS and fMRS spectra were analyzed in LCModel using a simulated basis set. Spectra were eddy current corrected and quantified using the unsuppressed water signal. MRS levels of glutamate were compared between groups using one-way ANCOVA controlling for age and sex. A linear mixed effects model controlling for age and sex was used to examine the effects of group, condition, and group*-condition interaction on fMRS levels of glutamate.

Results: For resting-state MRS, we observed a significant effect of group ($F(2,65) = 5.20$, $p = 0.0008$). Specifically, glutamate was significantly lower in PTSD vs. NT (p(Tukey) = 0.023) and in TE vs. NT (p(Tukey) = 0.019). For fMRS, the effect of condition and the group*condition interaction were not significant. However, we observed a significant effect of group ($p < 0.001$). Specifically, glutamate was lower across all conditions in PTSD vs. NT ($t = 3.79$, $p < 0.001$) and in TE vs. NT ($t = 2.95$, $p = 0.005$). The percentage change in glutamate was calculated relative to the MRS passive fixation and was largest for NT (5.0-5.9%) compared to PTSD (2.5-2.7%) and TE (1.5-2.2%).

Conclusions: We observed (1) lower glutamate in trauma-exposed people with and without PTSD compared to people without trauma and (2) increased glutamate during the task relative to passive visual fixation. Our findings are consistent with prior studies reporting lower glutamate in the anterior cingulate cortex of people with PTSD and add to the growing evidence for glutamatergic abnormalities associated with traumatic stress. In future work, we will examine the association between glutamate and brain activation from fMRI. Since fMRS and fMRI probe different aspects of neuronal firing and synaptic activity, the combined approach of these techniques could better characterize the neurobiological basis of working memory deficits in PTSD.

Keywords: PTSD, Functional Magnetic Resonance Spectroscopy, Glutamate, Working Memory

Disclosure: Nothing to disclose.

P65. Lesion Network Localization of Neuromodulation Targets for PTSD in Veterans

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Background: The optimal transcranial magnetic stimulation (TMS) target for posttraumatic stress disorder (PTSD) remains unknown, and clinical trials have yielded mixed results. Connectivity of symptom-causing lesions have been shown to predict efficacy of neuromodulation targets for the same symptoms, including depression, motor parkinsonism, cognitive symptoms of Parkinson's disease, and tics. PTSD risk has been shown to be reduced after amygdala lesions, but the amygdala is too deep to access directly with TMS. Thus, we used brain lesions to map a circuit-based TMS target for PTSD in Veterans. We analyzed three datasets to derive a circuit-based TMS target that is specific to PTSD in military Veterans.

Methods: First, in Veterans who sustained penetrating traumatic brain injury ($n = 193$), we tested whether PTSD risk was reduced after damage to specific brain regions (using voxel lesion symptom mapping) or specific brain circuitry (using lesion network mapping). We used split-half cross-validation to confirm significance. Second, we tested whether our "PTSD circuit" derived from brain lesions was abnormal in Veterans without brain lesions ($n = 180$) using resting-state functional connectivity (rsFC). Finally, we tested whether connectivity changes in this circuit were associated with improvement in PTSD symptoms following TMS ($n = 10$ active, 10 sham). We controlled for depression and tested for circuit specificity in all analyses.

Results: Lesion locations that reduce PTSD risk map to a common brain circuit that includes the medial prefrontal cortex (mPFC), amygdala, and anterior temporal lobe. Lesion network maps derived from half the patients predicted PTSD status in the other half ($p = 0.01$). This result survived even when excluding all lesions that overlap with the amygdala ($p < 0.05$), demonstrating that this is a circuit-level phenomenon. Connectivity within this circuit, but not other circuits, was associated with PTSD diagnosis in patients without brain lesions ($p < 0.01$) and with PTSD improvement following TMS ($p < 0.05$).

Conclusions: Lesion locations that protect against PTSD map to a common brain circuit. This circuit is abnormal in PTSD patients without brain lesions, associated with TMS-induced improvement in PTSD severity, and may serve as a neuromodulation target for Veterans with PTSD symptoms.

Keywords: PTSD, TMS, Circuitry-Based Approach, lesion

Disclosure: Magnus Medical: Consultant (Self), Brainsway Inc: Stock / Equity (Self)

P66. Effect of Glucocorticoid Treatment During Acute Infectious Illness on Hippocampal Function in Survivors

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Background: Neuropsychiatric symptoms afflict half of survivors of critical illnesses leading to substantial public health burden. Glucocorticoids are commonly administered to acutely ill patients for their cardiovascular and anti-inflammatory properties, but they also influence brain outcomes. Intriguingly, observational and randomized clinical trials show that glucocorticoid treatment during critical illness decreases the risk of post-traumatic stress disorder (PTSD) in survivors, but the mechanisms are unknown. We hypothesized that glucocorticoid treatment during illness might influence hippocampal-dependent emotional and/or cognitive processes in survivors.

Methods: We used cecal ligation and puncture (CLP) to induce systemic infection in male and female mice (N = 80 divided equally among groups) and treated them with corticosterone (16 mg/kg) or vehicle during the acute illness (Days 1-5). Illness severity was measured using locomotion and daily weights. Beginning on Day 14, we studied affective behavior and neuroendocrine function. The Open Field and Elevated Plus Maze were used to measure negative affect, while the Object Recognition test was used to measure memory. Basal and stress-induced (forced swim) ACTH and corticosterone were measured from plasma using an ELISA, and systemic inflammation was measured using splenic cell counts by flow cytometry. Dorsal and ventral hippocampi were dissected from half the mice and RNA was isolated and prepared for paired-end 50 bp RNA Sequencing, which was performed on an Illumina NovaSeq S4.

Results: Corticosterone prevented CLP-induced weight loss without significantly affecting sickness-induced locomotor behavior or overall survival. After a 14-day recovery, male and female CLP survivor mice showed persistent negative affect based on decreased exploration of open areas in the Open Field and Elevated Plus Maze, and this was not rescued by corticosterone treatment. On the other hand, corticosterone treatment during illness impaired survivors' performance on the Object Recognition test. CLP survivor mice showed evidence of enhanced central hypothalamic-pituitary-adrenal (HPA) axis activity based on elevated basal ACTH levels, but corticosterone treatment during illness had no effect on HPA axis function in survivors. There was also evidence of persistent systemic inflammation in CLP survivors based on splenic cell counts that was not rescued by corticosterone treatment.

RNA sequencing showed distinct neural signatures of CLP and corticosterone treatment. 103 genes were differentially expressed in CLP survivors and 2076 genes were differentially expressed after corticosterone treatment, with only 6 overlapping genes. CLP survivors showed persistent upregulation of inflammation- and immunity-related genes, which were not altered by corticosterone treatment. Correlation of behavior in the Elevated Plus Maze with gene expression revealed a set of 126 genes in the ventral hippocampus that related to affective behavior after CLP, most of them involved in neuroendocrine signaling rather than inflammatory or immune processes.

Conclusions: In summary, murine survivors of CLP-induced acute infectious illness showed persistent negative affect and heightened central HPA axis activation. While persistent systemic and neuro-inflammation was seen in CLP survivors, correlation of gene expression with behavior related altered neuroendocrine signaling in the ventral hippocampus, rather than neuroinflammation, to affect in CLP survivors. Corticosterone treatment during illness did not rescue negative affect in survivors, but it impaired object memory and altered the expression of 10% of the detectable transcripts in the dorsal and ventral hippocampus. These findings provide the first separation of mechanisms related to affective and cognitive impairments after acute illness. The data suggest that neuroendocrine signaling in the ventral hippocampus is important for emotional outcomes while highlighting a role for glucocorticoids in cognitive outcomes. Future work will pursue the resulting hypothesis that glucocorticoids may alter the risk for PTSD by altering hippocampal-dependent memory processes.

Keywords: Glucocorticoids, Hippocampus, Infection

Disclosure: Camurus AB: Contracted Research (Self), Recordati Rare Diseases: Advisory Board (Self), Chiasma Inc.: Advisory Board (Self)

P67. The Prediction of Long-Term PTSD Symptom Development in Military Personnel: Applying Machine Learning to Pre-Deployment Risk Factors

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Background: Active-duty army personnel are frequently exposed to traumatic events during deployments, yet only a minority of them develop mental health disorders such as posttraumatic stress disorder (PTSD). Why some are at increased risk for developing PTSD after deployment is still not fully understood. A large amount of literature has been published on the identification of risk factors for PTSD, but have not yet led to the development of effective pre-deployment screening tools. Machine learning might be a promising approach for developing better prediction models.

Methods: The present study utilized a random forest method to predict the development of PTSD symptoms up to ten years after deployment in the PRISMO cohort of Dutch Afghanistan veterans (N = 963). The dataset consisted of both psychological and biological pre-deployment variables.

Results: The predictive model had a performance well above chance (AUC = 0.71, sensitivity = 0.63, specificity = 0.69). Among the top five highest-ranked predictive features were self-reported symptoms (depression, anxiety and distrust and personal sensitivity) and lab markers (vasopressin and DEX-sensitivity). A random forest model using a dataset with only psychological predictors performed as well as the random forest model based on both psychological and biological information.

Conclusions: The results suggest that a random forest approach can be effective in the identification of important predictive markers to define novel risk mitigation interventions. As the model performance in the present study was modest and no external validation could be performed, more research is needed to increase the usability for pre-deployment screening.

Keywords: PTSD, Machine Learning, PTSD, Prediction, Causal Modeling, Trajectory Modeling, Cortisol

Disclosure: Nothing to disclose.

P68. Evaluating if CRP Contributes to Risk for Developing PTSD-Like Behaviors in Trauma-Exposed Mice

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

Methods: Avoidance and conditioned fear processes were tested in CRP null male and female C57BL6J mice. Male wild-type C57BL6J mice received an intra-jugular injection of 10^11 genome copies of AAV8.CRP or AAV8.Null and four weeks later were tested in the predator stress model for PTSD which assesses enduring avoidance behavior and trauma-specific fear responses 1-2 weeks after exposure to a feline predator.

Results: CRP null female mice have at baseline enhanced recall fear extinction (FCRP = 1.794, n = 15, p = 0.0053). Despite near three-fold protein level increases (17.63 pg/mL for AAV8.CRP vs. 6.34 pg/mL for AAV8.null), male mice with AAV8.CRP overexpression did not confer a higher risk for PTSD avoidance-like behaviors or alter cued fear extinction in males after predator stress.

Conclusions: Loss of CRP signaling supports increased fear extinction in females but not males. Males also do not show CRP overexpression effects, suggesting females may be more susceptible to CRP effects on PTSD-relevant behaviors. Future studies will examine how constitutive CRP expression contributes to fear behaviors after trauma, as well as how CRP overexpression contributes to trauma effects in female mice. Studies are ongoing regarding how CRP alters peripheral and central immune responses after trauma.

Keywords: C-Reactive Protein, Fear Conditioning and Extinction, Neuroimmunology, Stress and Anxiety Behavior

Disclosure: Nothing to disclose.

P69. Gut Feelings: Connectome-Microbiome Dynamics Associated With Anxiety

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Background: There has been an increasing recognition of the role of the gut-brain-axis in internalizing psychopathologies. The present study explored dynamic connectomes by formulating a joint analysis of brain/microbiome networks in participants with depression and obesity to understand post-intervention coupled temporal changes.

Methods: Participants were from a study evaluating the effectiveness of specialized behavioral therapy over 2 months. A subset (n = 26, 8:usual care, 18:intervention) with gut microbiome/neuroimaging data at baseline and 2 months were included in our analyses. For each subject, a brain and individual sample microbiome network at both time points were derived and represented as tensors. Joint tensor factorization was used to couple the decomposition of the tensors at the subject mode to identify shared variation in both brain/microbiome networks, and subject factors derived from the analysis were used to relate clinical scales to network changes.

Results: One subject factor was significantly correlated with GAD-7 decreases (rho=0.547, p = 0.007, q = 0.045). The associated brain network factor consisted of negative connections between limbic/default mode network regions. For the microbiome network factor, the predominant connectivity was from the Bacteroidaceae/Lachnospiraceae families. Additionally, we found that the coupled factorization provided the best clustering of participants, with 96% accuracy, followed by independent taxa (85%) and brain network (69%) factorizations.

Conclusions: Coupled tensor decomposition can be used to identify shared temporal changes in the brain/microbiome networks of participants undergoing treatment for comorbid obesity and depression, suggesting that incorporating heterogeneous data fusion uniquely reveals salient network features.

Keywords: Gut Microbiome, Brain Connectome, Depression, Obesity, Anxiety

Disclosure: KeyWise AI: Founder (Self), Embodied Labs, Blueprint, Milken Institute, Sage Therapeutics: Advisory Board (Self),

P70. A Neurosurgery Case Series on the Role of the Amygdala and Temporal Pole in Development and Maintenance of PTSD

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Background: A better understanding of the neurobiology of posttraumatic stress disorder (PTSD) could reveal novel neuromodulation targets and improve treatment outcomes. One such target could be the amygdala, a key region in threat neurocircuitry. An overreactive right (R) amygdala in combination with lower ventromedial prefrontal cortex (vmPFC) control have been related to PTSD. Moreover, brain injury inclusive of the amygdala has been shown to be protective against the development of PTSD; however, due to its invasive nature, neurosurgical interventions have not been studied for PTSD.

Methods: This unique case series reports prospective data of 10 epilepsy patients who underwent unilateral ablation for their treatment-refractory epilepsy. The data are presented as a case series because the differential nature of the ablations does not allow for group-level analyses. Before and >6 months after surgery PTSD symptoms were assessed with the Clinician Administered PTSD Scale (CAPS) for DSM-5, and functional MRI scans were collected at both timepoints to assess threat reactivity and functional connectivity (for N = 5). Neuroimaging analyses were performed in native space using Statistical Parametric Mapping 12 and the CONN Toolbox. Post-ablation scans were co-registered to pre-ablation scans of the same modality to assure all scans were in the same native space. Regions of interest (ROIs) were the L amygdala (CITI168 probabilistic atlas) and the vmPFC (WFU PickAtlas, BA25) and first created in standard MNI space. The pre-ablation structural image was normalized to MNI space and the invert normalization parameters were used to convert ROIs to native space. Mean ROIs were extracted using the REX toolbox. The mean difference for the contrast value for fearful vs neutral faces was calculated as a measure for threat reactivity. The CONN toolbox was used to calculate amygdala-vmPFC functional connectivity (FC) during threat reactivity.

Results: Five patients met for PTSD before or after ablation. Patients (Pt) 1 and 2 no longer met for PTSD after R amygdala ablation. Pt 2 showed a pre- to post-treatment decrease in left (L)

amygdala threat reactivity (-0.27), an increase in vmPFC reactivity (+0.14) and an increase in vmPFC-amygdala FC (+ 0.29). Pt 3 did not recover from PTSD after R amygdala + R temporal pole ablation. Additionally, she developed suicidal ideation post-surgery. She showed a minimal change in threat reactivity (-0.07 for the L amygdala and +0.04 for the vmPFC) and a decrease in vmPFC-amygdala FC (-0.36). Pt 4 was trauma exposed with no PTSD, but developed PTSD after R amygdala + temporal pole ablation. Pt 5 underwent prior surgery sparing her R amygdala. A car accident resulted in recurrence of seizures and development of PTSD. She received R amygdala open resection after which her PTSD ameliorated, but seizures continued. Five non-PTSD epilepsy patients (MRI data for N = 3) who underwent L amygdala ablations were evaluated as controls.

Conclusions: This case series support the R amygdala as critical for maintenance of PTSD symptoms, bolstering earlier work on the importance of the amygdala in PTSD treatment non-response, and supporting the R amygdala as a key neuromodulation target. The findings also suggest R temporal pole ablation is related to poor PTSD outcomes, following another case report and findings of lower activation and size in PTSD, suggesting the temporal pole is an important brain region for further study.

Keywords: Amygdala Ablation, Post Traumatic Stress Disorder, Epilepsy, Temporal Pole, Threat Reactivity

Disclosure: Nothing to disclose.

P71. Treating Transdiagnostic Dysphoria Using a Sequence of Virtual Reality and Two Accelerated Transcranial Magnetic Stimulation Protocols

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Background: Dysphoria is a transdiagnostic symptom of unease or dissatisfaction experienced across a range of diagnoses, including mood disorders and pain. Dysphoria may have underlying commonalities in mechanisms, and thus treatments targeting dysphoria may have broad impact. Traditionally, studies have focused on narrow diagnosis-specific inclusion criteria, while excluding co-morbid diagnosis. In clinical practice, co-morbid conditions involving dysphoria are quite common (e.g. depression and pain), so a transdiagnostic approach may better reflect clinical reality. The goal of this project was to pilot a sequenced approach for rapidly treating dysphoria. In the first step ("Phase 1"), we sought to determine the feasibility and effectiveness of Virtual Reality (VR) treatment sessions. In subsequent steps as indicated, we sought to determine the effectiveness, tolerability, and feasibility of Accelerated Transcranial Magnetic Stimulation (accel-TMS) for dysphoria starting with sessions stimulating the left dorsolateral prefrontal cortex ("Phase 2A"), sequentially followed by sessions stimulating the dorsomedial cortex ("Phase 2B").

Methods: Participants were age 18 years and above, recruited from the community. Inclusion criteria for dysphoria was depressive (PHQ-9 ≥ 10), anxiety (GAD-7 ≥ 10), PTSD (PCL-5 ≥ 45), or chronic pain (Average Pain Intensity ≥ 4/10 for > 3 months) symptoms. We operationally defined "response" as a 30% improvement on the primary symptom scale, with efficacy separately assessed by clinician-rated scales. Participants were excluded for medical contraindications to TMS and other major psychiatric diagnoses. Phase 1 was a series of VR mindfulness exercises, twice daily for 10 days. Those who did not respond to VR treatments went on to Phase 2A of accel-TMS (five sessions a day with 50 minutes breaks, for 5 days, totaling 25 sessions). Phase 2A involved stimulation of left dorsolateral prefrontal cortex (modified Beam F3), 110% hand motor threshold, 1800 pulses of iTBS. At one-week post treatment, participants with a response continued for 5 more sessions over 5 weeks. Participants advanced to Phase 2B treatments if they failed to respond to Phase 2A or if they remained symptomatic after Phase 2A as defined by inclusion criteria. Phase 2B was accel-TMS (five sessions a day with 50 minutes breaks, for 5 days, totaling 25 sessions), plus 5 more sessions over 5 weeks. Phase 2B involved stimulation of dorsomedial prefrontal cortex (midline, 25% of nasion-inion distance, from nasion), 110% foot motor threshold, 1800 pulses of theta-burst primed 10 Hz (600 pulses of iTBS followed by 1200 pulses of 10 Hz).

Results: This interim report is based on 20 participants enrolled, with 1 screen failure and 19 initiated for Phase 1. For Phase 1 (VR mindfulness), 10 participants completed the full 10 days, 3 participants completed 5 days, and 6 participants discontinued before 5 days. Reasons for dropping out of VR were no change in symptoms (n = 9), did not enjoy VR (n = 6), motion sickness (n = 1), and pain precluded continuing coming to lab for VR (n = 1). There were 5 participants that did not continue to Phase 2A (1 responder with improvement in chronic pain and 4 participants chose to not continue). There were 14 participants that continued to Phase 2A (3 responders completed the study and 3 are currently pending to complete Phase 2B). Eight participants went on to Phase 2B with two of eight (25%) demonstrating response. All participants completed full acute courses of Phase 2A and 2B without any major adverse events.

Conclusions: Virtual reality treatments were tolerated by most, but many 37% (7/19) felt the experience was negative. Of the 14 who went on to Phase 2A accel-TMS, all completed the full week (25 sessions) and tolerated treatment well. For both Phase 2A accel-TMS and subsequent Phase 2B accel-TMS, there was a modest degree of response (21%, 25%) despite only 1 week of treatment. Our enrolled participants had experienced multiple failed treatment trials in their current episode. This likely factored into perceptions of the VR treatments and modest TMS response rate. Future work is required to optimized progressive sequencing of treatment approaches to provide rapid relief to patients suffering from symptoms of dysphoria.

Keywords: Theta Burst Transcranial Magnetic Stimulation, Virtual Reality, Dysphoria

Disclosure: Neuronetics: Other Financial or Material Support (Self)

P72. Evidence of Between-Session but Limited Within-Session Psychophysiological Habituation in Response to Standardized, Context-Relevant Virtual Reality Exposure for PTSD

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Background: Virtual reality (VR) exposure therapy has been used for the treatment of posttraumatic stress disorder (PTSD) based on the idea that its immersive nature promotes the emotional engagement necessary for clinical improvement. However, VR, just like non-VR exposure is intense and emotionally demanding. Previously, we demonstrated that non-personalized, standardized VR warzone scenes which capture the context of the traumatic experience distinguishes Veterans with PTSD from those without and reduces PTSD symptom severity with high completion rates. Because clinical response to non-VR exposure is associated with

between-session habituation to trauma cues as compared to habituation within a therapy session, we examined between- and within-session psychophysiological habituation to standardized, warzone-related VR exposure to test whether standardized VR specifically captures clinically meaningful between-session emotional learning.

Methods: As part of a double-blind, randomized clinical trial (NCT03372460), Veterans with PTSD (n = 28) completed six, 25-minute sessions warzone virtual reality exposure over the course of 2-3 weeks. Within each session, participants completed three times the same virtual reality Iraq or Afghanistan Humvee driving scenario or "run". Psychophysiological habituation of arousal was quantified through skin conductance reactivity to VR events and measured continuously throughout each session.

Results: To test for the presence of between- and within-session habituation as well as their interaction, a repeated measures ANOVA included the variables Session (session 1-6 that occurred over 2-3 weeks reflecting between-session habituation) and Run (runs 1-3 that occurred within each session reflecting within-session habituation). Mauchly's sphericity test was significant ($p < 0.001$) and Greenhouse-Geisser values are reported to reflect adjusted degrees of freedom. The interaction Session*Run term was significant ($F(4.5,122.2)=2.46$, $p = 0.04$, observed power 0.73) as was the main effect of Session was significant ($F(2.9,79.2) =3.48$, $p = 0.02$, observed power 0.75), but not the main effect of Run ($F(1.3,35.8)=1.75$, $p = 0.19$, observed power 0.28). These results reflect within-session habituation in the first VR session only, but between-session habituation across all VR sessions.

Conclusions: These data show robust between-session habituation to non-personalized, warzone-related VR exposure. However, aside from within-session habituation in the first VR session, no further within-session habituation took place. This suggests that the reduction in arousal to the VR trauma context is due to learning over the course of multiple sessions consistent with traditional, non-VR exposure. The use of standardized, non-personalized VR environments might offer clinical benefit without the need to highly personalize the exposure experience, which in turn could improve treatment adherence and completion.

Keywords: Posttraumatic Stress Disorder, Virtual Reality, Safety Learning, Emotional Arousal, Skin Conductance Response

Disclosure: Roche: Consultant (Self)

P73. Does Protocol Matter for PTSD? A Multisite Analysis of Veterans Receiving Therapeutic Transcranial Magnetic Stimulation

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Background: Repetitive Transcranial magnetic stimulation (rTMS, hereafter TMS) is an evidence-based treatment for pharmacoresistant major depression (MDD). We recently demonstrated that standard parameters used to treat MDD can also effectively reduce symptoms of posttraumatic stress disorder (PTSD) in Veterans, yielding statistically significant and clinically meaningful improvements. However, prior PTSD TMS studies often utilized different device protocols and parameters, which fall under the broad categories of high frequency, left-sided stimulation and lower frequency right-sided stimulation, with more recent inclusion of theta burst stimulation (TBS). In MDD, TBS and 10Hz TMS the left dorsolateral prefrontal cortex (DLPFC) are non-inferior to each other, although from the limited data available higher frequency stimulation in PTSD appears to yield greater effect sizes. To this end, we examined clinical outcomes in a large, multisite registry study of TMS; based on our prior work, we hypothesized that high-frequency stimulation would demonstrate greater effectiveness than other TMS protocols.

Methods: VA Palo Alto/Stanford Institutional Review Board approved procedures related to this report. Training was standardized through VA Palo Alto and data gathered using a centralized VA REDCap database. Veterans were eligible for TMS if they met standard inclusion/clearance criteria, and other treatments were recommended to be stable for approximately 6 weeks prior to stimulation and held unchanged during 30 sessions of treatment, generally applied using individual measurements of head anatomy (Beam/F3).

Regarding TMS protocols, we compared the magnitude of clinical outcomes in patients treated with FDA-cleared vs. non-cleared (i.e., off-label) TMS parameters. FDA cleared parameters, at the time of this writing, include 10Hz for Magstim, Neuronetics or Magventure systems for 4s, 11-26s intertrain interval (ITI), 3000 pulses per session; Brainsway devices using 18Hz for 2s, 20s ITI, 1980 pulses; and intermittent theta burst delivering 50Hz triplets at 5Hz, 2s stimulation with 8s ITI, 600 pulses). All other utilized parameters were off-label. These included 5Hz TMS (120% MT, 3000 pulses/session), right-sided intermittent TBS (80% of MT, 1800 pulses/session), right-sided 1Hz TMS (120% of MT, up to 3000 pulses/session), and right-sided 1Hz dTMS.

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 and response (>10 point reduction in PCL, > 50% reduction in PHQ9). Outcomes were analyzed using paired-sample t-tests and missing data were excluded listwise. This data includes Veterans that received TMS from October 2017 through February 2022, which represents an expanded cohort from prior reports.

Results: The cohort included N = 1,256 Veterans who received care at 35 different VA TMS clinics with usable data. Of these, n = 883 (70.3%) received "on-label" FDA protocols; Representation was greatest for groups receiving 10Hz (n = 736), 5Hz (n = 66), left-sided iTBS (n = 72), and dTMS (n = 75); other protocols were more sparsely represented. There were no statistically significant differences in PTSD outcomes between on- and off-label TMS parameters, and ANOVA did not reveal significant differences between examined protocols (all ps > .1). TMS was associated with a statistically significant and clinically meaningful reduction in PTSD symptoms (Baseline mean (SD) = 45.0 (18.3) and post-treatment mean SD 28.6 (19.7) $p < .001$, $d = 1.04$). There were also no statistically significant differences in MDD outcomes between any of the examined protocols (all ps > .1) TMS was also associated with significant and meaningful reduction in MDD symptoms (Baseline score = 18.3 (5.1), post-treatment score = 10.9 (7.0) $p < .001$, $d = 1.21$). Sensitivity analyses evaluating outcomes in only those who received an adequate dose (defined as 30 TMS sessions) did not change results.

Conclusions: This large, multisite naturalistic data did not provide any clear indication that a single TMS protocol is superior for the treatment of PTSD. Similarly, no protocol outperformed another for MDD. This data shows, at the population level, that decisions regarding clinical parameters may be made based on convenience for the patient or clinic, and that they can be held in equipoise regarding their effectiveness. As previously demonstrated, TMS was able to provide significant and meaningful reductions in PTSD and MDD symptoms. Limitations are those inherent to naturalistic cohort studies and may not have captured multiple changes in protocols that occurred over time. These results represent population-level data without biomarkers, and it is possible that biological data may help whether individually customized parameters or targeting might improve outcomes. Within the off-label group, individual protocols were not evenly

represented, limiting statistical power. Accelerated and neuronavigated TMS were not well represented. Ongoing studies, nested within this coordinated VA network, are currently investigating biomarkers (fMRI, EEG) and their relationship with clinical outcomes. In summary, this data did not find any compelling evidence of superiority of any TMS protocol for PTSD or MDD, underscores existing data on the effectiveness of TMS in this patient population, and provides important information on protocol selection for treating physicians.

Keywords: Repetitive Transcranial Magnetic Stimulation, Post-traumatic Stress Disorder, Veterans

Disclosure: Neurolief, Wave Neuro: Other Financial or Material Support (Self)

P74. Paternal Deprivation Increases Social Vigilance in the Absence of Threat in Adult California Mice (Peromyscus Californicus)

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Background: Adverse early-life experiences increase susceptibility to developing mental health disorders, like anxiety disorders, which are often characterized by social avoidance behaviors. In addition to a reduction in time spent engaging with others, social avoidance also includes the inability to initiate interactions. This may be a consequence of less social motivation (i.e., desire to interact) or a result of distress experienced during social interactions. In rodents, social approach is reduced by social stressors (e.g., social defeat, predator stress) and is accompanied by increased social vigilance (i.e., looking at, but not approaching, an unfamiliar individual from a safe distance). In humans, reduced social approach coupled with increased social vigilance may predict the development of social anxiety disorders. Since interventions are successful in reducing social avoidance, determining which early-life adverse experiences are associated with social avoidance, and the trajectory of this behavior, is important for early detection and improving outcomes. To test the exploratory hypothesis that the early adverse experience of father absence leads to maladaptive social behavior, we utilized a unique model of early-life stress in biparental rodents, where the paternal male is permanently removed from their offspring (paternal deprivation [PD]) and examined the impacts of PD on the relationships among sociability, social anxiety, and social vigilance in adult offspring.

Methods: Adult male and female control-reared and PD-reared California mice (Peromyscus californicus) were behaviorally assessed in a three-chambered apparatus over the course of two days. Social vigilance and time spent in the chamber with a confined, non-threatening unfamiliar same-sex conspecific was measured during sociability (Day 1) and social anxiety (Day 2) testing. Statistical analyses included two-way analysis of variance (ANOVA) testing (rearing x sex; $n = 10$-11/group), followed by Sidak's post-hoc comparisons (when appropriate), and simple linear regressions. Statistical significance was reached when $p \leq 0.05$.

Results: Paternal deprivation did not significantly influence social vigilance in males or females during sociability testing on Day 1 (two-way ANOVA; rearing x sex: $F(1,37) = 2.768$, $p = 0.10$; sex: $F(1,37) = 3.962$, $p = 0.054$; rearing: $F(1,37) = 1.703$, $p = 0.199$). However, a marginally significant interaction between rearing and sex was observed in social vigilance behavior during social anxiety testing on Day 2 (two-way ANOVA; rearing x sex: $F(1,37) = 4.059$, $p = 0.051$). Sidak's multiple comparison's test revealed significantly less social vigilance in PD-reared males, compared to PD-reared females (padjusted=0.03). Social vigilance did not differ in control-reared offspring (padjusted=0.90).

A simple linear regression was used to predict social vigilance behavior during sociability testing on Day 1. Social vigilance explained a significant amount of variance in time spent interacting with the unfamiliar conspecific in PD-reared males ($F(1,6)=9.27$, $p = 0.022$, $R2 = .61$) and females ($F(1,9)=11.81$, $p = 0.007$, $R2 = .57$). The regression indicated that an increase in social vigilance corresponded with less time in the chamber with the unfamiliar same-sex conspecific. This relationship was absent in control-reared adult offspring on Day 1 sociability testing ($p > .05$, all comparisons). By Day 2, when mice were again presented with the now-familiar same-sex conspecific (social anxiety testing), social vigilance no longer explained the variance in time spent with the same-sex conspecific in any group ($p > .05$, all comparisons).

Conclusions: These analyses highlight the unanticipated relationship between the adverse early-life experience of paternal deprivation and social vigilance during a low-risk social interaction. Paternal deprivation may augment perceived threat-related mechanisms in both males and females, thus contributing to this maladaptive behavior. Importantly, the negative relationship between social vigilance and social interaction is reduced with repeat exposure to the same conspecific. Future work will focus on identifying pathways and manipulating targets to directly link alterations in social behaviors with the loss of the paternal male in a biparental rodent species. Collectively, this work adds to our understanding of the behavioral consequences of early adverse experiences and may advance therapeutic interventions.

Keywords: Early Life Stress, Social and Behavioral Deficits, Social Anxiety

Disclosure: Nothing to disclose.

P75. ENX-102, a GABA-A Alpha2,3,5 PAM That Blocks Alpha1, for the Treatment of Generalized Anxiety Disorder: A Phase 1 Single Ascending Dose Clinical Study

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Background: Generalized anxiety disorder (GAD) can manifest as excessive worry and/or tension associated with a variety of somatic symptoms and substantially impairs daily life. Nonselective gamma-aminobutyric acid type A (GABA-A) positive allosteric modulators (PAMs) such as benzodiazepines, are proven treatments for GAD, but their use is generally limited to short-term therapy due to the risk of drug dependence and significant side effects such as sedation as well as cognitive and motor impairment, consequences largely mediated by activation of alpha1 subunit–containing GABA-A receptors. ENX-102 is a GABA-A PAM that activates neurotransmission via GABA-A receptors containing alpha2, alpha3, or alpha5 subunits, while blocking 1. ENX-102 is an investigational new drug in development for the treatment of GAD. A Phase 1 clinical study was designed to evaluate the safety, tolerability, and pharmacokinetics (PK) of ENX-102 in healthy volunteers.

Methods: ENX-102 was evaluated in a randomized, double-blind, placebo-controlled, single ascending dose study in healthy male and female volunteers. The primary objective was to evaluate the safety and tolerability of ENX-102 after single oral administration of increasing doses in comparison to placebo. The secondary objective was to evaluate the PK of ENX-102 after single dose administration. After signing written informed consent, subjects were screened up to 28 days prior to Study Day 1 (day of

dosing). Eligible subjects were admitted to a clinical unit one day before dosing and remained confined for at least 4 days after dosing to monitor safety and PK and an outpatient follow-up visit occurred approximately 7 days after discharge. Eight (8) subjects were planned per dose cohort and were randomized to receive ENX-102 (N = 6/cohort) or placebo (N = 2/cohort). Within each cohort, 2 sentinel subjects were dosed and followed for at least 24 hours before the remainder of the cohort was dosed. A Dose Escalation Committee reviewed safety, tolerability, and PK data before escalating to the next higher dose.

Safety was measured by adverse events (AEs), vital signs, 12-lead electrocardiograms (ECGs), clinical laboratory tests (hematology, serum chemistry, coagulation, urinalysis), physical exams, and the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale. Safety measures were analyzed using descriptive statistics. Blood samples for PK analysis were collected pre-dose and then 15, 30, 45, 60, 90, and 120 minutes and 4, 6, 8, and 12 hours after dosing. Plasma concentration-time data for ENX-102 were analyzed using non-compartmental methods. PK parameters included maximum plasma concentration (Cmax), time to reach Cmax (Tmax), area under the plasma concentration-time curve (AUC), and half-life (t1/2).

Results: Of the fifty-six (56) healthy volunteers who participated, 30 (53.6%) were male and 26 (46.4%) were female, and 53 (94.6%) were White, 1 (2.4%) was Asian, 1 (2.4%) was Black, and 1 (2.4%) reported mixed races; none reported being Hispanic/Latino, though 19.6% declined to report ethnicity. Participants were randomized to receive ENX-102 or placebo (6 active:2 placebo per cohort) across 7 cohorts that evaluated doses of 0.5, 1.0, 1.5, 2.0, 3.0, 5.0 and 10.0 mg. All 14 subjects randomized to placebo completed the study and 41/42 (97.6%) subjects randomized to ENX-102 completed the study; one subject randomized to 10.0 mg ENX-102 withdrew consent after the inpatient portion of the study was completed and declined the follow-up visit.

There were no deaths or other serious adverse events in the study. There were no discontinuations due to adverse events. Treatment-emergent adverse events (TEAEs) were reported in 16/42 (38.1%) subjects randomized to ENX-102 and in 1/14 (7.1%) subjects randomized to placebo. Consistent with a central GABAergic mechanism of action, the most frequently reported adverse events in subjects receiving ENX-102 were nervous system disorders and included mild dizziness in 8/42 (19.0%), mild fatigue in 4/42 (9.5%), and mild to moderate somnolence in 3/42 (7.1%). Postural dizziness occurred in similar rates in subjects receiving ENX-102 (3/42; 7.1%) and placebo (1/14; 7.1%); instances of postural dizziness were mild to moderate in ENX-102-treated subjects and was moderate in the placebo-treated subject. Other TEAEs occurred in one subject each. None of the TEAEs were severe.

There were no notable, dose-related changes in physical exams, vital signs, ECGs, or clinical laboratory values. On the MOAA/S scale, no frank sedation was observed.

Pharmacokinetic analyses indicated increases in ENX-102 exposure with dose. Mean (standard deviation) Cmax values ranged from 7.013 ng/mL (1.2663) associated with the lowest dose of 0.5 mg to 95.633 ng/mL (11.7959) associated with the highest dose of 10 mg, and AUC to the last nonzero time point ranging from 244.296 h*ng/mL (55.1034) to 4400.502 h*ng/mL (1710.469). Tmax was reached between 1 and 4 hours. Mean t1/2 was approximately 60 hours.

Conclusions: ENX-102 was considered safe and well-tolerated in healthy volunteers. A maximally tolerated dose was not defined. ENX-102 exhibited predictable dose-related exposure with a long half-life consistent with once daily oral administration. The data support continued development of ENX-102 for the treatment of GAD. ENX-102 has advanced into a Phase 1b multiple ascending dose study.

Keywords: Phase 1, GABA-A, Positive Allosteric Modulators, Generalized Anxiety Disorder

Disclosures: Engrail Therapeutics: Employee(Self), Intra-Cellular Therapies: Stock / Equity(Self), Evolution Research Group: Consultant (Self)

P76. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Infection and "Long COVID" in Adolescents With Anxiety Disorders: A Prospective Longitudinal Study

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Background: The impact of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection on youth with anxiety disorders has not been prospectively examined. Further, there are limited prospective data on post-acute sequelae COVID-19 (PASC), including symptoms that constitute the long COVID neuropsychiatric syndrome. With this in mind, we examined the longitudinal course of anxiety and long COVID symptoms following SARS-CoV-2 infection in affected adolescents.

Methods: In December 2019, we began an IRB-approved, longitudinal study of adolescents aged 12-17 with DSM-5 primary anxiety disorders treated with either duloxetine or escitalopram. At each follow-up visit, affective and anxiety symptoms during the prior interval, week-by-week were assessed using the Longitudinal Interval Follow-up Examination (LIFE). Each follow-up interview included all items from the Generalized Anxiety Disorder-7 (GAD-7) and Quick Inventory of Depressive Symptoms (QIDS) scales each week and a weekly psychiatrist-rated Clinical Global Impression-Severity (CGI-S) scale. To examine pre- and post-SARS-CoV-2 infection differences in anxiety symptoms, the distribution of the difference in means for GAD-7/QIDS items and CGI-S were computed using Monte Carlo simulation from a Student-t distribution for the mean pre- and post-COVID symptom scores.

Results: Twenty-six patients contributed 1,308 weeks of data (1019 patient weeks from patients who did not contract COVID-19, 289 patient weeks from patients with COVID-19 infection, and 139 patient weeks post-COVID-19). Patients were, on average, 14.3 ± 1.64 years (14.8% male) and 3.8% Black and were followed for 50.3 ± 30.4 weeks (range: 7 to 96 weeks, median: 55.5 weeks). All patients met criteria for generalized anxiety disorder, 19% had separation anxiety disorder, 78% had social anxiety disorder, 30% had panic disorder, 26% had ADHD, and 37% had a history of a depressive disorder. All patients with COVID-19 were unvaccinated and experienced mild symptoms; none were hospitalized or received monoclonal antibodies. Post-COVID-19, anxiety symptoms significantly worsened for all GAD-7 subscales (all $p < 0.001$), and patients had significant syndromic worsening, reflected by CGI-S scores ($p < 0.001$). SARS-CoV-2 infection was also associated with within-patient worsening in long COVID symptoms (anergia, $\beta = 0.37$, $p < 0.001$; amotivation, $\beta = 0.57$, $p < 0.001$; concentration, $\beta = 0.35$, $p < 0.001$, and irritability, $\beta = 0.52$, $p < 0.001$).

Conclusions: This prospective study of the longitudinal impact of COVID-19 in pediatric anxiety disorders reveals that COVID-19 is associated with worsening anxiety symptoms and a disquieting 33% worsening in syndromic severity. Further, these data raise the possibility that, in anxious youth, COVID-19 is associated with a surfeit of neuropsychiatric symptoms, representing a long COVID neuropsychiatric syndrome like that described in adults.

Keywords: Adolescent Anxiety, COVID-19, Antidepressant Treatment Practice

Disclosures: Myriad Genetics: Other Financial or Material Support (Self), AbbVie: Contracted Research (Self), MedScape, Neuroscience Educational Institute: Speakers Bureau (Self), Yung Family Foundation: Grant(Self), Springer Publishing: Royalties (Self), Cerevel: Consultant(Self)

P77. Preclinical Evaluation of ENX-102, a GABAA α2,3,5 PAM That Blocks α1, in the Elevated Plus Maze and Pharmaco-EEG

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Background: Nonselective gamma-aminobutyric acid type A (GABAA) positive allosteric modulators (PAMs) such as benzodiazepines, are proven anxiolytics, but their clinical use is limited due to significant side effects, largely mediated by activation of α1 subunit–containing GABAA receptors. There have been several efforts to generate subtype selective (α2, α3, or α5 subunits) GABAA modulators driving the beneficial effects of GABA modulation without the undesired effects that result from the activation of α1 subunit-containing receptors.

ENX-102 is a GABAA PAM that activates neurotransmission via GABAA receptors containing α2, α3, and α5 subunits, while blocking α1. We evaluated the potential for anxiolysis of ENX-102 in rodent anxiety models such as the elevated plus maze (EPM) after acute and chronic dosing, and we characterized the sleep-wake electroencephalography (EEG) profile in rats induced by increasing doses of ENX-102.

Methods: The in vitro pharmacology of ENX-102 was assessed with the SyncroPatch platform in PAM mode on the human GABAA receptors α1/β3/γ2 α2/β2/γ2, α2/β3/γ2, α3/β3/γ2, and α5/β3/γ2.

Adult male SD rats (N = 130; N = 10 per condition) were used in the in vivo pharmacology studies. ENX-102 was evaluated in the EPM test which assesses anxiety. The following measures were automatically recorded: distance travelled, time spent in each arm, and entries into each arm. ENX-102 was orally dosed at 0.1, 0.3 and 1 mg/kg to target receptor occupancies of approximately 30%, 50% and 80% at 1 hr post-dosing, respectively. Acutely dosed rats were also tested 24 hrs post-dose. A different cohort was dosed chronically, once daily for fourteen days and tested 1 hr following the last dose to assess the anxiolytic properties of ENX-102 after continuous target engagement. Chlordiazepoxide was used as a positive control. Data were analyzed by ANOVA followed by post hoc analyses where appropriate.

Adult male SD rats (N = 12 in a cross-over design) were used in the pharmaco-EEG studies. Sleep-stage specific pharmaco-EEG signature of ENX-102 was assessed after orally dosing ENX-102 (0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg and 1 mg/kg) to target a broad range of receptor occupancies. Lorazepam (1 mg/kg) was used as a positive control. Rats were implanted with telemetry devices. Recordings started 2 hrs prior to compound administration and recorded continuously for 48 hrs after administration. Spectral analysis included quantifying the changes in spectral power for the traditional EEG bands (Delta-0.5-3.9 Hz, Theta-4-7.9 Hz, Alpha-8-11.9 Hz, Beta 12-29.9 Hz, Low Gamma 30-49.9 Hz, and High Gamma 50-100 Hz).

Sleep analysis was also conducted for 2 hrs prior to ENX-102 administration and for 12 hours after ENX-102, as well as 23-25 hrs and 46-48 hrs after ENX-102 was administered. Several variables were analyzed: sleep architecture as percent time in active awake, quiet wake, NREM, REM and latency to sleep. ANOVA with Dunnett's multiple comparisons was applied independently to each 15-minute bin to assess the drug effects on the power of different EEG spectral bands as well as across sleep stages.

All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: The functional in vitro activity of ENX-102 on GABAA receptors showed that ENX-102 is a subtype-specific GABAA PAM. ENX-102 did not drive Cl- flux on cloned human GABAA subtype α1/β3/γ2. ENX-102 displayed EC50 of 0.80 nM, 0.82 nM, 2.47 nM and 0.20 nM at GABAA subtypes α2/β2/γ2, α2/β3/γ2, α3/β3/γ2, and α5/β3/γ2, respectively; and Emax of 50%, 89%, 113% and 96% at GABAA subtypes α2/β2/γ2, α2/β3/γ2, α3/β3/γ2, and α5/β3/γ2, respectively.

In vivo characterization of ENX-102 in the EPM assay showed that ENX-102 increased distance traveled in the open arms demonstrating an anxiolytic-like profile. The effect was equivalent to what was demonstrated by the reference anxiolytic compound, chlordiazepoxide. Chronic dosing of ENX-102 showed a similar magnitude of anxiolytic effect. These data demonstrate strong anxiolytic activity in rodents that is maintained with chronic dosing.

The EEG profile induced in rats by dosing ENX-102 was also studied. ENX-102 induced EEG changes indicative of an anxiolytic profile and that mimic the spectral EEG signature that has been associated to other subtype-selective GABA modulators (see Christian et al., 2014). This spectral EEG signature can be used as an indication of central pathway engagement in rodents and as a translational bridge into clinical studies.

Conclusions: ENX-102 is anxiolytic-like in rodents after acute and sub-chronic (14 days) dosing without any loss of effect despite engaging the target for the entire duration of the study. Sleep-wake pattern and EEG profile analysis suggest a non-sedative anxiolytic profile of ENX-102. These data support the clinical study of ENX-102 in anxiety-related indications.

Keywords: Anxiety, GABA-A, Positive Allosteric Modulators, Elevated Plus Maze, EEG Biomarkers

Disclosure: Engrail Therapeutics: Employee (Self)

P78. The Role of Matrix Metalloproteinases 2 and 9 in the Nucleus Accumbens After an Exposure to an Acute Stress and a Stress-Conditioned Cue

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Background: There is a strong comorbidity between post-traumatic stress disorder (PSTD) and substance use disorders. For example, a person with PTSD is 2 to 4 times more likely to have a substance use disorder than a person without PTSD. Preclinical studies have shown that both addictive drug use and acute stress exposure produce long-lasting neuroadaptations at glutamatergic synapses in the nucleus accumbens core (NAcore) including an increase in the AMPA/NMDA ratio and dendritic spine density, and a downregulation of the glutamate transporter. Furthermore, like drug associated cues, a stress-conditioned stimulus (CS) is able to reinstate drug seeking behavior. Knowing that cue-induced drug seeking requires activation of NAcore matrix metalloproteinases (MMPs), we previously found that a stress-CS also increased MMP-2,9 activity in NAcore, and induced burying behavior in a defensive burying task (DBT). However, it is unknown whether the increase of MMPs is mediated by MMP-2, MMP-9 or both. In this study, we firstly sought to determine which MMP is involved in the stress-CS induced burying behavior. Then

we sought to quantify the MMPs activity in a cell-specific manner in NAcore. We hypothesized that the burying behavior is mediated by MMP-9, an MMP known to be involved in cue-induced drug seeking.

Methods: To quantify the effect of an acute stress on MMP-2,9 activity, Long Evans rats were microinjected with an MMP-9 inhibitor followed by FITC-quenched gelatin into the NAcore immediately before 30 min of acute restraint stress or 30 min in the home cage. Then, to evaluate the role of a stress-CS on the behavior and MMP-2,9 activity, 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. 3 weeks after the stress, rats were pretreated with MMP-2 or -9 inhibitors, then FITC-quenched gelatin was injected into the NAcore and the effect of the CS or a novel stimulus (NS) was tested in a DBT for 15 min. The burying and immobility behaviors, as well as MMP-2,9 activity were quantified. Finally, the MMP-2,9 activity was quantified in a cell specific manner. To this end, an AAV cre-dependent mCherry virus was used to transfect medium spiny neurons (MSN) in D1 and D2 cre-dependent rats.

Results: We found that an acute stress increased the MMP-2,9 activity in the NAcore. This increase is prevented by an MMP-9 inhibitor. Both MMP-2 and -9 inhibitors prevented the stress-CS induced burying behavior, without affecting the immobility. However, only the MMP-2 inhibitor significantly reduced MMP activity in NAcore. Finally, we found that the stress-CS significantly increased MMP-2,9 specifically around D1 but not D2 MSNs.

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: Matrix Metalloproteinases, Post Traumatic Stress Disorder, Nucleus Accumbens

Disclosure: Nothing to disclose.

P79. Simultaneous Targeting of NMDARs and 5-HT4Rs Exerts Additive Effects in Preventing Stress-Induced Hyponeophagia

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Background: Serotonin (5-HT) receptors and N-methyl-D-aspartate receptors (NMDARs) have both been implicated in the pathophysiology of affective and anxiety disorders. In particular, 5-HT type 4 receptor (5-HT4R) agonists and NMDAR antagonists have been suggested to act as rapid-acting antidepressants and resilience-enhancing prophylactic drugs. Here, we evaluated whether targeting both receptors through combined dosing of (R,S)-ketamine and prucalopride, a 5-HT4R agonist would have additive effects, resulting in reductions in stress-induced fear, behavioral despair, and hyponeophagia.

Methods: A single injection of saline, (R,S)-ketamine, prucalopride, or a combined dose of (R,S)-ketamine + prucalopride was administered before or after contextual fear conditioning (CFC) stress in male and female 129S6/SvEv mice. Drug efficacy was assayed using a variety of behavioral tests, including the forced swim test (FST), elevated plus maze (EPM), open field (OF), marble burying (MB), novelty-suppressed feeding (NSF), and contextual fear discrimination (CFD). All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the New York Psychiatric Institute (NYSPI). c-fos and parvalbumin (PV) expression in the hippocampus was assayed using immunohistochemistry. Generally, the effect of Drug was analyzed using an analysis of variance (ANOVA), using repeated measures where appropriate. Post-hoc Dunnett, Sidak, or Tukey tests were used where appropriate.

Results: A single dose combination of prophylactic (R,S)-ketamine + prucalopride (10 + 3 mg/kg) attenuated learned fear in male mice ($n = 6$-10 male mice per group; ANOVA; $p = 0.0035$) and decreased behavioral despair in both sexes ($n = 6$-10 male mice per group; ANOVA; $p < 0.0001$; $n = 6$-12 female mice per group; ANOVA, $p < 0.0001$). Combined administration of (R,S)-ketamine + prucalopride exerted an additive effect in preventing stress-induced hyponeophagia in both male and female mice, but not when administered separately ($n = 6$-10 male mice per group; ANOVA; $p = 0.0004$; $n = 6$-12 female mice per group; ANOVA, $p = 0.0467$). Prophylactic (R,S)-ketamine + prucalopride also exerted an additive effect of enhancing and facilitating contextual fear discrimination in male mice ($n = 6$-7 mice per group; RMANOVA; $p < 0.0001$). Combined, but not separate, administration of (R,S)-ketamine + prucalopride significantly increased neural activity and PV expression, as well as overlap, in ventral CA3 of the hippocampus ($n = 7$-8 mice per group; ANOVAs; $p = 0.0001$, $p = 0.0197$, $p = 0.0178$, respectively).

Conclusions: Our results indicate that simultaneously targeting NMDARs and 5-HT4Rs using a drug combination of (R,S)-ketamine + prucalopride exerts additional and distinct neural and behavioral effects in reducing a wide variety of stress-induced fear, behavioral despair, and hyponeophagia behaviors in both male and female mouse models of stress. Simultaneously targeting NMDARs and 5-HT4Rs is sufficient to enhance both excitatory and inhibitory signaling in specific subregions of the HPC, a brain region critically involved in stress processing and psychiatric disorders. Together, our findings demonstrate the potential of leveraging combinatorial pharmacological treatment to advance targeted therapies for stress-induced psychiatric disorders.

Keywords: (R,S)-ketamine, Prucalopride, NMDA Receptor, 5-HT4 receptor, Depression and Anxiety

Disclosure: Patent: Patent (Self)

P80. Orally Administered Heteroaromatic Salvinorin A Analogue (AK-1402) Elicits Antinociceptive and Anxiolytic-Like Effects in Mice

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Background: Despite the attribution of the analgesic and anxiolytic properties of Salvia divinorum to neoclerodane diterpene called Salvinorin A (SA), the limiting pharmacological profile and dearth of clinical applications of SA support the preliminary screening of its C(22)-fused-heteroaromatic analogue [2-O-salvinorin B benzo[b]thiophene-2-carboxylate (AK-1402)] in mice nociception and anxiety models while assessing potential mechanism of action.

Methods: Acute oral administration of AK-1402 (1, 3, or 10 mg/kg), morphine (MOR 5 mg/kg), diazepam (DZP 1 mg/kg), or vehicle 10 ml/kg in male and female Swiss mice [5-week-old with weight = 25 ± 3 g; $n = 8$ (4 mice per sex) randomly distributed into the treatment groups to the acetic acid-induced abdominal writhing, formalin-induced hind paw licking, the thermal reaction in the hotplate as well as aversive response in the elevated plus-maze (EPM), and light-dark box (LDB). Additionally, mice were pretreated intraperitoneally with naloxone (NAL 3 mg/kg; non-selective opioid receptor antagonist), naloxonazine (Nalzine10 mg/kg; selective μ-OR antagonist), and nor-binaltorphimine (nor-BNI 10 mg/kg; selective κ-OR antagonist), or vehicle before the

oral administration of AK-1402 10 mg/kg, MOR 5 mg/kg, DZP 1 mg/kg or vehicle 10 ml/kg and behavioral evaluations. The NIH Guidelines for the Care and Use of Laboratory Animals as approved by the Ethics Committee of the Federal University of Goiás (protocol number 104/08) were adhered to in all experimental procedures. Data were subjected to ANOVA followed by Dunnett's s or Bonferroni's post hoc tests and expressed as mean ± SEM ($p < 0.05$ was considered statistically significant).

Results: The oral administration of AK-1402 (3 and 10 mg/kg) and MOR 5 mg/kg reduced the abdominal writhing activity [$F(4, 39) = 61.6$], licking behavior at the first phase of formalin [$F(4, 25) = 19.8$, $p < 0.05$], and increased latency to thermal stimulus response on hot plate [$F(4,35) = 8.6$, $p < 0.05$], thereby showing antinociceptive property. Like DZP 1 mg/kg, an increase in the time spent and entry into the open arm of EPM [$F(4, 35) = 12.5$ and $F(4, 35) = 8.3$, respectively; $p < 0.05$] as well as the transition and time spent at the light area of LDB [$F(4, 35) = 10.1$ and $F(4, 35) = 9.3$, respectively; $p < 0.05$] suggest the attenuation of aversive-like response by AK-1402 at the dose of 10 mg/kg. The blockade of the antinociceptive and anxiolytic-like properties following NAL, nor-BNI, and/or FLU pretreatments suggests opioid receptors and benzodiazepine site involvement in the activities of AK-1402.

Conclusions: The antinociceptive and anxiolytic-like effects of AK-1402 are promising and demand additional pharmacological characterizations.

Keywords: Salvinorin A, AK-1402, Behavioral Model, Opioid System, Gabaergic System

Disclosure: Nothing to disclose.

P81. Association of Early Life Stress and Dietary Factors With Mitochondrial DNA Deletions in Healthy Young Adults

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Background: Increasing evidence indicates that mitochondria respond to psychosocial and environmental stressors. In a recent study, elevated mitochondrial DNA (mtDNA) deletions were found in the postmortem brains of individuals with psychiatric disorders, but no prior work has examined the impact of early life stress (ELS) and health behaviors in adulthood on mtDNA deletions. This study assessed mtDNA deletions, diet, and cardiometabolic risk in peripheral blood mononuclear cells (PBMCs) in healthy young adults, with and without ELS.

Methods: Participants ($N = 181$; 69% female) ages 18-40 were recruited from community. Participants with ELS ($n = 108$) experienced childhood maltreatment and parental loss. Controls ($n = 73$) had no maltreatment, loss, or psychiatric disorders. Standardized interviews and self-reports assessed demographics, adversity, psychiatric history, cardiometabolic risk factors, and dietary intake using the Healthy Eating Index. PBMCs were isolated for sequencing of mtDNA deletions. Splice-Break pipeline with long-range PCR and next-generation sequencing was utilized to quantify mtDNA deletions, with statistical control for coverage depth. mtDNA deletions were log transformed for analysis due to skew.

Results: In a linear model adjusted for age, sex, and coverage depth, participants with ELS demonstrated significantly more unique mtDNA deletions ($F(4,176) = 19.2$, $p < .001$) than participants without ELS. Current psychiatric disorders, adult stressors, and current perceived stress were each associated with deletions ($p's < .05$) but did not account for ELS associations with unique mtDNA deletions. A healthier diet was associated with fewer unique mtDNA deletions ($p = 0.046$) in a linear model adjusting for age, sex and coverage depth, though diet did not account for the association of childhood adversity and mtDNA deletions ($p < .001$). There was no association between a composite metabolic risk score and mtDNA deletion burden ($p = 0.50$).

Conclusions: In this sample, participants with ELS demonstrated higher numbers of unique deletions, regardless of psychiatric status. Additionally, diet, but not cardiometabolic risk, predicted mtDNA deletions in healthy young adults. These preliminary findings add to recent evidence indicating that mtDNA is impacted by childhood adversity, psychiatric disorders, and health behaviors. Next steps include additional assessments of relationships with mitochondrial structure and function, metabolic hormones, and type and timing of adversity.

Keywords: Mitochondrial DNA, Early Life Stress (ELS), Diet

Disclosure: Nothing to disclose.

P82. Fear Potentiated Startle to an Unconditioned Aversive Stimulus Predicts Individual Differences in Physiological Conditioned Fear and Self-Reported Fearful Traits in a Posttraumatic Stress Sample

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Background: Posttraumatic stress symptoms (PTSS) are characterized, in part, by pathological fear, anxiety, and stress reactivity (FASR). Distinct aspects of FASR are indexed using physiological measures of acute conditioned fear responding and self-report measures of dispositional fearful reactivity. Elevated physiological or self-reported FASR has been observed in some, but not all, PTSS samples and may contribute to variability in clinical presentation and treatment response.

Unconditioned physiological responding (UCR) to a naturally aversive stimulus may contribute to individual differences in FASR. Studies in rodents, healthy humans, and humans with fear- and-anxiety-related disorders have found that UCR predicts physiological responding to a conditioned fear cue during fear acquisition. In a recent fear-potentiated startle (FPS) study of trauma-exposed individuals with low PTSS (Kreutzmann et al., 2021), UCR predicted FPS to conditioned fear and safety cues during acquisition. Some prior studies have found that physiological stress responding is related to self-reported FASR. In a transdiagnostic study of fear and anxiety disorders (Marin et al., 2020), UCR predicted a composite score of self-reported dispositional reactivity to stress. Here, we extend prior work by testing the association of UCR with both physiological and self-reported fearful reactivity in a trauma-exposed sample with a continuum of PTSS severity. We hypothesized that higher UCR would be positively correlated with FPS to conditioned fear cues during acquisition and extinction, with FPS to conditioned safety cues during acquisition and extinction, and with self-reported dispositional FASR. We also explored associations of UCR with PTSS severity.

Methods: Participants were 79 trauma-exposed adults (61 female) with a continuum of PTSS severity. PTSS severity was quantified as total score on the Clinician Administered PTSD Scale (CAPS) for DSM-5. A composite score of self-reported Fear Anxiety and Stress Reactivity (FASR) was derived by averaging z-scores from four self-report measures that index distinct aspects of dispositional FASR and previously have been associated with PTSS and fear conditioning: Fear Survey Schedule-II, Anxiety Sensitivity Index-3, State-Trait Anxiety Inventory–Trait Anxiety Scale, and 10-

item Connor-Davidson Resilience Scale. FPS was recorded during fear Acquisition and Extinction. The auditory startle probe was a burst of white noise. The unconditioned stimulus (US) was an airblast to the larynx and the conditioned stimuli were two colored shapes. The conditioned fear cue (CS+) was paired with the US during Acquisition and the conditioned safety cue (CS-) was never paired with the US. Acquisition consisted of 12 trials of each CS, with the US following each CS + trial. Extinction consisted of 16 trials of each CS type. Mean FPS for UCR was calculated using all 12 US presentations during Acquisition. Mean FPS for CS + and CS- during Acquisition and Extinction were calculated after omitting the first trial of each CS type for each phase. Six separate linear regressions tested the correlation of UCR with FPS to CS + during Acquisition, FPS to CS + during Extinction, FPS to CS- during Acquisition, FPS to CS- during Extinction, FASR score, and total CAPS. All analyses were performed both with and without biological sex as a covariate.

Results: UCR was positively correlated with CS + during Acquisition ($R2 = 0.24$; $p < 0.0001$), CS + during Extinction ($R2 = 0.13$; $p = 0.0028$), CS- during Acquisition ($R2 = 0.14$; $p = 0.0015$), CS- during Extinction ($R2 = 0.21$; $p < 0.0001$), and FASR ($R2 = 0.15$; $p = 0.0004$). UCR was not correlated with total CAPS ($R2 = 0.03$; $p = 0.1352$). All significant correlations survived Bonferroni correction and remained Bonferroni significant when controlling for sex. Female sex was associated with greater total CAPS scores ($B = 0.66$, $p = 0.0184$), but this association did not survive Bonferroni correction.

Conclusions: Higher UCR was associated with greater conditioned FPS to four distinct conditions (CS + Acquisition, CS + Extinction, CS- Acquisition, CS- Extinction) and with a composite measure of self-reported dispositional FASR in this trauma-exposed sample with a continuum of PTSS severity. Thus, in trauma-exposed individuals, elevated UCR to naturally aversive stimuli may lead to elevated physiological fear reactivity to a broad array of conditioned stimuli and to an increased tendency to feel fearful and anxious in response to stress. Conditioned fear extinction is the basis of prolonged exposure therapy for PTSD and inability to inhibit fear in the presence of conditioned safety cues is theorized to represent a distinct phenotype of PTSD with regard to treatment responsivity or resistance. Thus, future studies are needed to determine whether elevated UCR predicts differences in treatment response in PTSD.

Keywords: Post Traumatic Stress Disorder, Fear-Potentiated Startle, Fear Conditioning and Extinction, Unconditioned Responses, Anxiety and Stress

Disclosure: Nothing to disclose.

P83. Neighborhood Poverty is Associated With Reduced Acoustic Startle in Individuals With Fewer PTSD Symptoms

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Background: Neighborhood poverty is associated with exposure to chronic stressors including environmental stressors like noise and pollution and traumatic events like police violence and other violent crimes. Recent work from our group has shown that neighborhood poverty is associated with worse posttraumatic stress disorder (PTSD) symptoms, both in chronically trauma-exposed Black women and in acutely traumatized men and women of varied race. PTSD is also characterized by dysregulation of autonomic function. For example, individuals with PTSD take longer to habituate to a startle tone than those without PTSD in the aftermath of a traumatic event. Furthermore, several studies demonstrate increased startle magnitude in individuals with PTSD as compared to individuals without PTSD. Despite the relation between neighborhood poverty and PTSD symptoms, little is known about how neighborhood poverty relates to psychophysiological functions that are dysregulated in PTSD. Thus, in the current study we determined associations between neighborhood poverty and acoustic startle in a fear potentiated startle paradigm. We hypothesized that individuals living in areas with high rates of poverty with high PTSD symptoms would show the highest startle magnitude. We also explored the interactions of PTSD symptom subclusters with rates of neighborhood poverty on startle magnitude.

Methods: Participants ($N = 64$, 70% female) were enrolled between 2013 and 2014 in the waiting rooms of a large publicly funded hospital in Atlanta, GA. Participants were recruited out of medical clinics, including primary care or obstetric and gynecology waiting rooms. The sample was 93.8% Black, 3.1% white, 1.6% Latinx, and 1.6% mixed. Basic sociodemographic information including zip code where the participant lived was collected. PTSD symptoms were assessed with the modified PTSD Symptom Scale (PSS). Neighborhood poverty was assessed using the 2014 5-year American Community Survey, an annual survey that determines socio-demographic information (such as poverty) for a given area within the US. Neighborhood poverty was defined as the percentage of individuals living below the federally determined poverty line in a participant's zip code. Lastly, startle magnitude was determined using an acoustic startle paradigm. Startle magnitude was defined as the average eye blink electromyogram (EMG) response to 106 decibel white noise probes across 12 trials of the task. Moderation analyses were used to determine the effect of neighborhood poverty on startle magnitude and whether there was a moderating role of PTSD symptoms.

Results: The model with total PTSD symptoms as the moderator accounted for 13.45% of the variance in startle magnitude, $F(3,60) = 3.11$, $MSE = 1080.49$, $p = 0.03$. Neighborhood poverty significantly predicted startle magnitude, where living in areas with more poverty predicted lower startle magnitude ($B = -132.27$, $SE = 54.56$, $p = .02$). There was also an interaction between neighborhood poverty and PTSD symptoms on startle magnitude ($B = 6.19$, $SE = 3.15$, $\Delta R^2 = .06$, $p = .05$). Follow up analyses were run to determine conditional effects of PTSD symptoms. Results showed that the effect of neighborhood poverty on startle magnitude was only significant at the 16th percentile of PTSD symptoms ($B = -136.08$, $SE = 52.39$, $p = .02$), but not at the 50th ($B = -70.42$, $SE = 39.49$, $p = .08$) or 84th percentiles of PTSD symptoms ($B = 13.70$, $SE = 53.09$, $p = .80$). For symptom subcluster analyses, the model with hyperarousal symptoms as the moderator accounted for 11.88% of the variance in startle magnitude, $F(3,60) = 2.70$, $MSE = 1100.11$, $p = .05$. In this model, neighborhood poverty significantly predicted startle magnitude ($B = -124.33$, $SE = 56.70$, $p = .03$), though the interaction between hyperarousal symptoms and neighborhood poverty was not significant ($B = 14.88$, $SE = 8.69$, $\Delta R^2 = .04$, $p = .09$). For intrusive symptoms, the model accounted for 7.86% of the variance in startle magnitude and was not significant, $F(3,60) = 1.7$, $MSE = 1150.34$, $p = .18$. Finally, for avoidance and numbing symptoms, the model predicted 12.54% of the variance in startle magnitude, $F(3,60) = 2.87$, $MSE = 1091.84$, $p = .04$. Neighborhood poverty significantly predicted startle magnitude, ($B = -132.77$, $SE = 53.11$, $p = .02$) and the interaction between avoidance and numbing symptoms and neighborhood poverty was significant ($B = 14.04$, $SE = 6.80$, $\Delta R^2 = .06$, $p = .04$). Follow up analyses revealed that neighborhood poverty predicted startle magnitude at the 16th ($B = -132.77$, $SE = 53.11$, $p = .02$) and 50th ($B = -83.63$, $SE = 40.97$, $p = .05$) percentiles of avoidance and numbing symptoms, but not at the 84th percentile of avoidance and numbing symptoms ($B = 2.02$, $SE = 48.97$, $p = .97$).

Conclusions: Our results indicate that neighborhood poverty is associated with blunted startle magnitude only for those with few PTSD symptoms. This effect seems to be driven by the avoidance and numbing symptom cluster of PTSD. It is possible that individuals living in areas with high rates of poverty are more habituated to loud noises, potentially due to greater exposure to loud noise in their everyday environments, resulting in decreased responses to acoustic startle probes. This habituation may no longer be present when an individual has greater PTSD symptoms. Overall, results imply that neighborhood poverty can impact basic autonomic functioning. Future studies should further explore the role of different types of PTSD symptoms and their interaction with neighborhood poverty in impacting psychophysiology.

Keywords: Acoustic Startle, PTSD, Neighborhood Poverty

Disclosure: Nothing to disclose.

P84. HIV Interacts With PTSD to Impact Fear Extinction and Estrogen Receptor Expression in Trauma-Exposed Black Women

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Background: People living with HIV experience high rates of trauma exposure over their lifetime, which increases individual risk for the development of posttraumatic stress disorder (PTSD) and psychophysiological hyperarousal, including deficits in fear extinction. Additionally, under-resourced and minoritized Black women living in urban environments tend to be exposed to high levels of trauma, suffer from chronic PTSD, and are at increased risk for HIV infection. Because evidence-based PTSD treatments, such as prolonged exposure, rely on fear extinction, it is important to understand how HIV serostatus interacts with PTSD to impact fear extinction. We hypothesized that individuals with both HIV and PTSD would show greater impairments in fear extinction. Additionally, it is important to understand the biological mechanisms by which HIV and PTSD may impact fear extinction processes. Estradiol (E2) and its receptors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) affect neurobiological processes underlaying fear extinction. More specifically, preclinical and translational studies in women indicate that limbic regions of the brain critical for fear extinction and learning process are densely populated with ERα and ERβ and show that E2 impacts fear extinction processes. Thus, in the current study we also assessed the impacts of HIV and PTSD on the ratio of peripheral gene expression of ERα to peripheral gene expression of ERβ. We hypothesized that both HIV and PTSD would be associated with differences in ERα to ERβ gene expression ratios.

Methods: Women (18-65 yrs) recruited from the Women's Interagency HIV Study (WIHS) in Atlanta, GA ($n = 70$, 26 without HIV, 44 with HIV) provided informed consent and underwent a semi-structured interview to assess adult (Traumatic Events Inventory; TEI) and childhood trauma (Childhood Trauma Questionnaire; CTQ) exposure, as well as PTSD using the PTSD Checklist for DSM-5 (PCL-5). Participants also underwent a fear-potentiated startle (FPS) paradigm to assess fear extinction. Blood samples were collected within two weeks of the FPS paradigm to assess peripheral gene expression of ERα and ERβ. A ratio of ERα to ERβ gene expression was calculated. ANCOVAs assessing the effects of HIV, PTSD, and their interaction on fear extinction and ERα/ERβ expression controlled for age, income, education, and adult trauma exposure were conducted.

Results: PTSD diagnosis ($p = 0.021$; $\eta 2 = 0.088$) and HIV status ($p = 0.046$; $\eta 2 = 0.067$) were both associated with deficits in the percent decrease of fear extinction. There was also a significant interaction of HIV serostatus and PTSD diagnosis ($p = 0.023$; $\eta 2 = 0.082$) on fear extinction, as HIV was associated with impaired fear extinction only in women with a PTSD diagnosis ($p = 0.026$; partial eta2 = 0.078). Although there were no main effects of PTSD or HIV status on ERα/ERβ expression, there was a significant interaction between PTSD and HIV ($p = 0.006$; $\eta 2 = 0.142$). ERα/ERβ expression was significantly lower in women without PTSD and with HIV ($p = 0.007$), and women with HIV and without PTSD ($p < 0.001$) compared to women without either HIV or PTSD.

Conclusions: Our findings suggest HIV impacts fear psychophysiology in women with a PTSD diagnosis, demonstrating deficits in fear extinction. Given the use of prolonged exposure as a prominent evidence-based therapy for PTSD, these findings are clinically relevant, suggesting that HIV may impair the efficacy of such treatment in women living with HIV. Our findings also suggest that HIV and PTSD impact ERα/ERβ expression in women, but not in a synergistic manner. Future studies are necessary to determine how alterations in expression of ERα/ERβ may contribute to deficits in fear extinction in women living with HIV, including inflammatory pathways implicated in psychophysiological hyperarousal in trauma-exposed individuals.

Keywords: PTSD, HIV, Fear Extinction, Estrogen Receptor, Women's Mental Health

Disclosure: Nothing to disclose.

P85. Maternal-Infant Emotional Connection is Related to Reduced Physiological Stress Response in Mothers and Their Babies: Preliminary Results From a Trauma-Exposed Sample of Primarily Black Mother-Infant Dyads

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Background: Trauma and posttraumatic stress disorder (PTSD) related risks are transmitted across generations, yet little is known about early pathways of risk or resilience in the context of maternal PTSD. Infancy is a critical time for socioemotional development. Emotional connection (EC) between mother and infant is particularly important for an infant's secure attachment and ability to regulate stress. Infant cardiac reactivity and low vagal tone, measured with heart-rate (HR) and respiratory sinus arrhythmia (RSA), are significantly impacted by parenting behaviors and may be an important early biomarker of risk for later vulnerability to trauma-related psychopathology. Additionally, heightened sympathetic arousal is a biomarker of PTSD, and maternal emotional reactivity may impact a mother's ability to sensitively respond to their infant's needs. However, no prior studies have examined how mother-infant EC relates to maternal and infant stress response or its relation to maternal PTSD. Thus, examining physiological reactivity patterns to stress in newborns and their mothers in a sample of women with high levels of PTSD symptoms and trauma exposure will aid in our understanding of potential pathways through which maternal PTSD impacts early emotional and stress regulation development in children at risk for negative outcomes.

Methods: The current pilot study measured maternal PTSD symptoms, mother-infant emotional connection (EC), and maternal and infant stress response to the Double Still Face Paradigm (DSFP, 4m) in 20 mother-infant dyads (mean maternal age = 27; infant age = 4 months; 91% Black or African American) recruited from an urban safety net public hospital with high levels of trauma

exposure and PTSD (45% met for probable PTSD diagnosis). DSFP is a laboratory stressor task used to assess infant response to maternal disengagement. Dyads completed a behavioral task assessing baseline EC and post-stressor EC using the Welch Emotional Connection Scale, which includes measurement of attraction, vocal, facial, and sensitivity scales and an overall EC score. PTSD symptoms were assessed using the PTSD Checklist for DSM-5. Infant respiratory sinus arrhythmia (RSA) and heart rate (HR) was measured during DSFP. Maternal skin conductance response (SCR) during DSFP was also obtained on a subsample of participants (n = 8). Bivariate correlations between maternal PTSD, baseline mother-infant EC, and physiological response to stress were analyzed. For infant stress response, baseline, initiation of first recovery phase, and final recovery phase time points were used. Bivariate correlations between PTSD symptoms and post-stressor EC were also run.

Results: Bivariate correlation analyses indicated higher mother-infant EC was significantly associated with higher infant RSA response during initiation of the first recovery phase of DSFP (r = .48, p = .034), but not baseline (r = -.15, p = .53) or final recovery (r = -.33, p = .19). There were no significant associations between mother-infant EC and HR. Additionally, mother-infant EC was inversely related to levels of maximum maternal skin conductance response during the DSFP (r = -.85, p = .008, n = 8). No significant correlational associations between PTSD symptoms and baseline mother-infant EC or infant RSA or HR were found. However, higher levels of PTSD symptoms were related to lower post-stressor mother-infant EC for facial and sensitivity subscales (r = -.54, p = .031 and r = -.52, p = .041, respectively; n = 16).

Conclusions: Results suggest that EC in mother-infant dyads relates to how both mothers and their infants respond to stress and point to an important potential pathway through which intergenerational trauma and PTSD-related risk or resilience may occur.

Keywords: Posttraumatic Stress Disorder, Intergenerational Transmission of Trauma, Psychophysiology

Disclosure: Nothing to disclose.

P86. Quality of Sleep Moderates Cardiovagal Reactivity to the Trier Social Stress in Black Women With PTSD

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Background: Increased rates of trauma exposure are strongly associated with poverty. Women exposed to trauma develop post-traumatic stress disorder PTSD at twice the rate observed in than men. Previous research suggests that presence of PTSD is associated with poor sleep and greater risk of cardiometabolic diseases. However, given the possible link between poor sleep and cardiometabolic diseases, it remains unclear whether sleep disturbance is the mechanism through which PTSD leads to greater cardiovascular disease risk. In this study, we examined the impact of PTSD diagnosis and symptom severity on cardiovascular and autonomic responses to an acute psychological stress. In addition, we examined the effect of sleep quality and comorbid depression on these responses. We hypothesized that women with a diagnosis of PTSD will have exaggerated blood pressure, heart rate and sympathetic reactivity to the Trier Social Stress Test (TSST), and that these responses will be moderated by sleep quality.

Methods: To test this hypothesis, we recruited 74 black women (age between 31 and 63 years old) with a history of trauma exposure and type 2 diabetes from a large county hospital in the southeastern United States. We measured cardiovascular and sympathovagal response during the TSST in women with (n = 45) and without (n = 29) a current diagnosis of PTSD. Categorical diagnosis of PTSD and PTSD symptom severity were determined using the Clinician Administered PTSD Scale (CAPS), depressive symptom severity was assessed with the Beck Depression Inventory (BDI) and quality of sleep was assessed with the Pittsburgh Sleep Quality Index (PSQI). Baseline systolic and diastolic blood pressure (BP) was measured via automated SphygmoCor. Participants underwent the 20-minute Tier Social Stress Test (TSST) and were seated in a quiet room before and after the standardized psychosocial stressor test. Heart rate (HR) and Low-Frequency to High Frequency Ratio (LF/HF) were obtained via 3-lead ECG measured before, during and after TSST. Pearson correlation was used to probe for the relationship between CAPS, BDI and PSQI scores. BP, HR and LF/HF reactivity was analyzed using repeated measures ANOVA with time as within factor and PTSD diagnostic as between factor. PSQI and BDI were covariates in the analysis.

Results: We found significant correlation between PSQI and CAPS severity (r = 0.47, p = <0.001) and between PSQI and BDI (r = 0.58, p = <0.001. Heart rate response to TSST was significantly higher in the PTSD group vs. the non-PTSD group (time, p = <0.001; group, p = 0.02). LF/HF response was also significantly higher in those with PTSD vs. those without (time, p = 0.46; group, p = 0.05). However, systolic blood pressure response was not different between groups (time, p = <0.001; group, p = 0.22). Given the strong correlation between PSQI and BDI, we chose to control for one of the two variables in the repeated measures ANOVA. Sleep was selected because of the literature supporting the link between sleep deprivation and exaggerated sympathetic response to acute stress in women. When controlling for sleep, the difference in heart rate (group, p = 0.05) and LF/HF (group, p = 0.044) reactivity between the group was affected.

Conclusions: Our results show that PTSD status is associated with heart rate over-reactivity and autonomic imbalance during a standardized psychosocial stressor in this sample of traumatized black women with type 2 diabetes. Furthermore, we observed that sleep quality moderates that response. This suggests that sleep disturbances are a hallmark of PTSD and might be the factor driving the reported sympathetic overactivity to acute stress described in PTSD. Therefore, improvement of sleep quality may serve as a target for interventions aiming at reducing cardiovascular risk associated with PTSD.

Funding: Supported by R01MH099211 (CFG).

Keywords: PTSD, Women, Sleep, Heart Rate Variability, Heart Rate

Disclosure: Nothing to disclose.

P87. Plasticity in Dissociable Neural Circuits Supports the Divergent Consequences of Severe Stress

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Background: Acute severe stress is able to produce an array of changes in fear and anxiety-like behavior, including defensive responses to stress-associated stimuli, heightened reactions to novel aversive events, and increases in anxiety-like behavior. Whether these diverse consequences of severe stress arise from plasticity in a common circuit, or if distinct circuits support the

various consequences of severe stress, remains unknown. Addressing this question is fundamental to understanding how the pathological consequences of stress might be mitigated. Here, utilizing a combination of region-specific protein synthesis inhibition, chemogenetics, and activity-dependent cell tagging, we show that stress-induced plasticity within the ventral hippocampus (vHC) and basolateral amygdala (BLA) support dissociable consequences of severe stress.

Methods: Experiment 1 examined the role of stress-induced protein synthesis in the vHC and BLA on subsequent behavior. Mice were exposed to a an acute severe stressor in which they received 10 foot-shocks, or not, and protein synthesis in either structure was blocked just after. A week later, associative fear of the stressor was examined by placing the animals back in the stressor environment and measuring freezing, anxiety-like behavior was assessed in a light-dark test, and learning about novel aversive events was assessed by exposing them to a loud auditory stressor in a distinct conditioning chamber. Experiment 2 then assessed how the BLA and vHC support the expression of post-stress behavioral changes. An identical behavior procedure to that of Experiment 1 was used, but chemogenetics were utilized to inhibit the vHC or BLA during the expression of post-stress behavioral phenotypes. Lastly, Experiment 3 utilized a transgenic activity-dependent tagging approach to permanently label stress-reactive cells and then looked at their reactivation in response to subsequent events utilizing of immunohistochemistry. n = 8-12/group. Male mice were used for these experiments.

Results: In Experiment 1, blockade of stress-induced protein synthesis in the BLA profoundly reduced subsequent associative freezing in the stressor context and mitigated heightened responses to a novel stressor, but had no impact on the anxiety-like behavior of the same animals. Conversely, blockade of stress-induced protein synthesis in the vHC attenuated changes in anxiety-like behavior but had no impact on responses to novel stressors and had minimal impact on associative freezing. In Experiment 2, using chemogenetics, we demonstrated that neural activity in BLA and vHC have similar dissociable contributions to the expression of these post-stress phenotypes. Lastly, in Experiment 3, we found that stress reactive ensembles in the BLA (but not the vHC) are more likely to be reactivated by novel stressors, suggesting that stress-induced plasticity specifically within this cell population supports the heightened response to novel stressors

Conclusions: Collectively, these results indicate that plasticity in separate BLA and vHC circuits support distinct impacts of stress on behavior, and ongoing research aims to elaborate on the unique role of stress-reactive ensembles. Critically, these results suggest that the impacts of severe stress are biologically divergent. If true, this might indicate that interventions geared towards one these post-stress phenotypes (e.g., associative memories) in conditions like PTSD may not affect other post-stress phenotypes (e.g., anxiety). Moreover, by targeting the specific cells within these networks that are activated by stress, we might be able to mitigate post-stress phenotypes.

Keywords: Fear, Anxiety, Amygdala, Hippocampus

Disclosure: Nothing to disclose.

P88. Examining the Role of BNST CRF in Models of Aversive Learning

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Background: Anxiety disorders affect roughly one third of the population and a core symptom of anxiety and trauma-related disorders is excessive and generalized fear. While identifying and responding to threats that predict danger is essential for survival, persistent apprehension about future threats can become maladaptive. Recent work suggests that the predictability of if and when danger will occur plays a critical role in development of pathological anxiety. Recent studies in rodents have shown that unpredictable, but not predictable threat engages the bed nucleus of the stria terminalis (BNST), a part of the extended amygdala that has long been studied with respect to stress, anxiety and drugs of abuse.

Methods: We used multiple approaches to probe this question. To determine if CRF in the BNST regulates fear and antecedent behaviors, we used viral deletion of CRF coupled to behavior in the mouse brain. To determine activity profiles of neurons and modulators, we used fiber photometry and miniscope recordings in mice.

Results: First we found that partially (PRF), but not fully reinforced fear (FRF) conditioning led to exaggerated acoustic startle response in males but not females. Next, we used multi-region fiber photometry to characterize BNST cell body and terminal dynamics in the periaqueductal gray, a midbrain structure that regulates adaptive motor responses. We found that during fear conditioning, BNST cell body and terminal activity did not differ between fear groups, but that differences emerged during fear recall 24 hours later. Interestingly, the size of BNST responses during conditioning positively correlated with recall freezing behavior in PRF but not FRF mice.

We next were curious about the modulatory signaling that drove these behaviors. Extensive work from Mike Davis and others over the years have suggested that the neuropeptide Corticotropin Releasing Factor (CRF) played an important role in this behavior. Thus, we aimed to determine the role of BNST CRF neurons in partially reinforced fear in male and female mice. Using a transgenic floxed CRF line, we found that knockdown of CRF in the BNST selectively enhanced PRF in females but not males. Next, we implanted GRIN lenses and used miniature head-mounted microscopy to record individual BNSTCRF cells during behavior. We found that in both sexes, BNSTCRF activity was higher after conditioning in partially reinforced compared to fully reinforced fear mice, and that cells responded differently during fear recall across groups and sexes. Finally, using fiber photometry coupled to a novel CRF biosensor, we identified differences in CRF release in the PAG but not BNST during fear recall across groups.

Conclusions: Overall, we find sex-specific effects of BNSTCRF signaling in partially and fully reinforced fear, suggesting potential sex-dependent treatment strategies based on CRF modulation. Notably we find that deletion of BNST CRF leads to enhanced post fear startle in male mice exposed to reliable fear, suggesting fear evoked release of CRF constrains arousal.

Keywords: Amygdala, Neuropeptides, Acoustic Startle Response

Disclosure: Nothing to disclose.

P89. Adolescent Chronic Social Defeat Produces Robust Social Avoidance and Changes in Neural Activation in the Adult Mouse

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Background: Adolescence is a time of monumental cognitive and neurobiological changes. Trauma during this time period can in turn alter normal brain development and result in lifelong psychiatric disorders. While models of early life neglect in rodents do exist, models of early life trauma are nearly absent. In order to

better understand the neurobiological underpinnings of early life trauma on the adult brain, we have developed a model of adolescent chronic social defeat in male mice.

Methods: Adolescent chronic social defeat stress (aCSDS): On the first day of aCSDS, an experimental C57BL/6J (C57) male mouse (P27 + /-2 days) is placed directly into an adult (>8 weeks) CFW male resident's territory for a 3-minute aggressive encounter (ended after 30 bites). After the aggressive encounter, the experimental C57 mouse is housed adjacent to that resident for 24 hours until the aggressive encounter on the next day. This procedure of defeat and housing is repeated with a novel resident each day for 10 days. Non-defeated control C57 mice are housed in divided home cages identical to those used for defeat next to an unfamiliar control C57 mouse. On the tenth day of aCSDS, defeated and control C57 mice are housed side-by-side in divided cages that allow both olfactory and visual contact until adulthood.

Open Field Social Interaction with CFW male (OFSI): After 1 hour of habituation to the testing room, C57 male mice (P50 for late adolescent testing, P70 for adult testing) are placed opposite an inverted black wire cup in a large (44 x 44 cm) arena. After 150 seconds of exploration of the arena and empty cup, the C57 is placed back in the arena with the cup now containing a novel nonaggressive CFW male inside and allowed to explore for 150 seconds. All interactions are captured and scored with Ethovision. Social interaction ratio is calculated as time spent with the empty cup divided by time spent with the cup with a CFW inside.

Home Cage Social Interaction with ovariectomized CFW female (HCSI): Adolescent (~P50) C57 male mice remain in their home cage and an ovariectomized CFW female is placed inside the home cage for 90 seconds. If any biting is observed from either male or female, interaction is immediately ended, and the female is replaced with another female. The interaction is recorded at 30 fps and later analyzed for specific behaviors with DeepLabCut.

Whole Brain c-fos imaging: After OFSI, mice are placed in a clean empty cage in a quiet room for 1 hour and then perfused with 1x PBS followed by 4% PFA. Brains are removed and placed in PFA overnight then stored in 0.02% sodium azide PBS. Only brains without any visible redness or damage were used for tissue clearing followed by whole brain c-fos labeling and imaging (LifeCanvas Technologies).

Results: After chronic defeat exposure in early adolescence, mice display robust social avoidance that is maintained throughout late adolescence and adulthood (t-test for OFSI social interaction ratio with nonaggressive male CFW; $n = 40$ defeated, 38 control male mice; in late adolescence (~P50), $p < 0.0001$; in adulthood (>P70), $p < 0.0001$). This social avoidance behavior also generalizes to female mice of the same strain as those used as aggressors during defeat (HCSI with female CFW at ~P50 characterized with DeepLabCut, $n = 14$ defeated, 14 control; t-test for reductions in following ($p < 0.001$) and contact ($p < 0.01$), increases in defensive posturing ($p < 0.001$) and freezing ($p < 0.05$) in defeated mice compared to controls). Re-exposure to a nonaggressive male mouse during adulthood drives dramatic increases in c-fos activity, with changes in network connectivity, in the brains of male mice defeated as adolescents, as revealed by whole brain c-fos imaging. Significant increases in c-fos expression in comparison to control mice were found in hippocampus (CA3), medial and lateral entorhinal cortex and anterior insula ($n = 6$ defeated brains, 6 control brains, t-test for comparison of defeated and control brains, $p < 0.05$).

Conclusions: Our aCSDS model produces robust and long-lasting deficits in social interaction which allows us to examine the neural circuits underlying avoidant behavior, a hallmark of neuropsychiatric disorders such as generalized anxiety and PTSD, in the aftermath of adolescent social defeat stress. One of the strongest areas of activation after adult social interaction in mice defeated as adolescents is CA3, a region implicated in pattern completion. Intriguingly, changes in hippocampal volume and connectivity are among the most robust findings in human imaging studies of adults that experienced trauma during childhood and adolescence. We are currently using 10x RNA sequencing to compare differential gene expression in CA3 of adult mice defeated as juveniles to that of CA3 in adult humans with a history of early life trauma. By using this reverse translational approach, we hope to better understand how circuits are altered after early life trauma, with the hopes of developing more effective treatments for psychiatric disorders resulting from early life trauma.

Keywords: Adolescent Stress, Chronic Social Defeat Stress, Early Trauma

Disclosure: Nothing to disclose.

P90. Norepinephrine Dynamics Represent Threat Prediction Errors Under Uncertainty

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Background: Posttraumatic stress disorder involves an internal state of hypervigilance to reminders of prior traumatic experiences. Norepinephrine (NE) has been proposed as a mediator of this process, since excessive NE release after stress induces anxiety in animals and exacerbates PTSD symptoms in humans. Understanding the moment to moment processing of traumatic memories and how they alter threat computation via internal states thus requires direct measurement of NE over the course of traumatic memory expression.

Methods: We expressed the fluorescent norepinephrine sensor GRAB-NE2h in the mouse medial prefrontal cortex (mPFC) using AAV9. We measured NE release via fiber photometry during fear conditioning while varying the delay between cues and outcomes (in groups of $n = 10$-13 animals). NE dynamics were measured over the course of 20 training trials and 20 extinction trials. Reinforcement learning models were fit to cue- and footshock-induced NE release over the course of learning and extinction. Models included 1) temporal difference learning, 2) TD learning with belief states (Starkweather et al 2017), and 3) TD learning with state uncertainty (Mikhael et al 2022).

Results: NE is released during footshock, and NE release during threat-predictive cues increases with repeated cue/shock associations ($p = 0.0026$, $t = 3.882$, forward 1st training vs 11th training cue). Cue-evoked NE persists in the absence of cue-shock pairings ($p < 0.0001$, $t = 7.606$, forward 1st training vs 1st extinction cue)). Trace fear conditioning also increases cue-evoked NE release in 5 second-trace conditioning($p = 0.0011$, $t = 4.385$, 1st training vs 1st testing cue) but not 15s ($p = 0.0806$, $t = 1.968$, 1st training vs 1st testing cue) or 30s trace conditioning ($p = 0.4501$, $t = 0.7896$, 1st training vs 1st testing cue), and testing cue evoked NE decreases with increased trace length (Trace: $F(2,30)=7.136$, $p = 0.0029$). When modeling trace conditioning using TD learning under state uncertainty, the prediction error term recapitulated several important features of NE release, such as sustained cue-evoked release, cue-offset evoked release, and release suppression during trace periods.

Conclusions: Our results suggest that prefrontal norepinephrine represents a more precise threat prediction error signal than has previously been described. Reminders of a traumatic event induce NE release that scales with the intensity of the threat memory. Reinforcement learning models of threat required modification to incorporate constant feedback from the environment and temporal uncertainty to reproduce observed patterns of NE release. Thus, NE appears to multiplex threat and uncertainty during retrieval of fear memories, offering a computationally rich signal for navigating dangerous environments.

Keywords: Norepinephrine, Anxiety Circuitry, Reinforcement Learning, Optical Biosensors, Uncertainty

Disclosures: Transcend Therapeutics, Freedom Biosciences: Contracted Research (Self)

P91. Cell-Type-Specific Dopamine Signaling in Ventral Hippocampus Tracks Anxiety-Related Behaviors

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Background: Despite accumulating evidence for both the ventral hippocampus (vHipp) and the mesocorticolimbic dopamine system in encoding anxiety-relevant information, surprisingly little is known about how dopamine signaling selectively affects vHipp representations of emotionally-salient stimuli to guide approach/avoidance behaviors. To address these shortcomings, we here study dopaminoceptive neurons in mouse vHipp – which can be segregated based on their expression of either the dopamine D1 or D2 receptor – to delineate a model of dopamine action in vHipp.

Methods: We capitalized on D1-Cre and D2-Cre mouse lines to visualize, record and manipulate vHipp D1 and D2 cells. The reporters lines were used for anatomy, snRNA-sequencing, slice electrophysiology, and cell-type-specific in vivo calcium imaging, chemogenetics and optogenetics. Fluorescent dopamine sensors were used to record dopamine release in vHipp.

Results: At the histological level, D1- and D2-expressing cells exhibit a precise topographical organization across vHipp subfields, which we further dissected using RNA-sequencing of single, sorted nuclei from D1 and D2 cells. We also confirmed dopamine's pharmacological effects on the electrophysiological properties of these cell types. Functionally, we found that anxiogenic environments and approach/avoidance conflicts trigger distinct patterns of calcium activity in D1 vs. D2 vHipp neurons in concert with dopamine release in in this region. Bidirectional chemogenetic and optogenetic manipulation of D1 or D2 vHipp neuron activity causally demonstrated their opposite roles in mediating anxiety and approach/avoidance behaviors. Intriguingly, vHipp dopaminoceptive mechanisms also contribute to cocaine-related behaviors, suggesting drug-induced plasticity in this circuit as well.

Conclusions: Together, we propose that dopamine dynamics in vHipp operate as a feedback loop that bidirectionally tracks anxiety levels to gate exploratory behaviors through differential recruitment of vHipp D1 and D2 neurons, which in turn mediate opposite approach/avoidance and anxiety-like responses. This work paves the way for further studies of dopamine signal processing in limbic regions, and underscores the complexity of the circuit mechanisms that govern affective states.

Keywords: Anxiety, Dopamine, In vivo Imaging

Disclosure: Nothing to disclose.

P92. Early Life Adversity in Mice Leads to Heightened Threat Reactivity and Sex-Differences in Activity of Corticotrophin Releasing Hormone Neurons in the Central Amygdala

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Background: Experiencing early life adversity (ELA), such as resource scarcity, is associated with an increased risk for anxiety-related disorders. Females are twice as likely to develop anxiety-related disorders compared to males. A core feature of these disorders is enhanced reactivity to real and perceived threat. Sex biases in risk and symptom presentation may be related to changes in corticotropin-releasing hormone (Crh), a neuropeptide that is released in response to stress and is important for driving hyperarousal associated with threat reactivity. In males, ELA has been associated with changes in Crh levels in the amygdala, however, the effects on females and the impact on threat reactivity is still largely unexplored.

Methods: We used the limited bedding and nesting (LBN) model of ELA in mice to test its effects on fear-potentiated startle, a translationally relevant behavioral phenotype used to assess threat reactivity, and the activity of Crh+ cells in the amygdala. Mice underwent LBN rearing from postnatal day 4 (PD4) to PD11 and were tested on the fear-potentiated startle task as adults ~PD70. The magnitude of startle was measured during both moderate- and high- threat anticipation states, in which mice were presented with the startling 50ms white noise either randomly (moderate anticipation) or preceded by a tone previously associated with a foot shock (high anticipation). The activity of Crh+ neurons in the central amygdala was measured using two methods. First, the number of active Crh+ neurons was quantified post-testing using cFos as a marker of neuronal activity. In a separate cohort of mice, in-vivo Ca2+ of Crh+ neurons was measured during fear-potentiated startle using fiber photometry. Two-way ANOVAs were used to test for main effects of rearing (control x LBN), sex (male x female) and an interaction on the startle response to the random and cued white noise ($n = 15$-17/group), on the percent of Crh+ neurons co-expressing cFos ($n = 6$-7/group), and on fiber photometry z-score and AUC calculations ($n = 5$-7/group).

Results: We found that LBN enhanced acoustic startle both when the startling white noise was preceded by the conditioned tone (rearing: $F(1,57)=7.37$, $p = 0.008$, eta2partial $= 0.11$) and when presented randomly (rearing: $F(1,57)=9.61$, $p = 0.003$, eta2partial $= 0.14$) indicating heightened threat reactivity across both high and moderate anticipatory states. This effect was more robustly observed in LBN females, who show greater change in startle compared to control females. LBN female mice also had increased co-labeling of cFos+ and Crh+ in the central amygdala (interaction: $F(1,23)=4.29$, $p = 0.04$), eta2partial $= 0.15$) and sustained Ca2+ activity of Crh+ neurons during the conditioned tone (interaction: $F(1,23)=4.63$, $p = 0.04$), eta2partial $= 0.16$), suggesting a role of Crh+ central amygdala neurons in threat anticipation.

Conclusions: The current work identifies a potential sex-specific mechanism for enhanced threat responding as a result of ELA. Ongoing studies will lay the groundwork for understanding sex differences in pathology and will contribute to the improvement of individualized treatment and interventions informed by the prior experience and sex of the individual.

Keywords: Early-Life Adversity, Fear-Potentiated Startle, Corticotropin-Releasing Hormone, Sex-Differences

Disclosure: Nothing to disclose.

P93. CRF1 Receptor-Expressing Central Amygdala Projections to Lateral Hypothalamus Mediate Stress-Induced Escalation of Alcohol Self-Administration and Anxiety-Like Behavior

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Background: In humans, stressful events may elicit avoidance coping (i.e., persistent avoidance of stress-associated stimuli) in

some individuals, which is associated with negative consequences such as increased alcohol drinking and increased anxiety. Using rats, our lab has shown that predator odor (i.e., bobcat urine) stress produces persistent avoidance of a stress-paired context in a subset of subjects, termed 'Avoiders'. Interestingly, Avoider rats display long-lasting increases in alcohol self-administration (SA) following stress exposure, a phenomenon that is absent in stress-exposed Non-Avoiders, and that recapitulates findings in humans. Our published work show that antagonism of CRF1 receptors in the central amygdala (CeA) rescues stress-induced escalation of alcohol SA and blunts avoidance of a stress-paired context in Avoider rats. In addition, we have demonstrated that avoidance of a stress-paired context is mediated by CeA neurons that project to the lateral hypothalamus (LH), a brain region that is known to modulate motivated behaviors. The main goal of this study is to test the hypothesis that stress-induced escalation of alcohol SA and avoidance behavior is supported by activation of CRF1 receptor-expressing CeA-LH neurons.

Methods: All experiments were performed in male and female CRF1-Cre-tdTomato rats that were generated in our lab. In Experiment 1, we used a combination of retrograde tracing and c-Fos immunohistochemistry to test the hypothesis that stress produces more c-Fos+ CRF1 CeA-LH neurons in Avoiders compared to Non-Avoiders (N = 11-14 rats/group). In Experiment 2, we used ex vivo slice electrophysiology to characterize the synaptic transmission and intrinsic properties of CRF1 + CeA-LH neurons in stressed Avoiders and Non-Avoiders, and unstressed Controls (N = 38-42 cells/group). In Experiment 3, we used chemogenetics to test the hypothesis that in vivo inhibition of CRF1 CeA-LH neurons rescues stress-induced escalation of alcohol SA, avoidance of a stress-paired context, and anxiety-like behavior as measured using the elevated plus maze (N = 7-11 rats/group). In Experiment 4, to inform future hypotheses, we used a Cre-dependent viral-mediated anterograde tracing approach to identify additional brain areas that receive projections from CRF1 + CeA neurons (N = 4 rats). Data were analyzed using multifactorial ANOVAs and Tukey's post-hoc tests where appropriate.

Results: In Experiment 1, stress increased the number of c-Fos + CRF1 CeA-LH neurons in Avoider rats ($p < 0.01$). In Experiment 2, we found that stress blunts sIPSC frequencies in CRF1 CeA-LH neurons ($p < 0.05$), which may contribute to increased excitability of these neurons. In Experiment 3, chemogenetic inhibition of CRF1 CeA-LH neurons rescued stress-induced escalation of alcohol SA ($p < 0.05$), anxiety-like behavior, but not avoidance of a stress-paired context. In Experiment 4, Cre-mediated anterograde tracing showed that CRF1 CeA neurons project to a variety of brain areas (in addition to the LH), including the nucleus accumbens, insula, lateral septum, bed nucleus of stria terminalis, lateral preoptic area, ventromedial hypothalamus, medial amygdala, lateral habenula, paraventricular nucleus of the thalamus, zona inserta, piriform cortex, and periaqueductal gray. In all experiments, no sex differences were detected.

Conclusions: These results suggest that increased excitability of CRF1-expressing CeA-LH neurons supports escalated alcohol consumption and anxiety-like behavior in Avoider rats following stress exposure. Current work is focused on elucidating the extended neurocircuitry that contributes to this phenomenon. Future work focused on identifying the molecular mechanisms that modulate the activity CRF1 CeA-LH neurons and related neurocircuitry may unveil novel targets for the management of alcohol misuse and anxiety related to stress.

Keywords: Anxiety and Stress, Alcohol, Central Amygdala

Disclosure: Roche In Vitro Diagnostics: Consultant (Spouse)

P94. Suppressing Fear in the Presence of a Safety Cue Requires Infralimbic Cortical Signaling to Central Amygdala

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Background: Stressful events can have lasting and impactful effects on behavior, especially by disrupting normal regulation of fear and reward processing. Post-traumatic stress disorder (PTSD) has become a growing public health concern, with a lifetime prevalence of ~6.4% of the US population. Increasing evidence suggests PTSD may alter the ability to discriminate among different stimuli, in particular where PTSD disrupts the ability to use signals that indicate safety, and overgeneralize the impact of fear signals.

Our lab uses a unique rodent behavioral task that allows us to examine stimulus discrimination alongside regulation of fear and reward processing behaviors. Male Long Evans rats show significant discrimination among cues representing reward, fear and safety. Specifically, they spend more time reward seeking during a reward cue predicting sucrose compared to a fear or safety cue, and more time freezing during a fear cue predicting footshock compared to a reward or safety cue. Critically, they also show suppressed freezing levels when the fear cue is presented concurrently with the safety cue but without footshock (fear+safety cue). Prior stress exposure reduces this fear suppression effect.

Our prior work has shown areas of the prefrontal cortex and amygdala contribute to this learning. However, the precise connections amongst these subregions in the ability to express learned safety have not been previously tested.

Methods: Adult male Long Evans rats (n = 25-30) were used in the present study since we have previously shown that female rats do not learn to suppress fear in the presence of a safety cue. Using a dual virus approach, we expressed mCherry-tagged hM4D(Gi) receptors in the infralimbic cortex (IL) targeting either basolateral amygdala (BLA) projecting or central amygdala (CeA) projecting neurons for CNO-induced (3mg/kg, i.p.) neuronal inhibition. All rats underwent stimulus discrimination training where a reward cue was paired with sucrose, a fear cue paired with footshock, a safety cue paired with neither sucrose or footshock, and a compound fear+safety cue without footshock. All rats received vehicle i.p. injections across the first 3 discrimination sessions. Then, using a within subjects design, rats received either vehicle or CNO during the 4th and 5th discrimination sessions. Freezing and reward seeking behaviors were quantified to each cue and collapsed across the 4th and 5th discrimination sessions followed with 2-way repeated measures ANOVAs comparing drug and cue.

Results: We show that the infralimbic projection to the central amygdala is necessary for suppressing freezing in the presence of the safety cue, while the projection to the basolateral amygdala is not. Specifically, only CNO inhibition of IL-CeA neurons resulted in a failure to suppress freezing during the fear+safety cue compared to the fear cue ($p > 0.05$). In contrast, freezing was significantly lower to the fear+safety cue than the fear cue in the same rats (n = 5) under vehicle conditions ($p < 0.05$), and for rats (n = 6) with unconfirmed mCherry expression injected with either CNO ($p < 0.01$) or vehicle ($p < 0.05$).

Conclusions: The loss of discriminative fear regulation seen specifically during IL-CeA inhibition is similar to the disruption seen in PTSD individuals that fail to regulate fear in the presence of a safety cue.

Keywords: Safety Learning, Fear Regulation, Amygdala, Medial Prefrontal Cortex

Disclosure: Nothing to disclose.

P95. Visualizing the Longitudinal Development of Stress-Induced Anhedonia From Representations of Valence in the PFC

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Background: A critical issue within the mental health field is the lack of granularity in diagnostic practices. For example, a patient that is sleeping and eating too much may be prescribed the same antidepressant as a patient who is sleeping and eating too little. This may explain the low rates of efficacy for first line antidepressants and begs further dissection beyond the DSM. Anhedonia, described as the inability to experience pleasure, is a core feature expressed in both major depressive disorder and schizophrenia. Anhedonia is linked to a dysregulation within the brain reward pathways that includes the medial prefrontal cortex (mPFC), which is highly involved in emotional and valence processing, critical for encoding hedonic values. Dopamine (DA) transmission tightly regulates mPFC cortical activity and associated behavior and is implicated in anhedonia. However, it remains unknown how DA modulates mPFC valence-specific neuronal population activity during stress-induced anhedonia.

Methods: We implemented learned helplessness (LH) and chronic mild stress (CMS) protocols to induce anhedonia in mice. We designed an anhedonic phenotype score for individual mice. To investigate the effects of DA modulation within mPFC valence-specific neuronal populations during anhedonia, we performed longitudinal in vivo 2-photon calcium imaging with optogenetics techniques to photostimulate DA ventral tegmental area (VTA) inputs in the mPFC while measuring mPFC population activity and dynamics during a Pavlovian conditioning task in mice exposed to LH and CMS. Both males and females were used in this study.

Results: Our preliminary findings showed a significant reduction in reward consumption and sociability in LH mice (Pearson Correlation, $r = -0.69$, $p = 0.03$), but not CMS mice (Pearson Correlation, $r = -0.16$, $p = 0.58$), suggesting a difference in behavioral phenotypes depending on the stress. Following initial exposure to CMS, we observed a significant decrease in mPFC activity during aversive trials in susceptible mice compared to resilient and control groups (One-way ANOVA ($F(2,398)=13.66$, $p < 0.001$; Tukey-Kramer post hoc Susceptible/Resilient, $p = 0.004$, Susceptible/Control, $p < 0.001$, Resilient/Control, $p = 0.18$). Interestingly, susceptible mice revealed a significant increase in activity during reward trials compared to resilient groups (One-way ANOVA ($F(2,398)=7.26$, $p = 0.0008$; Tukey-Kramer post hoc Susceptible/Resilient, $p = 0.002$, Susceptible/Control, $p = 0.999$, Resilient/Control, $p = 0.003$), but no change in control mice.

Conclusions: These results indicate that anhedonic states influence mPFC valence encoding properties, and that acute, severe stressors can lead to distinct etiologies from chronic, mild stress. Altogether, these experiments point to the need for increased granularity in the measurement of both behavior and neural activity, as these factors can decode the induction conditions of stress-induced anhedonia, as well as the underlying biological pathology, propelling us towards a future of individualized medicine.

Keywords: Valence, Anxiety and Depression, Medial Prefrontal Cortex

Disclosure: Nothing to disclose.

P96. The Hippocampal Spatial Code is Dynamically Altered During Context Fear Discrimination

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Background: Assessing a situation as either threatening or safe is critical for our survival. However, this process can be impaired in individuals with post-traumatic stress disorder (PTSD). Consequently, neutral environments may not be discriminated from a traumatic memory and lead to re-experiencing the psychological distress of the original trauma. One theory for the pathophysiology of PTSD is impaired context processing. Prior work indicates that contexts are represented in the hippocampus (HPC) by ensembles of 'place cells' that collectively construct a spatial map. Although the HPC is essential for episodic memory, the dynamics of the HPC spatial representation during the formation of an aversive memory, and the retrieval of a threatening or neutral memory, are not fully understood.

Methods: To investigate context fear discrimination we used one-photon microendoscope calcium imaging in freely behaving male C57Bl/J6 mice ($n = 8$-11) during a novel context memory retrieval task. Mice received surgical AAV injection to express the calcium indicator GCaMP6f in CaMKII+ cells of HPC CA1; a GRIN lens was later implanted for imaging. A customized fear conditioning apparatus composed of audio speakers and a cylindrical LED screen (90 cm dia.) presented two different contexts, A and B, while mice remained in the same physical space. On Days 1 and 2, mice were placed in neutral context B in the AM and fear conditioned with footshock in context A in the PM. On Day 3 mice were placed in the apparatus for 12 minutes and context A and B presentations were repeatedly alternated at 2-minute intervals. Throughout the task HPC activity and freezing behaviour were recorded. To investigate spatial encoding, spike likelihoods were calculated from neuronal calcium transients and place cell rate maps were generated. For each mouse, neuronal activity was correlated with a population vector of the mouse's place cell ensemble activity. This population vector correlation was used to assess the strength of the HPC spatial map at a given moment. A separate group of non-conditioned control mice ($n = 4$) underwent similar behavioural and CA1 recording procedures. Data analysis was performed with Matlab and statistical significance was calculated using Goodness-of-fit tests, non-parametric tests, Pearson correlation, and within-subject comparisons to shuffled controls.

Results: We report three primary findings in our fear conditioning task: (1) On Day 1 fear conditioned mice significantly increased freezing ($p < 0.01$ Friedman) and were different from non-conditioned controls ($p < 0.01$ Kruskal-Wallis). Conditioned and non-conditioned mice had significantly different freezing on Day 2 conditioning ($p < 0.01$ Kruskal-Wallis). On Day 1 the HPC spatial map during fear conditioned context A was significantly remapped compared to neutral context B ($p < 0.01$ Kolmogorov-Smirnov; $p < 0.05$ Wilcoxon signed-rank for context A). (2) During Day 3 context "teleportation" freezing behaviour significantly changed during test ($p < 0.01$ Friedman) and was different between conditioned and control mice ($p < 0.01$ Kruskal-Wallis). The HPC spatial maps of contexts A and B were significantly altered during context transitions ($p < 0.05$ Wilcoxon signed-rank for conditioned mice). (3) During the time surrounding context transitions, stronger context A spatial maps were correlated with greater freezing and stronger context B maps were associated with less freezing (Pearson correlation $p < 0.05$).

Conclusions: These results reveal the temporal relationship between the HPC spatial map and memory formation/retrieval

processes. Despite an unchanged context, formation of an aversive memory destabilized and altered the spatial map. Moreover, context alteration in the "teleportation" studies illustrated the dynamic relationship between spatial map strength and fear memory expression. These studies bridge the field of spatial navigation with emotional behaviour and introduce a task to identify circuits of safe and dangerous context representations that may guide the development of targeted therapeutics for PTSD.

Keywords: Contextual Fear, Fear Generalization, Hippocampus, PTSD, Spatial Memory

Disclosure: Nothing to disclose.

P97. Circuit-Reorganization and Transformation for Observational Contextual Fear Memory

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Background: The formation and maintenance of long-term memories for episodes are important for daily life. A feature of episodic memory is autobiographical; requires own experience accompanied with representation of the self in an episode and preserves at first-person viewpoint, which allow to distinguish own episode from other's episode. While it has been speculated that the self-reference effect may be closely related to the transformation process from episodic memory to semantic memory in human, it remains unknown about the neural circuit mechanisms for the time-limited self-reference effect on memory recall.

Methods: To direct examine the self-reference effect on fear memory recall in mice, we have established observational contextual fear conditioning (observational CFC) paradigm and examined the neural circuit mechanisms for recent (1 day) and remote memory (28 days) recall for observational CFC in the observer side and demonstrator side. Because, the observer mice showed the freezing behavior in the observer side but not in demonstrator side at recent time point, while the observer showed the freezing behavior in both at remote time point. Importantly, the enhanced freezing response in the demonstrator side is not due to the fear generalization. This behavioral model is suitable to evaluate the time-limited self-reference effect on observational fear memory recall. 10-15 weeks C57BL6J mice were used in the observer and the demonstrator. Experimenters were blinded with respect to the genotype of the mice and the experimental designs were counterbalanced. Equal numbers of animals from each experimental group (i.e. genotype, sex, age) were assigned. Sample size determination: Using data from our previous reports (Terranova et al., 2022, Neuron), we performed a power analysis with effect size at 0.9 and alpha value of 0.05 yielding a group size of 10 mice. Based on our previous studies (Kitamura et al., 2017, Science, Terranova et al., 2022, Neuron), we determined 6 mice per a group for histology. Statistical analysis: Comparisons between two-group were analyzed by unpaired t test or paired t test, if normally distributed, otherwise by Wilcoxon sum rank or signed-rank test. Multiple group comparisons were assessed using a one-way, two-way, or repeated-measures analysis of variance (ANOVA), followed by the post-hoc test when significant main effects were detected. The null hypothesis was rejected at the $P < 0.05$ level.

Results: We found that dorsal hippocampal activity is crucial for the recent memory recall of observational CFC in the observer side ($U = 13$, $P = 0.004$, Mann-Whitney U test), while medial prefrontal cortical (mPFC) activity is essential for the remote memory recall of observational CFC in both observer and demonstrator sides ($t19 = 2.42$, $P = 0.03$, unpaired t-test). Consistently, the recent memory recall of observational CFC in the observer side increased Arc expression in dCA1 ($t8 = 2.69$, $P = 0.03$, unpaired t-test). but not mPFC ($t8 = 0.74$, $P = 0.48$, unpaired t-test), while the remote memory recall of observational CFC increased Arc expression in mPFC in both observer ($t8 = 3.13$, $P = 0.01$, unpaired t-test) and demonstrator side ($t10 = 3.32$, $P = 0.01$, unpaired t-test), but not dCA1. Optogenetic terminal inhibition further demonstrated that mPFC-BLA pathway is crucial for remote memory recall of observational CFC.

Conclusions: Observational CFC memory is long-lastingly maintained. While observational CFC requires the dorsal HPC (dHPC) at recent time point, observational CFC requires the mPFC-basolateral amygdala (BLA) pathway at remote time point. Furthermore, we revealed that recent observational CFC memory can be only egocentrically retrieved, while remote observational CFC memory can be retrieved as both egocentric and allocentric recall depending on the animal's context. Finally, we demonstrated that mPFC neural activity during observational CFC is necessary for the successful recall of both egocentric and allocentric observational CFC memory at the remote time point.

Keywords: Dorsal Hippocampus, Medial Prefrontal Cortex, Observational Learning, Fear Conditioning, Mouse Models

Disclosure: Nothing to disclose.

P98. Cholinergic Interneuron Control of Cue-Dependent Salience and Aversive Learning

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Background: Surviving harmful environmental stimuli requires encoding for appropriate response to future threat. The Nucleus Accumbens (NAc) is a hub for processing motivational information, including aversive events, and dictates response to these salient events. Highly salient, aversive events promote phasic changes in striatal cholinergic interneuron (ChI) firing, which may contribute to learning. In addition, substance P is known to enhance ChI activity over the timescale of seconds to minutes, an increase that is observed following a pause in activity in response to aversive stimuli. Subsequent downstream signaling by enhanced ChI activity through substance P activation promotes plasticity on NAc medium spiny neurons (MSNs) via muscarinic 1 receptors, which may provide a synaptic substrate for learning about these salient, aversive events. Therefore, we aimed to test the hypothesis that enhanced acetylcholine release by substance P predicts and supports associative learning of salient aversive events.

Methods: A Pavlovian fear conditioning paradigm was used to study aversive learning in mice and freezing in response to the associative cue was used as the primary measure of learning. To determine selective release dynamics of acetylcholine, mice were injected with an adeno-associated virus expressing the genetically encoded acetylcholine sensor GRAB-ACh3.0 in the NAc core and in vivo signals were measured using fiber photometry in wild-type C57Bl/6J mice during various phases of fear conditioning and recall. All GRAB-ACh signals were normalized by z-scoring and changes were quantified using the area under the curve. To determine acetylcholine responses to salient environmental features, mice received foot shocks at varying intensities (0.1-1.0 mA) at random intervals. To further test the role of acetylcholine in salience, a subset of mice underwent latent inhibition procedures to diminish salience of aversive cues. To assess the role of substance P in

mediating acetylcholine signals and aversive learning, mice were injected with L-733,060, an antagonist of the primary receptor for substance P, the neurokinin 1 receptor (NK1R). In addition, a ChI-Cre transgenic mice were injected with a NK1R guide RNA in the presence of Cas9 for ChI-selective, conditional knockdown of the NK1R. Lastly, to determine the necessity of acetylcholine signaling in cue-dependent learning, the NAc of mice were directly infused with pirenzepine, a muscarinic 1 receptor-like antagonist via a NAc-implanted cannula.

Results: Cue-dependent fear conditioning produced a multiphasic response including activity in response to a novel cue, a decrease in release following a foot shock (pause), and a lasting increase in activity (1-2 sec) following the pause. Shock alone produced an identical pause followed by an increase in release as was observed during conditioning, which indicates salient stimuli in the absence of cues produces these phasic changes in acetylcholine. Increasing shock intensity caused increased acetylcholine release ($F(3,28) = 3.420$, $p < 0.05$) and the response diminished over subsequent cue exposures ($F(1,28) = 6.793$, $p < 0.05$), suggesting this enhanced acetylcholine release signals salience. NK1R antagonist treatment blocked the increase in activity at 0.6 mA ($t(9) = 2.017$, $p < 0.05$), indicating substance P signaling may be responsible for encoding salience of the aversive stimulus. Cue recall was significantly diminished by NK1R treatment ($t(40) = 3.801$, $p < 0.001$), NK1R CRISPR knockout ($t(26)=3.332$, $p < 0.01$), and latent inhibition ($t(10) = 2.170$, $p < 0.05$). In all treatment cases, motor responses did not explain the change in freezing. In addition, latent inhibition ($t(10) = 3.886$, $p < 0.01$) and NK1R antagonist treatment ($t(13) = 2.140$, $p < 0.05$) suppressed rebound cholinergic activity without affecting the pause during cue recall. Lastly, pirenzepine diminished cue recall when administered directly in the NAc prior to conditioning ($t(17) = 3.253$, $p < 0.01$), but not prior to cue recall ($t(11) = 1.220$, $p > 0.05$).

Conclusions: These results provide evidence for a NAc core microcircuit that requires substance P and acetylcholine release for plasticity underlying cue-dependent aversive learning. Additionally, this work implicates substance P signaling, and not necessarily intrinsic mechanisms, in the ChI firing rebound during aversive events and cue learning, which signals salient environmental features. This work reveals a mechanism for a peptide-acetylcholine microcircuit that shapes response to aversive events by dictating salience of stimuli. These results could have broader implications for processing stressful events driving anxiety and depression.

Keywords: Salience, Acetylcholine, Neuropeptides, Aversive Learning, Ventral Striatum

Disclosure: Nothing to disclose.

P99. Decoding Stress Susceptibility From Activity in Amygdala-Ventral Hippocampal Network

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Background: Major depressive disorder is characterized by network-level functional alterations, with the amygdala (AMY)-ventral hippocampus (vHPC) pathway as well as their connected regions being central components. However, how changes in emotional state are represented in the activity patterns of single neurons and populations in the AMY-vHPC network remains unclear.

Methods: Here, using high-density Neuropixels probes, we recorded single unit activity across the AMY-vHPC network, including AMY and vHPC subregions, as well as medial prefrontal cortex (mPFC), and thalamus. This allowed us to investigate how stress modulates representations of emotional information in the AMY-vHPC circuit and the effects on reward-seeking behavior. Mice (males and females, total $n = 60$) were first subjected to chronic social defeat stress, a well-validated rodent paradigm that stratifies stressed mice into those that exhibit a depression-like behavior (susceptible) vs. those that do not (resilient). After defeat, we recorded single unit activity across a AMY-vHPC-mPFC-thalamic network as mice performed a head-fixed sucrose preference test where they freely choose between consuming water vs. sucrose rewards, a classic test of anhedonia-related behavior. Statistical significance was assessed using two-way ANOVA followed by post-hoc tests where appropriate. If data were significantly non-normal or variances were significantly unequal, non-parametric tests including Kruskal-Wallis, Mann-Whitney, and Wilcoxon signed-rank tests were used.

Results: Our results show that sucrose preference after defeat was correlated with social interaction ratio ($P = 0.0002$), a standard measure of stress susceptibility. At the single neuron level, we found that spiking activity was greatly elevated during reward consumption in control (non-stressed) mice across the AMY-vHPC -mPFC-thalamic network, but stressed mice showed region-specific changes in firing patterns, including decreased activity in AMY and vHPC neurons ($P < 0.0001$), and elevated firing rates in mPFC neurons ($P < 0.0001$). Interestingly, despite reduced sucrose preference behaviorally in the stressed mice, neurons in AMY and vHPC showed increased reward selectivity. To probe this phenomenon further at the population level, we used support vector machine classifiers to decode reward representations. We found that in stressed mice, future reward choice (sucrose or water) could be decoded seconds before the choice was made in the AMY, mPFC, and vHPC neurons ($P < 0.0001$), suggesting a rigidity in future reward representations. Furthermore, in susceptible mice, neurons in these regions showed smaller change in decoding accuracy from pre- to post-reward ($P < 0.0001$), suggesting reduced reward sensitivity in comparison to controls and resilient mice. We next investigated how stress alters interaction between brain regions. We found that coordinated activity between AMY and vHPC neurons is correlated with individual animal's sucrose preference ($P = 0.003$), suggesting that reduced AMY-vHPC interaction may underlie anhedonia. In line with this, chemogenetic activation of AMY-vHPC pathway rescued anhedonic behavior in susceptible mice ($P = 0.01$).

Conclusions: Together, our results show that chronic stress alters single neuron firing patterns and population representations of reward in the AMY-vHPC network, and these changes may be responsible for ultimately driving anhedonic behavior following stress.

Keywords: Mood Disorders, Chronic Social Defeat Stress, Neural Circuit and Animal Behavior, Neural Decoding, Reward Representation

Disclosure: Nothing to disclose.

P100. A Thalamo-Ventral Hippocampal Circuit That Supports Anxiety-Related Behavior

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Background: Recent work has revealed the hippocampus (HPC), which has classically been implicated in learning, for its role in encoding emotionally charged environments and generating appropriate behavioral responses. More specifically, emerging evidence from our lab has identified HPC neurons in the ventral

pole that project to the hypothalamus encode anxiogenic environments and bidirectionally modulate avoidance behavior. Yet how and which inputs are involved in the generation of anxiety-related representations in the ventral HPC remain unknown. Using a whole-brain cell-type specific anatomical screen, we identified the anterior portion of the paraventricular nucleus of the thalamus (PVT), an area implicated in the processing of salient emotional stimuli, as a putative source of anxiety-related information to the HPC. In this study, we dissect the anatomical organization of the PVT-vHPC circuit and its contribution to anxiety-related behavior.

Methods: We used a Cre-dependent strategy to label vHPC-projecting cells of the paraventricular thalamus (PVT) with the inhibitory DREADD receptor hM4Di. We injected retroAAV-Cre in vHPC and a Cre-dependent chemogenetic inhibitor, hM4Di, into PVT, both bilaterally. Four weeks later we injected animals with CNO (3mg/kg) 15 minutes before running the elevated plus maze and open field test. To assess the collateral projections of vHPC-projecting cells in PVT, we used anatomical tracers (a Cre-dependent expression of Synaptophysin-mRuby in PVT and retroAAV-Cre in vHPC) and viral barcoding approaches. Four weeks later, animals were sacrificed, brains extracted, followed by whole-brain histology to identify axon fibers to other brain regions. We then sought to identify how PVT input differs from other inputs to vHPC during behavior with dual fiber photometry. We injected a retrograde jGcAMP8m in vHPC then implanted optical fibers over both the PVT and Basal-lateral amygdala (BLA) to record BLA-vHPC and PVT-vHPC neurons simultaneously. Four weeks later we recorded calcium signals in PVT and BLA while animals explored the elevated plus maze and open field.

Results: Inhibition of PVT-vHPC pathway resulted in reduced time in the open and center of the elevated plus maze ($p < 0.05$) and reduced time in the center of the open field test ($p < 0.05$). In our synaptophysin experiment we identified axon fibers to the nucleus accumbens, central amygdala, bed nucleus of the stria terminalis, and zona incerta. Dual fiber photometry recordings revealed differential responses of BLA-vHPC vs aPVT-vHPC in the elevated plus maze and open field test, indicating these two areas convey different information to vCA1 during exploration of anxiogenic environments.

Conclusions: Here, we identify a role for PVT-vHPC projections in modulating anxiety-related behavior. These PVT-vHPC projections collateralize to the amygdala and nucleus accumbens suggesting that broadcasting PVT-vHPC neurons may play an important role in orchestrating behavioral responses to threatening stimuli. Responses in PVT-vHPC neurons to anxiogenic compartments of the elevated plus maze differed from that of BLA-vHPC, which showed consistent increases in activity when animals explored anxiogenic areas. This indicates differential, specialized processing of anxiogenic information PVT-vHPC neurons when compared to the well-studied BLA-vHPC pathway.

Keywords: Ventral Hippocampus, Paraventricular Nucleus of the Thalamus, Anxiety Circuitry

Disclosure: Nothing to disclose.

P101. Dopamine Release at the Time of a Predicted Aversive Outcome Causally Controls the Trajectory and Expression of Conditioned Behavior

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Background: The execution of adaptive behavior depends on the ability of organisms to predict potential threats in their environment. To this end, animals learn to predict when aversive stimuli will occur and learn what actions are necessary to avoid contexts and situations with potential negative outcomes. However, equally important, is the ability to learn when aversive stimuli are not likely to be presented. This balance allows animals to avoid potential negative outcomes while still exploring their environment when it is safe and unlikely to result in harm. Dopamine release in the nucleus accumbens (NAc) has been causally linked to adaptive aversive learning and its dysregulation is a core phenotypic characteristic of anxiety and stress disorders. Thus, understanding the role of dopamine in aversive learning is important for both understanding neuromodulatory signaling in the brain as well as how its dysregulation is important in psychiatric disease states.

Methods: We employed an aversive conditioned inhibition design combined with fiber photometry and optogenetics to test the role of NAc dopamine in aversive learning. In this task, a target cue was presented in isolation (to predict the presentation of a footshock) or in combination with a secondary cue (which predicted that footshock would be omitted on the current trial). We recorded dopamine response to the cues that predict the presence and absence of aversive footshocks using a fluorescent dopamine sensor (dLight1.1). Then, we optogenetically manipulated the dopamine responses during the absent footshocks to show causal relationship between NAc dopamine and conditioned inhibition learning. Finally, we used a theory-driven computational model of associative learning (Kutlu-Calipari-Schmajuk, KCS, model) in order to explain our results and derive novel predictions regarding the role of dopamine in aversive learning.

Results: Using optical approaches to directly record and manipulate dopamine release in the NAc in awake and behaving animals, we show that dopamine responses evoked at the time of omitted aversive outcomes are causal to the expression of conditioned responses. The magnitude of the dopamine response at the time of an omitted footshock scaled positively with the prediction of the aversive outcome on that trial; however, this effect was only apparent at the time of the omitted outcome, but not in response to the predictive cue itself. Importantly, we show, via optogenetic manipulation of this signal, that dopamine in this context is not transmitting an error-based signal, as enhancing this signal disrupted learning rather than enhancing it. Finally, using the KCS model, we showed that these effects can be explained by dopamine signaling the perceived saliency of predicted aversive events, but not prediction errors.

Conclusions: Together, we show that NAc core dopamine responses to expected but omitted aversive stimuli causally determine associative learning for aversive stimuli in mice. These results add to the growing literature supporting the dopaminergic encoding of perceived saliency by dopamine in the NAc. Our conclusions regarding the dopaminergic information encoding in associative learning also have important clinical implications for anxiety and stress disorders. In sum, these results have far-reaching implications for the theory of learning and memory, the understanding of the mesolimbic neurocircuitry, and the psychopathology of anxiety and stress disorders.

Keywords: Dopamine, Safety Learning, Aversive Learning

Disclosure: Nothing to disclose.

P102. Ultrasonic Vocalization Patterns Reveal a Sex-Dependent Dimension of Fear Conditioning

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Background: Pavlovian fear conditioning is a widely used paradigm for investigating the neural mechanisms that mediate aversive learning. However, the behavioral outcome metric has historically been the singular "freezing" response, despite the complex nature of the task and multi-dimensional fear responses animals can exhibit. Particularly in light of increased consideration of sex as a biological variable, expanding the repertoire of our behavioral observations in this classic task will be critical to a more nuanced understanding of learning and memory processes.

Methods: Male (n = 100) and female (n = 100) Sprague Dawley rats underwent auditory cued fear conditioning in standard chambers, with either 1.0mA, 0.5mA, 0.3mA, or no unconditioned stimulus. Some animals also underwent extinction learning and recall procedures on consecutive days. Locomotor behavior was recorded using overhead cameras and tracked using Ethovision software. Vocalizations were recorded and analyzed using DeepSqueak software. Some animals were euthanized 90 minutes after fear conditioning concluded, and areas of the prefrontal cortex, periaqueductal gray (PAG), and spinal cord were immunostained for c-fos expression.

Results: We observed significant main effects of sex and shock intensity in the number of alarm calls elicited during fear conditioning. Males exhibited significantly more alarm calls than females, and these calls also persisted for longer across the training session in males compared to females. The propensity to make alarm calls tracked shock intensity and corresponded to freezing behavior in males but not females. Alarm calls emerged later when CS-US presentations were explicitly unpaired than when paired, suggesting that alarm calls may reflect associative learning. However, alarm calls could be observed during ITIs as well as during CS presentation. We also observed sex differences in correlations between c-fos activity and both conditioned and unconditioned responses.

Conclusions: This work provides novel evidence that males and females process a Pavlovian fear conditioning session differently, both in several dimensions of behavior, as well as in the activity of key brain regions. Our data hold important implications for both basic scientists who use Pavlovian fear conditioning to study learning and memory processes, as well as for translational neuroscience hoping to use rodent models to understand aversive learning-related disorders like PTSD.

Keywords: Fear Conditioning, Sex Differences, Ultrasonic Vocalization, Periaqueductal Grey (PAG), Prefrontal Cortex

Disclosure: Nothing to disclose.

P103. Investigating the Role of Nucleus of the Solitary Tract Projections to the Central Amygdala in Mediating Hyperarousal Symptoms After Traumatic Experience

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Background: Persistent hyperarousal symptoms, including exaggerated startle responses and increased sympathetic tone, are a hallmark of post-traumatic stress disorder. We hypothesize that interoception of sympathetic activity directly regulates threat responses to promote and maintain hyperarousal. Noradrenergic neurons in the nucleus of the solitary tract (NTS) receive ascending input from major organ systems and send noradrenergic/glutamatergic projections to the central amygdala (CeA), a key region for orchestrating responses to threats. We propose that traumatic experience alters NTS activity to promote exaggerated threat responses.

Methods: Hyperarousal symptoms were induced with the Traumatic Experience with Reminders of Stress (TERS) paradigm, consisting of a single exposure to a traumatic footshock (10 s, 2 mA) followed by six brief, intermittent exposures to a contextual reminder of the traumatic experience over 24 days. Male and female C57Bl6/J mice were randomly assigned to Control (n = 8) or TERS (n = 22) groups. Acoustic startle responses, resting heart rate, and sociability were measured before and after the trauma paradigm.

Population activity of NTS noradrenergic neurons was examined by injection of AAV5-CAG-FLEX-GCaMP6f in the NTS of TH-Cre mice (n = 2) and implantation of an optical fiber for photometry.

Chemogenetic activation of NTS noradrenergic input to the CeA was achieved by injection of AAV5-hSyn-DIO-hM3dGq in the NTS of TH-Cre mice (n = 2) and local application of DREADD agonist deschloroclozapine/vehicle via bilateral cannulae in the CeA.

Results: Mice that underwent the TERS paradigm had enhanced acoustic startle (p = .006, Welch's t-test) and elevated heart rate (p = .009, Mann-Whitney test) compared to unshocked controls. Based on the acoustic startle response, the traumatic exposure group can be subdivided into a susceptible group with significantly elevated startle responses, and a resilient group with startle responses like control. Sociability did not differ between groups (social interaction events p = .56; social interaction time p = .86, Welch's t-tests). No sex differences were observed in any measure.

Preliminary fiber photometry data suggest that startling and aversive stimuli elicit calcium responses in NTS noradrenergic neurons. Additionally, chemogenetic activation of NTS noradrenergic axon terminals in the CeA potentiates the acoustic startle response in naive mice (p = .05, paired t-test).

Conclusions: Traumatic experience, paired with situational reminders, elicits hyperarousal symptoms in male and female mice. Noradrenergic neurons within the NTS are likely activated during salient or aversive stimuli, and activation of noradrenergic NTS projections in the CeA potentiates startle. These findings suggest that the NTS may regulate threat responses by its projections to the central amygdala.

Keywords: Hyperarousal, Central Nucleus of the Amygdala, Noradrenergic System, Nucleus Tractus Solitarii

Disclosure: Nothing to disclose.

P104. Offensive Attack Initiation Triggered by an Amygdala CRH + Cell Activity Signature

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Background: Stress-induced psychopathologies like posttraumatic stress disorder can severely disrupt emotion regulation and increase reactivity – in some patients, this manifests as an increase in aggression. The central amygdala (CeA) is a point of intersection for neural circuits that modulate both threat responding and aggression. In our present work, we employed mouse models of naturalistic agonistic behavior to examine the neural bases of offensive (i.e., territorial) and self-defensive attacks. We used single-cell calcium imaging and chemogenetics to evaluate CeA neurons that express the well-characterized stress-signaling neuropeptide, corticotropin releasing hormone (Crh).

Methods: Male CRH-ires-Cre mice received intra-CeA AAV5-EF1a-DIO-GCaMP6s and were implanted with a gradient index lens to record calcium-dependent activity in Crh+ CeA cells. Cell activity (n = 16-32 cells/mouse) was video-recorded using a

miniature microscope during 5-minute home-cage offensive aggressive encounters with a submissive intruder mouse or during 1-minute self-defensive interactions with an aggressive conspecific. Crh+ CeA cell ensembles were identified based on their activity (3 z-score peak event threshold) relative to offensive attack initiation. Additional CRH-ires-Cre males received intra-CeA AAV5-hSyn-DIO-hM4D(Gi)-mCherry to drive inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) or control virus in Crh+ CeA neurons. Five minutes before offensive or self-defensive aggression trials, mice were injected systemically with vehicle or deschloroclozapine (0.02 mg/kg). Behavioral videos were collected from each trial and analyzed using a supervised machine learning pipeline. Specifically, animal tracking was conducted in multi-animal deeplabcut (maDLC; Lauer et al. 2022). Pose estimations generated by maDLC served as the input for SimBA (simple behavioral analysis; Nilsson et al. bioRxiv) which employed random forest classification to identify social approach, social contact and attacks. Sequences of classified behaviors were identified to quantify behavioral transition states, e.g., the probability of transitioning from social approach to social contact to attack.

Results: Crh+ CeA cells (n = 85) were classified into three ensembles – 41% were active in the 10 seconds before offensive attack initiation, 32% were active during attacks, and 27% were inactive during aggression. Chemogenetic inhibition of Crh+ CeA cells selectively prevented offensive attacks without affecting social approach, contact, or adaptive self-defensive bites toward an attacking intruder.

Conclusions: CeA Crh+ cell activity is necessary for the transition from social contact to the initiation of offensive attacks. CeA Crh+ cell activity was not necessary for self-defensive bites, suggesting distinct neural circuitry underlying offensive vs. self-defensive aggression. All-optical closed-loop approaches are presently being used to block attack initiation using Crh+ CeA cell activity to remotely trigger real-time inhibitory optogenetics.

Keywords: Aggression, Amygdala, Corticotropin-Releasing Factor (CRF), in Vivo Calcium Imaging, Chemogenetics

Disclosure: Nothing to disclose.

P105. Basolateral Amygdala Reactivity and Functional Connectivity Over Time Following Trauma: Evidence for a Dynamic Model

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Background: Amygdala hyperreactivity early post-trauma is a demonstrable physiological correlate of future posttraumatic stress disorder (PTSD). Two areas of the amygdala are particularly known to play a part in the threat response and PTSD – the basolateral amygdala (BLA) which is involved in threat processing and memory, and the central amygdala (CeA) which is involved in autonomic threat responses. However, there is little evidence regarding how the dynamics of amygdala reactivity over time relate to longitudinal PTSD symptomology. Two opposing hypotheses are that (1) trait differences in amygdala threat response predict PTSD and persist over time, or (2) dynamic changes in threat response over time impact symptoms. Exploring how reactivity and functional connectivity in the BLA and CeA persists or changes over time following trauma may allow for better understanding of the physiology of PTSD and future treatment options.

Methods: As part of a larger multisite study, "AURORA", participants were enrolled in the emergency department (ED) within 72 hours of a traumatic event. Participants experienced an event that involved actual or threatened serious injury, sexual violence, or death, either by direct experience, witnessing it, or learning about it. Participants were between the ages of 18-75 and English speaking. PTSD symptoms were assessed using the PTSD Symptom Checklist for the DSM-5 (PCL-5) at 2 weeks and 6 months post-trauma. Left and right basolateral amygdala (BLA) and central amygdala (CeA) responses to threat (i.e., fearful>neutral faces) during fMRI were collected 2 weeks (N = 305, 199 female) and 6 months post-trauma (N = 100, 69 female). Contrast values for fearful > neutral faces for the four regions of interest (ROIs) were tested for correlations with 2-week and 6-month PTSD symptoms. Follow-up task-based functional connectivity (FC; fearful>neutral faces) was analyzed for regions showing significant associations in the ROI analyses using Conn Toolbox with a voxel threshold of 0.005 to allow for a final FWE-corrected cluster threshold of 0.05.

Results: Left BLA response at 6 months post-trauma negatively correlated with both 2-week ($r = -.21$, $p = .04$) and 6-month PTSD symptom severity ($r = -.27$, $p = .006$). Similarly, a decrease in left BLA reactivity from 2 weeks to 6 months predicted 6-month PTSD symptom severity ($r = -.20$, $p = .04$). There were no correlations between PCL-5 scores and CeA or right BLA reactivity at either 2 weeks or 6 months. Follow-up FC analyses were then performed with the left BLA as a seed region. At 2 weeks, current PTSD symptoms were associated with more positive FC with the cerebellum and more negative FC with the anterior and posterior cingulate, the left middle frontal gyrus, and the left supramarginal gyrus. At 6 months, current PTSD symptoms were associated with more positive FC with the posterior cingulate gyrus, the left inferior temporal gyrus, and the left cerebellum, and more negative FC with the right temporal pole and the right superior frontal gyrus.

Conclusions: Findings suggest that PTSD symptoms persist in individuals who show a strong decrease in left BLA reactivity to threat over the months following trauma, supporting a dynamic model of threat response changes post-trauma in PTSD. The left BLA may experience a form of exhaustion in those with chronic PTSD, potentially due to the downregulation of norepinephrine or glutamate receptors in response to chronic stress. FC results suggest positive left BLA-cerebellum connectivity is consistently linked with higher PTSD severity across time points, while connectivity associated with higher PTSD severity between the left BLA and areas involved in the default mode network (posterior cingulate gyrus; anterior cingulate) starts as negative at 2 weeks and changes to positive by 6 months. This suggests the default mode network may play a major role in left BLA reactivity changes for those with higher PTSD severity. Findings support the importance of affective dynamics in PTSD risk and the need for further longitudinal neuroimaging studies.

Keywords: PTSD, fMRI, Basolateral Amygdala, Central Amygdala, Longitudinal MRI

Disclosure: Nothing to disclose.

P106. Serotonergic Effects of Viloxazine Measured Using 5-HT2 Agonist Radioligand, [11C]CIMBI-36, in Cynomolgus Monkeys: A PET Imaging Approach

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Background: Viloxazine ER (viloxazine extended-release capsules; Qelbree®), is a novel, non-stimulant FDA-approved treatment for

attention-deficit/hyperactivity disorder (ADHD) in children (≥6 years) and adults. Viloxazine therapeutic effects in ADHD have been ascribed to norepinephrine reuptake inhibition; however, its moderate potency (Ki=0.630 μM) compared to traditional NRIs (e.g. atomoxetine Ki=0.005 μM) for binding in cells expressing human norepinephrine transporters (NET) suggests alternate mechanisms of action may be involved. Emerging in vitro data show viloxazine has moderate affinity for human isoforms of serotonin (5HT)- receptors [5-HT2C (Ki=1.5 μM), 5-HT7 (Ki=1.9 μM) and 5-HT2B (Ki=3.4 μM)], but no expected clinically relevant effect at 5-HT reuptake transporters SERT (Ki =17.3 μM). Moreover, newly completed in vivo microdialysis experiments in rats are showing viloxazine significantly increases 5-HT levels in the rat prefrontal cortex at interstitial fluid (ISF) concentrations associated with clinically relevant plasma concentrations, an effect that cannot be explained by the NET inhibition alone. To build upon these newly emerging data and further elucidate viloxazine's effects on serotonergic neurotransmission in vivo, we conducted a positron emission tomography (PET) imaging study in cynomolgus monkeys. For this study, the radioligand agonist (for 5-HT2A and 5-HT2C), [11C]CIMBI-36, was used to assess the ability of viloxazine to induce changes in extracellular serotonin levels in cortical regions and to occupy 5-HT2C receptors in the choroid plexus

Methods: Four anesthetized cynomolgus monkeys were administered bolus infusions of viloxazine (3, 6, and 12 mg/kg), with a 6-week resting period between scans. PET scans were performed at baseline and 30 minutes after infusion (1-2 scans per dose, 123 minutes per scan). [11C]CIMBI-36 was administered via bolus infusion immediately prior to initiation of the scanning period. During image acquisition, arterial blood samples were drawn at eight timepoints 2-90 minutes post-scan initiation for quantification of [11C]CIMBI-36 metabolite. Plasma concentrations of viloxazine were measured in blood samples collected at four timepoints 15-120 minutes post-administration of viloxazine. PET images were analyzed using spatial processing to enable automated definition of regions of interest (ROIs). ROIs analyzed included frontal cortex, choroid plexus, and cerebellum. The choroid plexus was manually delineated. To estimate total volume of distribution (VT) or the ratio of [11C] CIMBI-36 in a target region, time-activity curves were estimated from the data using the established multilinear analysis (MA1) modeling method. From the calculated VT values, non-displaceable binding potential (BPND) was computed for each ROI using the cerebellum as a reference region. The effect of viloxazine on the displacement of the radioligand at 5-HT receptors is evaluated based on the change in BPND between baseline and post viloxazine treatment.

Results: Administration of 3 mg/kg (n = 1), 6 mg/kg (n = 2), and 12 mg/kg (n = 2) of viloxazine produced respective reductions in BPND in the choroid plexus of 60%, 76% and 100% (the latter representing complete blockade of [11C]CIMBI-36 binding). These same doses induced changes in BPND of 25%, 15-32%, and 38%-42%, respectively in the cortical regions. At 3 mg/kg, the unbound viloxazine plasma concentration (Cmax) was 3.9 μM, which is close to the range of 2.2-3.3 μM unbound viloxazine plasma concentration (Cmax) observed at the therapeutic dose of 400 mg/day viloxazine ER in children between 6 to 17 years old.

Conclusions: Our results establish a dose-dependent binding effect for viloxazine at the 5-HT2C receptor in the choroid plexus. The 60% displacement of the radioligand by viloxazine at a clinically relevant dose (3 mg/kg) may be attributable to direct occupancy of the 5-HT2C receptor by viloxazine. In the cortical regions, a dose-dependent displacement of [11C]CIMBI-36 by viloxazine was also seen. The effect of viloxazine in the cortical regions (rich in 5-HT2A receptors) may be attributable to either direct occupancy of 5-HT2A or due to increased release of synaptic 5-HT. An estimated EC50 value of viloxazine for the changes in BPND at the 5-H2A receptor is significantly higher than the unbound plasma concentrations of viloxazine at all doses tested. This suggests that the observed effect on radioligand binding may result from a viloxazine-mediated increase in endogenous 5-HT release rather than direct binding to the 5-HT2A receptor in the cortex. Overall, our experiments suggest that, at clinically relevant doses, viloxazine increases serotonergic neurotransmission in the PFC and acts on the 5-HT2C receptor, which could play a role in its efficacy in the treatment of ADHD.

Keywords: ADHD, Serotonin, PET imaging, PFC, Viloxazine

Disclosure: Supernus Pharmaceuticals Inc.: Employee (Self)

P107. Pubertal Stress Alters Future Maternal Behavior in Mice Through Lasting Disruptions to Chromatin and Transcriptional Landscapes in the Hypothalamus

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Background: Adverse childhood experiences, specifically during the pubertal transition, are one of the greatest predictors for affective dysfunction in women. As puberty is marked by dynamic hormonal changes and ensuing reorganization of the brain, it represents a window of sex-specific vulnerability to adverse experiences. We have previously shown that stress during puberty alters the hypothalamic-pituitary-adrenal (HPA) stress axis response in adult female mice only during pregnancy and postpartum. In humans, pubertal adversity led to a blunted HPA response to a maternal separation stressor, which was associated with increased postnatal depression score. We previously examined puberty-stress reprogramming in the paraventricular nucleus (PVN) of the hypothalamus, which initiates the HPA axis response. We found that pubertal stress led to an altered chromatin landscape and transcriptome phenotype in the PVN. Further, we found that the pregnancy-associated hormone allopregnanolone is necessary and sufficient to produce the blunted stress response in pubertally stressed females. It is possible that pubertal stress creates a disorganization of maternal responsiveness and vulnerability to affective dysfunction. To extend our understanding of the risks posed by pubertal stress to alter future behavior, we have begun to examine various aspects of forming and performing maternal behavior. Here, we examined pup-directed behavior both before (pre-maternal) and after pregnancy, as well as further investigated the lasting programming of the stress response by pubertal stress. We hypothesized that pubertal stress would lead to both deficits in the ability to form and display maternal behavior and alterations in the chromatin and transcriptional landscapes of maternal- and stress-related brain regions.

Methods: Mice were exposed to chronic variable stress (CVS) from postnatal day 21–34 using our previously established paradigm. CVS consisted of two stressors (tactile, olfactory, or auditory) per day. In adulthood, virgin females were either left undisturbed (no exposure) or were exposed to novel pups for 2h per day for 4 days (maternal exposure). All females were tested for pup retrieval in a maze 24h following the last maternal exposure, after which they were left undisturbed for two weeks. The pup retrieval task was repeated, and brains were collected. Pup-directed behavior was analzyed both during maternal exposures and during the pup retrieval task (n = 34-35/group). To examine molecular consequences associated with maternal behavior, the medial preoptic area (mPOA) of the hypothalamus was extracted from the brains, RNA was isolated, and quantitative PCR was used to measure gene expression (n = 9-10/group). In another set of mice, males and females were exposed to CVS as above and were left undisturbed until adulthood when pharmacological treatment and brain collection occurred. Mice were given either

allopregnanolone (100ng/200nl per side) or vehicle (25% w/v HP-β-CD) via intra-PVN cannulae 2h before brain collection. RNA from PVNs was isolated and gene expression was measured using quantitative real-time PCR (n = 2-6/group). Data were analyzed by ANOVA with Tukey post-hoc tests when necessary or Chi square.

Results: Adult, pubertally stressed females were more likely to show aggression towards pups throughout exposures and behavioral testing than were control females ($X2(1,69) = 6.031$, $p = 0.014$). In the mPOA, there was a significant interaction between pubertal stress and maternal exposure on expression of Crebbp ($p = 0.0034$). Specifically, there was upregulation of Crebbp in the mPOA of pubertally stressed females who had no maternal exposure compared to control females who had no exposure ($p = 0.0225$). This upregulation was associated with poor performance on the memory task, indicating that pubertal stress disrupted how Crebbp canonically influences memory. In the PVN, preliminary findings from intra-PVN allopregnanolone administration recapitulated prior findings showing that pubertally stressed mice respond differently to allopregnanolone than do control mice at the level of gene expression.

Conclusions: These results provide novel insight into the impact of adversity during puberty on lifelong risk for altered behavior. We found that stress during puberty led to disrupted pup-directed behavior and altered transcription in hypothalamic brain regions that regulate maternal and stress responses. Multiple areas of the hypothalamus are sensitive to pubertal stress, suggesting potentially extensive and lasting consequences of adversity during this developmental stage. Previous and ongoing work implicates disrupted histone acetylation as a potential mechanism of this lasting effect. This translationally-relevant mouse model provides the opportunity to understand the molecular underpinnings of risk for behavioral and hormonal stress dysregulation following pubertal stress.

Keywords: Epigenetics, Adolescent Stress, Risk and Resilience

Disclosure: Nothing to disclose.

P108. Sexual Dimorphisms in Neuronal Structure, Function, and Behavior in a Model System of Autism Spectrum Disorders

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Background: Autism Spectrum Disorders (ASD) are showing increasing prevalence in North America. Individuals are commonly given a standard diagnosis of ASD, despite symptom presentation and severity varying upon the spectrum, and sexual dimorphisms in age of onset, prevalence, etiology, and presentation are also observed. Animal models are often used to study specific aspects of ASD, however females have been historically underutilized, leading to a dearth of knowledge of the possible sex-dependent mechanisms that may underlie the disorders. In this study, we therefore aimed to characterize sexual dimorphisms in neuronal structure and function in the valproic acid model, a model often used for the study of idiopathic ASD.

Methods: Pregnant Sprague Dawley rats were injected with VPA (500 mg/kg) or saline i.p. on gestational day 12.5. To assess alterations in neuronal structure and function in vitro, cortical and hippocampal (HIP) tissues were dissected from postnatal day 0-1 male and female pups. On DIV 21, neurons were stained with microtubule associated protein-2 (MAP-2) for Sholl analysis, or neuronal systems activity evaluated on a multi-electrode array (MEA) (Axion Biosystems) for 30 min. To evaluate sexual dimorphisms in behavior, adolescent male and female rats (PND 31-38) underwent behavioral testing in the three-chamber social test, novel object recognition (NOR), object location (OL), and elevated plus maze (EPM). Following testing, rats were bilaterally implanted with stainless steel electrodes into the medial prefrontal cortex (PFC) and dorsal HIP. Once recovered, local field potential recordings were performed in awake, freely moving animals, for 30 min (N = 10 rats/group). Chronux software for MATLAB was used to evaluate the spectral power at each frequency band within each region.

Results: : Sholl analysis revealed sex-dependent VPA-induced alterations in the mean number of intersections in both the cortical and HIP neurons that were dependent upon the distance from the soma. Only female VPA cortical neurons exhibited reduced length compared to sex-matched controls ($p = 0.029$). When neuronal activity was evaluated, sex-specific alterations in systems function were evident in both the VPA group and control neurons. Control male-derived cortical neurons displayed elevated mean firing and bursting compared to female-derived control neurons ($p < 0.001$), although this was reversed in the VPA neurons ($p < 0.001$). VPA neurons from either sex also displayed elevated firing and bursting compared to their sex-matched controls ($p < 0.001$). Cortical neurons derived from males had a significantly greater synchrony index compared to those from females ($p < 0.001$), although this effect was lost between sexes in the VPA group. Overall, VPA neurons displayed significantly greater synchrony compared to controls [$F(1,62)=58.06$, $p < 0.001$]. In the HIP neurons, the control firing rate of male-derived neurons was greater than those from females ($p < 0.001$), an effect lost with VPA exposure. Only female-derived VPA cells displayed increased firing compared to their sex-matched controls ($p < 0.001$). Unlike the cortex, bursting activity was decreased in male-derived VPA HIP neurons ($p < 0.001$) and increased for the female VPA group ($p < 0.001$) compared to sex-matched controls. No significant group differences were observed for synchrony index. In vivo, behavioural testing in adolescence revealed sex-specific ASD-like characteristics in the VPA group. VPA-exposed female rats displayed greater anxiety in the EPM compared to sex-matched controls ($p = 0.005$), showed deficits in the NOR task ($p < 0.001$), and displayed a deficit in social index scores ($p = 0.007$) although they were more sociable than VPA-treated male rats ($p = 0.014$). VPA males, in contrast, showed deficits in both the OL task ($p = 0.012$) and social index score ($p = 0.036$) compared to male controls. Following behavioural testing neuronal oscillatory activity was examined. Female VPA rats displayed significantly greater PFC delta power ($p < 0.001$) and reduced theta power ($p < 0.001$) compared to female controls and had higher delta ($p < 0.001$) and reduced theta ($p < 0.001$) power compared to VPA males. In high frequency bands, VPA females displayed lower beta, low gamma, and high gamma ($p < 0.001$, $p = 0.012$, and $p < 0.001$ respectively) compared to VPA-exposed males, effects that were not evident between the sexes in the controls. In the HIP, differences were observed selectively in the low frequency bands, delta, and theta, in which VPA females displayed significantly greater delta power ($p = 0.037$) compared to VPA males and had lower theta power ($p = 0.008$) to control females. When coherence was examined between the PFC and dHIP, VPA females had lower delta coherence ($p = 0.029$), while VPA males exhibited no change compared to sex-matched controls.

Conclusions: These preclinical findings identify key sex differences in neuronal structure and function in vitro, and in PFC and HIP systems function and behavior in vivo, in a model commonly used to study ASD. Notably, the impact of VPA exposure in the PFC and HIP was more substantial females exposed to VPA. Overall, these findings demonstrate clear sexual dimorphisms in the VPA model that may have relevance to the sexual dimorphisms observed in ASD. This work also highlights the critical need for employing both sexes when utilizing animal models in the study of neuropsychiatric disorders.

Keywords: Autism, Neuronal Oscillations, Dendritic Arborization, Behavior, Systems Neuroscience

Disclosure: Nothing to disclose.

P109. The Paraventricular Nucleus of the Thalamus Contributes to Early-Life Adversity-Induced Disruptions in Reward-Related Behaviors

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Background: Early-life adversity (ELA) is associated with poor cognitive and emotional health, including an increased risk for a variety of affective disorders, such as depression and substance use disorders. Many of these disorders are characterized by impairments in reward-related behaviors, and we find that these same deficits are provoked by rodent models of ELA. However, the brain regions and processes underlying these long-term consequences of ELA remain largely unknown. The paraventricular nucleus of the thalamus (PVT) is an important node of the reward circuit that encodes remote emotionally salient experiences to influence future motivated behaviors. We hypothesize that the PVT encodes adverse experiences as remote as the early postnatal period in mice, and that ELA-engaged PVT neurons subsequently contribute to alterations in reward-related behaviors in adults.

Methods: We employ TRAP2 mice, which we exposed to a week of simulated ELA in a limited-resource cage between postnatal days 2-9. We induced the TRAP2 system using tamoxifen on P6, triggering Cre-dependent recombination in neurons activated during P6-P8. This leads to permanent labeling of neurons activated during this time frame. We validated our findings using routine cFos immunohistochemistry in WT mice. We then chemogenetically inhibited these ELA-engaged neurons during an adult reward-seeking task with the goal of ameliorating ELA-induced changes in reward-seeking behaviors.

Results: ELA robustly and selectively activates significantly more PVT neurons than typical rearing conditions ($p = 0.0154$, unpaired t-test; $N = 24$), and a large proportion of these ELA-engaged PVT neurons express CRFR1 (40% vs 20% in controls, $p < 0.001$, unpaired t-test; $N = 12$). Silencing ELA-engaged PVT neurons during reward-related tasks in adult female mice ameliorates the observed ELA-induced changes in reward-seeking behaviors ($N = 16$).

Conclusions: The PVT is robustly and almost uniquely activated in response to emotionally salient events in neonatal mice, and inhibition of these ELA-engaged neurons ameliorates ELA-induced changes in reward-seeking. The PVT is thus poised as a potential contributor to deficits in reward-related behaviors following ELA.

Keywords: Paraventricular Nucleus of the Thalamus, Early-Life Adversity, Affective Disorders, Reward Circuitry, Motivated Behaviors

Disclosure: Nothing to disclose.

P110. Adolescent Social Isolation Disrupts Developmental Profiles of GABAergic and Glutamatergic Gene Expression in the Reward Circuitry of Males but Not Females

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Background: Adolescence is a period of reward development and social experience in adolescence has robust effects on reward behaviors. Reduced social connections increases the risk for substance use disorder. Our lab and others have shown that adolescent social isolation (SI) alters cocaine-related behaviors and transcription in adulthood, but little is known regarding how SI disrupts development of the reward circuitry to influence behavior.

Methods: We hypothesized that SI disrupts developmental profiles of GABAergic and glutamatergic systems in 3 brain regions (prefrontal cortex - PFC, basolateral amygdala - BLA and ventral hippocampus – vHIP) to influence adult behavior. Animals were isolated from ~postnatal (P)22 – P42 and then group housed. Male and female mice were euthanized on the day of isolation (P22), mid-isolation (P32), rehousing (P42), and on P72. Punches of PFC, BLA and vHIP were collected for RT-qPCR ($n = 6$-8 animals per group; Total: ~150 animals) of 24 GABAergic and glutamatergic genes. Reward-related behaviors including palatable food consumption and cocaine conditioned place preference were also assessed in a subset of adult males and females.

Results: Preliminary analysis of behavior suggests an increased preference for palatable food and cocaine in males. Three-way ANOVA for sex, SI and age revealed main effects or interactions ($p < 0.05$) for glutamatergic receptor subunits (Gria1 and 2; Grin1, 2a, 2b and 2c) and the glutamate transporter (Slc17a6) in all 3 brain regions. Although region specific effects were observed, post hoc analysis revealed that expression of many glutamatergic genes was enhanced by SI in males on P32. However, GABAergic genes were only affected in the PFC. Three-way ANOVA revealed a significant Sex X Age X SI interaction for GABAA receptor subunits (Gabra1, Gabrg1; $p < 0.05$), GABA transporter (Slc32a1; $p = 0.006$) and Gad2 ($p = 0.001$). Post hoc analysis revealed a significant decrease in expression on P32 in males.

Conclusions: These data suggest that SI results in a sex-specific enhancement of the excitatory system across the reward circuitry in males and a concomitant suppression of the GABAergic system (PFC only). These finding provide a sex-specific mechanism by which SI may reprogram the reward circuitry to influence behavior in adults

Keywords: Mesolimbic Reward Circuitry, Gene Expression, Stress in Adolescence, GABA-A Receptors, NMDA Glutamate Receptors

Disclosure: Nothing to disclose.

P111. Physical and Functional Convergence of the Autism Risk Genes SCN2A and ANK2 in Neocortical Pyramidal Cell Dendrites

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Background: Dysfunction in sodium channels and their ankyrin scaffolding partners have both been implicated in neurodevelopmental disorders, including autism spectrum disorder (ASD). In particular, the genes SCN2A, which encodes the sodium channel NaV1.2, and ANK2, which encodes ankyrin-B, have strong ASD association. Recent studies indicate that ASD-associated haploinsufficiency in Scn2a impairs dendritic excitability and synaptic function in neocortical pyramidal cells, but how NaV1.2 is anchored within dendritic regions is unknown.

Methods: Here, we paired cellular and molecular biology with electrophysiology and two-photon imaging to demonstrate that the protein products of these two ASD risk genes, SCN2A and ANK2, interact in neocortical pyramidal cell dendrites to mutually regulate dendritic excitability.

Results: Using a novel epitope-tagged NaV1.2, we found that NaV1.2 co-localizes with ankyrin-B in the dendrites of mature neocortical neurons. Removal of ankyrin-B eliminated NaV1.2 dendritic localization. Furthermore, dendritic ankyrin-B loss was not compensated for by other ankyrin family members, indicating that ankyrin-B has a unique scaffolding role in this neuronal compartment. Ex vivo studies revealed that Ank2 haploinsufficiency results in intrinsic and synaptic dendritic deficits that closely phenocopy those observed in Scn2a heterozygous neurons.

Conclusions: Thus, these findings suggest that deficits in dendritic excitability may be a common point of convergence in ASD, with direct convergence between two high-risk genes SCN2A and ANK2.

Keywords: SCN2A, Dendrites, Excitatory Synapses, Autism Spectrum Disorders

Disclosure: Nothing to disclose.

P112. The Synaptic Proteome of Autism Spectrum Disorder Across Postnatal Development in Human Visual Cortex

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Background: Despite diversity in symptoms and severity in autism spectrum disorders (ASD), spine alterations are observed in many genetic (ASD) models and subject tissue. Genetic studies of ASD have identified rare variants and common loci that implicate the synaptic protein networks crucial for spine formation and stabilization. Additionally, transcriptomic studies of cortical regions in ASD have found altered synaptic gene expression. However, transcriptomic changes are not always well correlated with synaptic protein levels and a survey of the synaptic proteome in ASD subject tissue has not yet been performed. Thus, the extent to which transcriptomic alterations manifest at the synapse in ASD subjects and contribute to spine alterations is currently unknown. Here, in a preliminary study, we assayed synaptic protein levels in primary visual cortex tissue from postnatal ASD and matched neurotypical subjects.

Methods: Postmortem primary visual cortex tissue from 31 pairs of ASD and neurotypical subjects (ages 4-33) matched for sex, age and postmortem interval were obtained from the University of Maryland branch of the NIH NeuroBioBank. Synaptosome enrichments were prepared with SynPER. Synaptosomes were digested with trypsin, TMT-labeled, fractionated, and analyzed on an Orbitrap Eclipse with SPS and real time search. Peptide and protein identification and quantification were performed in Proteome Discoverer 2.5.

Results: 7,577 proteins were identified in synaptosome enrichments across all subjects. Of these, 4,601 (including 115 ASD risk gene products, SFARI) were quantified with > 50% present call and included for statistical comparison with age and diagnosis. Synaptosome levels of 1,462 proteins were significantly associated with age ($q > 0.05$); with 860 decreasing during postnatal development and 539 increasing. Interestingly, the 860 proteins that decreased with age were significantly enriched for the GO term synapse in SYNGO ($q = 5.82e\text{-}15$, relative to the 7,577 proteins quantified) while the proteins that increased with age were not. Similarly, the proteins that decreased with age included 40 ASD risk genes while increasing proteins included only 4 ASD risk genes. Of the ASD risk genes assayed, only one, Receptor-type tyrosine-protein phosphatase F differed significantly between ASD and neurotypical subjects ($FC = 1.2$, $q > 0.05$). We are currently assaying protein expression and phosphorylation in this cohort.

Conclusions: Synaptic protein levels are robustly altered across postnatal development in the human primary visual cortex. The decreased synaptosome levels of canonical synaptic proteins and ASD risk genes likely reflects the rearrangement and loss of synaptic proteins that accompanies synaptic pruning in postnatal development and the importance of ASD genes in prenatal synaptic development. Our findings further suggest a role for Receptor-type tyrosine-protein phosphatase F, which may be involved in postsynaptic organization and regulation of tyrosine kinases, in postnatal synaptic alterations in ASD.

Keywords: Autism, Proteomics, Postmortem Brain Tissue

Disclosure: Nothing to disclose.

P113. Hippocampal Mossy Cells Are Regionally Reduced in Adolescent and Adult 22q11.2 Deletion Syndrome Model Mice

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Background: Localized hyperactivity of the anterior hippocampal formation is a reproducible finding in patients with early psychosis. Identifying causes and consequences of regional hippocampal pathology at molecular and cellular levels of resolution is challenging in living humans, supporting the use of animal models of aberrant neurodevelopment predisposing to psychotic disorders, such as 22q11.2 deletion syndrome. Using a mouse model of 22q11.2 deletion syndrome, we tested whether mossy cells (MCs), a glutamatergic neuron in the dentate gyrus (DG) that is vulnerable to loss in other disorders of hippocampal hyperexcitability such as temporal lobe epilepsy, are reduced in number in adolescent and adult mice. We then performed RNA-sequencing to explore molecular pathways with potential relevance to localized MC changes.

Methods: Df(h22q11)/+ and D2-Cre mice were purchased from Taconic Biosciences and UC Davis, respectively, and bred in the Lewis Lab at Vanderbilt. Male and female D2-Cre;22q/+ or D2-Cre;+/+ were sacrificed at P28 (adolescent) or at >12 weeks of age (adult) followed by immunohistochemistry for the MC marker GluR2/3, confocal microscopy, and manual cell counting, performed blind to genotype. Statistical analysis of cell counts was performed using t test (2-sided or 1-sided if testing for predicted reduction) or ANOVA with Sidak's multiple comparisons test. RNA-sequencing was performed by the Vanderbilt VANTAGE genomics core using RNA extracted from 12-week-old Df(h22q11)/+ and +/+ littermate ventral hippocampal tissue. Library preparation was performed utilizing a ribo-depletion total RNA library preparation kit and sequencing performed at Paired-End 150 bp on the Illumina NovaSeq 6000 followed by differential gene expression analysis and p value correction for multiple comparisons.

Results: Mossy cell number was reduced in the ventral but not dorsal DG of adult D2-Cre;22q/+ mice compared to D2-Cre;+/+ littermates (region x genotype interaction: $F(1,19) = 9.087$, $p = 0.007$; post-tests between genotypes: dorsal region: $p = 0.87$; ventral region: $p = 0.001$; $N = 10\text{-}11$/group). However, in this group of mice there was no reduction in DG granule cell density ($p = 0.82$) or DG parvalbumin+ interneuron number ($p = 0.73$). We next explored whether this reduction was also

present during adolescent development, and found ventral MCs were reduced in 28-day-old D2-Cre;22q/+ mice versus +/+ littermates ($p = 0.03$, $N = 6$/group, 1-sided t test). RNA-sequencing from adult ventral hippocampal tissue ($N = 3$ Df(h22q11)/+, 3 +/+ mice) showed a significant reduction in 18 of 27 genes within the Df(h22q11)/+ deletion. This analysis also identified significant reduction in Fos and JunB, two members of the activator protein-1 (AP-1) family of transcription factors not encoded within the Df(h22q11)/+ deletion region. Immunohistochemistry revealed that both Fos and JunB were expressed in ventral DG MCs in wildtype mice.

Conclusions: These studies show that MCs are reduced in number in mice with a genetic deletion orthologous to the human 22q11.2 deletion syndrome. This deficit occurs at least as early as P28, arguing that the loss is not due to cell death in later development. Several questions arise from this study: 1. Is the reduction in ventral MCs due to a problem with progenitor populations and/or cell-autonomous developmental dysfunction? 2. Do abnormalities in DG networks early in development, such as excitation-inhibition imbalance, result in cell non-autonomous deficits in MC maturation or stability? 3. Are AP-1 transcriptional networks actually perturbed in MCs as opposed to other cell types? If so, is this cell autonomous or non-autonomous? Taken together, regardless of whether the loss of ventral MCs per se contributes to cognitive or other aspects of 22q11.2 deletion syndrome, exploring the mechanisms underlying MC regional loss may yield a more general understanding of hippocampal molecular and cellular pathology in conditions predisposing to psychosis.

Keywords: Hippocampus, 22q11 Deletion Syndrome, Dentate Gyrus, RNA-Sequencing, Psychosis

Disclosure: Nothing to disclose.

P114. Understanding Primate Amygdala Development: A View From Microglia

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Background: The amygdala of nonhuman primates is remarkably similar to that of humans, developing over a lengthy postnatal period. This long development means that environmental events may influence neuronal growth and connectivity during early life. Our group and others–including those working with human postmortem tissue–have shown that throughout life, the primate amygdala has a repository of immature post-mitotic neurons (the paralaminar nucleus, PL) that surrounds and interdigitates with the main basal nucleus (Bpc). While most PL neurons are immature at birth, by adolescence a proportion have developed mature profiles suggesting ongoing differentiation. The Bpc in contrast is largely populated by mature neurons based on immunocytochemical markers. While we are beginning to understand normal neural maturation in the PL, the role of microglia, the brain's immune cells, in PL development is unknown. However, microglia are critical for differentiation of precursor cells to neurons, clearing excess neuroblasts and pruning synaptic contacts. Each of these functions is associated with different morphological and molecular signatures. Microglia phagocytose unneeded neural precursors (large microglia soma, short thick processes, clustered), prune neural synapses (small microglia soma, ramified processes, relatively dispersed), and release growth factors that assist in neural differentiation.

Methods: As a first step in characterizing the role of microglia in the developing PL, we assessed microglia in 3-month-old (infant) and 4-year-old (adolescent) macaques ($n = 4$/group) in both the PL and adjacent Bpc. We immunostained 1:12 sections through the amygdala for Iba1 (ionized calcium-binding adaptor molecule 1), which is a marker for microglia. The region of the PL and Bpc were identified using adjacent sections stained for mature and immature neurons. High-power photomicrographs were then taken at similar levels across cases, in a blinded fashion. We then analyzed morphologic features (microglia density, 'clustering', and soma size/shape) using FIJI/Image J. Studies of microglia branching (Sholl) and immunostaining for chemical markers for synaptogenesis are ongoing.

Results: The average density of microglia in the PL was 37% greater in adolescents (390 microglia/mm2) compared to infants (267 microglia/mm2; $p = 0.0143$), with no differences in density across medial, central, and lateral PL. The Bpc microglia density was also greater in adolescents (341 microglia/mm2) compared to infants (290 microglia/mm2), although by a lesser percentage: 16% ($p = 0.0143$).

The clustering (spacing) index, which measures microglial distribution while accounting for density (calculated as: (average nearest neighbor distance)2 *microglia density) was similar between the infant and adolescent groups in the PL ($p = 0.395$). In contrast, in adolescent Bpc the spacing index (0.473) was 10% less relative to infants (0.530), indicating closer spacing ($p = 0.0277$).

Conclusions: These preliminary data show an increase in microglia numbers from infancy to adolescence in both the PL and Bpc. There is also increased clustering of Bpc microglia in adolescence, suggesting potential increased microglia interactions with neurons. Ongoing data analyses will determine whether differences in increasing microglia densities in each region are associated evidence of regional synaptogenesis.

Keywords: Paralaminar Nucleus, Immune Markers, Cytokines, Synapses, Schizophrenia, Autism, Basal Nucleus

Disclosure: Nothing to disclose.

P115. Preliminary Observations From an Open Phase II Trial of Cannabidiol in Children With Autism Spectrum Disorder

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Background: Autism spectrum disorder (ASD) is common (~2.3% of children) and lacks specific pharmacologic treatments. Anecdotal reports and one placebo-controlled trial (of a 20:1 ratio of cannabidiol (CBD) and THC; Aran et al., Molecular Autism, 2021) suggest CBD may be helpful for some autistic children. CBD has been approved by the US FDA to treat refractory seizure syndromes in children and was well tolerated. We undertook an ongoing open trial of pharmacologic grade FDA-approved CBD without THC in children and adolescents without seizures and at least average intellectual ability as a basis for hypothesis generation and selection of dose and outcomes in future controlled trials. CBD is being provided at no cost by JAZZ Pharmaceuticals, which has had no had a role in designing the study, nor in analyzing or reporting results.

Methods: Nineteen youth, ages 7-17 ($M = 11.3 \pm 2.9$) with ASD completed an ongoing, 6-week Phase 2 open trial of 98% CBD (100 mg/mL) at 3, 6 or 9 mg/kg/d; target $N = 30$ [NCT03900923]. Dose was determined per a Bayesian optimal interval design starting at the 6 mg/kg/d dose with an initial expectation of 60% response rate. Inclusion criteria were a confirmed ASD diagnosis, verbal fluency, IQ ≥ 80, Social Responsiveness Scale (SRS-2) Total

T-score ≥ 66, and Clinical Global Impression Scale–Severity (CGI-S) score ≥ 4 on an individualized target symptom domain identified at baseline by clinician consensus from informant report, rating scales, and clinical observation. Response was defined as CGI–Improvement (CGI-I) score ≤ 2 in the target domain. Adverse events (AEs) were assessed at weeks 2, 4 and 6 by clinician administered UKU Side Effects Rating Scale – Patient Version-5 to dyads, and at weeks 1, 3, 5 by coordinator phone calls to parents. Clinician consensus determined relatedness of AEs to treatment. In response to COVID-19, all screening procedures and assessments (excluding physical exams and blood testing to monitor safety) were conducted via secure virtual teleconferencing. Surveys were administered securely via REDCap software. Plasma CBD levels and clinical labs were obtained at the last session.

Results: Demographics: We enrolled 18 males and 1 female. Full-scale IQ scores ranged from 81 to 136 ($M = 104 \pm 17$). Seventeen had co-occurring psychiatric diagnoses: ADHD–Combined ($n = 12$), ADHD–Inattentive ($n = 4$), OCD ($n = 4$), Anxiety ($n = 3$), and Other–Specified Disruptive and Impulse Control Disorder ($n = 2$). Participants' racial and ethnic identities included white ($n = 12$), white/Hispanic ($n = 5$), Asian ($n = 2$), Black/African American ($n = 1$), multiracial ($n = 2$), unknown race/Hispanic ($n = 1$), and unknown race/ethnicity ($n = 1$).

All 19 enrolled participants completed the trial; overall, eight were classified as responders (42%); response rate was related to dose, ranging from 17% at 3 mg/kg/d ($n = 6$), to 44% on 6 mg/kg/d ($n = 9$) and 75% on 9 mg/kg/d ($n = 4$; this phase is ongoing). Dose correlated with CGI-I ($r(18) = -0.43$, $p = .05$). CGI–S scores improved significantly from pre-($M = 4.68 \pm 0.48$) to post-treatment ($M = 3.95 \pm 0.78$), paired $t(18) = 3.98$, $p = <.001$; Cohen's $d = 0.92$; 95% CI [0.37, 1.44]. In a post-hoc analysis, we noted that none of the five participants taking stimulants for ADHD responded to CBD, while 8 of 11 (73%) who have ADHD but were not being treated with a stimulant responded (Fisher exact $p = 0.013$).

A total of 83 AEs were reported; 79 were considered mild, 3 mild-moderate, and 1 moderate. Of 30 AEs judged by clinician consensus to be related to CBD, none were both related and unexpected. There were 17 unexpected AEs, all deemed unrelated. Liver function lab tests and complete blood counts were unaffected by CBD. Plasma CBD levels after 6-week treatment ranged widely from 2.4 ng/ml to 430.6 ng/ml and were related to dose ($r(18) = 0.46$, $p = 0.05$) but not to CGI-I ($r(18) = -0.19$).

Conclusions: CBD is well tolerated and seems to be beneficial for autistic youth although with a lower overall response rate than expected. We speculate placebo effects may have diminished based on informal tests conducted by families, facilitated by CBD legalization, although mean CBD doses in the community are 2 to 3 mg/kg/day (DiLiberto et al., 2022). Our experience suggests that higher doses are more likely to be beneficial and that benefits, when they emerge, do so over several weeks. Unexpectedly, we observed a high response rate (73%) in youth with comorbid ADHD not being treated with stimulants. These results resonate with Seeman' 2016 observation (in Translational Psychiatry) that CBD has partial agonist properties at striatal D2 receptors, comparable to aripiprazole, one of two atypical neuroleptics approved to treat irritability in pediatric autism. We plan to continue enrollment until our IRB-approved accrual of 30 is reached, with a focus on ASD + ADHD comorbidity in the remaining participants.

Keywords: Delta9-Tetrahydrocannabinol, Cannabidiol, Autism Spectrum Disorder and Related Syndromes, Phase 2, Attention Deficit Hyperactivity Disorder

Disclosures: BOL Pharma: Advisory Board (Self), Frontiers Media: Honoraria (Self)

P116. Pharmacological Interventions for the Treatment of Tourette's Syndrome in Youth: A Systematic Review and Network Meta-Analysis

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Background: Tourette's syndrome (TS) is a neurodevelopmental disorder characterized by several motor, and at least one phonic, tics which onset during childhood or adolescence and last longer than 1 year. Practice guidelines from the American Academy of Neurology (AAN), the American Academy of Child and Adolescent Psychiatry (AACAP), and the European Society for the Study of Tourette Syndrome (ESTSS) agree that medications should be offered for individuals whose TS symptoms are moderate to severe, persistent, and associated with impairment. However, there is no agreement regarding what medications should be used as first-, second- or third-line treatments. The inconsistencies in treatment recommendations arise from the fact that pharmacological randomized double-blinded controlled trials (RDBCTs) in TS are scarce and based on small sample sizes, which creates difficulties when trying to determinate the head-to-head effects of different medications. Network meta-analyses facilitate the estimation of the comparative efficacy of two or more interventions, even when they have not been investigated in head-to-head RDBCTs and are required to inform treatment guidelines as the highest level of evidence. To fill this gap, we conducted a systematic review and network meta-analysis of pharmacological treatments evaluated in RDBCTs for the treatment of youth with TS.

Methods: We searched PubMed, the Cochrane Central Register of Controlled Trials, Web of Science, Embase, PsycINFO, and the WHO International Trials Registry Platform, including ClinicalTrials.gov, from the date of database inception to November 19, 2021. We also hand-searched the US Food and Drug Administration, European Medicines Agency, and relevant drug manufacturer's websites, as well as references of previous systematic reviews and guidelines, to look for additional studies. We included randomized, double-blinded controlled trials (RDBCT) that enrolled children and adolescents (aged ≥ 4 and ≤ 17 years), adults (≥ 18 years), or both with a primary diagnosis of TS according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-III/III-R/IV/IV-TR/5), the International Classification of Diseases (ICD-9/10/11) or the Chinese Classification of Mental Disorders 3rd edition (CCMD-3). We did not restrict eligibility based on medications provided they were administered as monotherapy. For the outcome, we considered efficacy, measured as change in tic symptom severity; tolerability, measured as the proportions of participants who discontinued the study due to adverse events; and acceptability, measured as the proportions of participants who discontinued due to any reason. Pairs of researchers independently reviewed the records to select the studies and extracted data. We performed random-effects network meta-analysis in a frequentist framework. We assumed a common heterogeneity variance τ^2 across the various treatment comparisons. We quantified heterogeneity as low, moderate or high by comparing τ^2 with its empirical distribution. We evaluated inconsistency globally (design-by-treatment interaction) and locally (separate indirect from direct evidence, SIDE). All analyses were done in R with packages meta or netmeta. We assessed risk of bias of RDBCTs with the Cochrane tool. We plotted comparison-adjusted funnel plots to investigate publication bias. We assessed

certainty of findings from the network meta-analyses with Confidence in Network Meta-Analysis (CINeMA) framework.

Results: Of the 12,088 records screened, 39 RDBCTs involving 4,578 participants (mean age [SD] 11.83 [4.55]; 80% male) were included in the network meta-analyses. Assessments of heterogeneity, inconsistency, and publication bias were incorporated in the ratings of quality of evidence. In terms of efficacy, aripiprazole (SMD -0.60; 95% CI -0.83, -0.38), haloperidol (SMD -0.51; 95% CI -0.88, -0.14), olanzapine (SMD – 0.83; 95% CI -1.49, -0.18), pimozide (SMD -0.48; 95% CI -0.84, -0.12), risperidone (SMD -0.66; 95% CI -0.98), clonidine (SMD -0.20; 95% CI -0.37, -0.02) and ecopipam (SMD – 0.34; -0.63, -0.06) led to larger changes in tic symptom severity than placebo (moderate certainty of evidence). There were no significant differences between antipsychotic medications in head-to-head comparisons (low to very low certainty of evidence), however aripiprazole (SMD -040; 95% CI -0.69, -0.12) and risperidone (SMD -0.46; 95% CI -0.82, -0.11) were more efficacious than clonidine (moderate certainty of evidence). In terms of tolerability and acceptability, there were no relevant findings for any of the efficacious medications against each other or placebo (low to very low certainty of evidence).

Conclusions: Overall, our results support that antipsychotics are the most efficacious medications in the management of TS. Because there were no differences in efficacy between any pairs of antipsychotics based on the currently available data, the choice of antipsychotic should be guided mainly by their tolerability. Nevertheless, whether antipsychotics should be used as first-line pharmacological interventions in the treatments of TS is debatable given they are potentially toxic and may lead to somatic and serious adverse events, particularly in vulnerable populations such as children and adolescents. Although alfa2-agonists are less efficacious, their benefit-to-risk ratio may be higher because of less worrisome adverse events.

Keywords: Tourette Syndrome, Psychopharmacotherapy, Meta-Analysis

Disclosure: Nothing to disclose.

P117. An Open-Label, Tolerability and Efficacy Study of ZYN002 (Cannabidiol) Administered as a Transdermal Gel to Children and Adolescents With 22q11.2 Deletion Syndrome (INSPIRE)

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Background: 22q11.2 deletion syndrome (22q) is caused by a microdeletion of region 11.2 on the long arm of chromosome 22 and is the most common recurrent contiguous gene deletion syndrome, estimated to occur in about 1 in 4000 live births. 22q is associated with a range of developmental anomalies including congenital heart defects, palate and pharyngeal defects and immunodeficiency. Behavioral problems, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and mood disorders occur frequently in children with 22q. ZYN002 is a pharmaceutically produced cannabidiol transdermal gel in development for treatment of the behavioral symptoms in 22q, Fragile X syndrome and ASD. INSPIRE was an open-label, phase 2 trial to evaluate the safety/tolerability and efficacy of ZYN002, in children and adolescents ages 4 to <18 years, in the treatment of behavioral and anxiety-related symptoms in 22q.

Methods: Males and females with 22q confirmed by genetic testing, with or without autistic features, a Clinical Global Impression-Severity (CGI-S) score ≥4 and a Pediatric Anxiety Rating Score-Revised (PARS-R) score ≥10 were enrolled. Patients weighing ≤35 kg received 250 mg/day and those weighing >35 kg received 500 mg/day of ZYN002 in divided doses every 12 hours added to current stable therapy for 14 weeks. Patients with <25% improvement from baseline in the Aberrant Behavior Checklist-Community (ABC-C) Irritability subscale at week 6 could have their dose increased to either 500 mg/day or 750 mg/day based upon weight. Safety assessments included adverse events, vital signs, laboratories, and electrocardiograms (ECGs). Efficacy assessments included change from baseline on the Anxiety, Depression and Mood Scale (ADAMS), ABC-C, PARS-R, CGI-Improvement (CGI-I), and the Children's Sleep Habits Questionnaire (CSHQ). A qualitative caregiver reported behavioral problems survey was also collected.

Results: Twenty patients, 60% males, with a mean age of 9.9 years (5 to 15 years) were enrolled. Seventeen patients completed and 3 withdrew during the treatment period. Sixteen patients had evaluable efficacy assessments at week 14 and 13 patients entered a six-month extension period. Statistically significant improvements were seen in the ADAMS, ABC-C and PARS-R scales. Mean change and mean/median percent improvement from baseline on the ADAMS were as follows: Total Score -18.4, 45.3%/43.0%, $p = 0.0005$; General Anxiety -5.4, 43.6%/48.8%, $p = 0.0005$; Depressed Mood -4.3, 50.3%/52.8%, $p = 0.0033$; Social Avoidance -4.4, 41.3%/50.0%, $p = 0.0084$; Obsessive/Compulsive Behavior -1.9, 64%/66.7%, $p = 0.0037$; Manic/Hyperactive Behavior -3.1, 38.2%/27.4%, $p = 0.0032$. Mean change and mean/median percent improvement from baseline on the ABC-C were as follows: Social Withdrawal -6.4, 27.6%/46.4%, $p = 0.011$; Inappropriate Speech -1.8, 18.3%/50.0%, $p = 0.0166$; Stereotypic Behavior -2.3, 52.1%/58.3%, $p = 0.0155$; Irritability -8.4, 36.3%/39.6%, $p = 0.0055$; Hyperactivity -7.6, 16.5%/38.1%, $p = 0.0091$. Mean change and mean/median percent improvement from baseline on the PARS-R was: Total Score -6.2, 40.6%/40.0%, $p = 0.0005$. Twelve of 16 patients (75%) were rated as "improved", "much improved" or "very much improved" at week 14, with 62.5% being "much improved" or "very much improved" on the CGI-I. Three patients reported treatment related adverse events which were all mild application site adverse events which were transient and resolved with continued dosing. One patient discontinued treatment due to adverse events not related to ZYN002. No serious adverse events or clinically significant changes in vital signs, ECGs or laboratories were reported.

Conclusions: INSPIRE provides initial evidence suggesting a positive risk–benefit profile for ZYN002 in improving behavioral and anxiety-related symptoms in children and adolescents with 22q when added on top of stable standard of care. Further studies are warranted.

Keywords: 22q11 deletion syndrome, Adolescence, neurodevelopmental disorders, cannabidiol, CBDV, translational models, clinical trials

Disclosure: Zynerba: Consultant (Self)

P118. Molecular Variation in Fragile X Syndrome: Impact on Drug Target Engagement

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Background: Fragile X syndrome (FXS) is the most common single gene cause of autism and the most inherited form of developmental disability. Despite the available of fragile X knockout (KO) animal models, the translation of preclinical treatment success has

not yet resulted in successful approved treatments in humans with FXS. We have discovered molecular variation in fragile X messenger ribonucleoprotein (FMRP) expression in males with FXS described with clinical genetic testing as having a fully methylated full mutation (CGG expansion) in the FMR1 gene. We have added peripheral blood FMRP assay to recent target engagement drug trials in FXS as a means to characterize patient-specific molecular pathology. We describe initial experience with this approach in placebo controlled trial of single dose baclofen (GABA B agonist) and two weeks daily dosing of BAER-101 (selective GABA A alpha 2,3 agonist) in humans with FXS.

Methods: Utilizing a Luminex-based system we obtained peripheral blood FMRP levels in subjects with FXS enrolling in two Phase Ib target engagement studies in adolescents and adults with FXS. We then analyzed the outcome results of each trial using molecular FMRP level as a quantitative means to subgroup patients. The first trial was a double-blind, placebo-controlled crossover single dose study of acamprosate, lovastatin, minocycline, and baclofen in adolescents and adults with FXS. The second trial of a double-blind, placebo-controlled, crossover, two-week treatment period study of the selective GABA A alpha 2,3 agonist BAER-101 in adults with FXS. Both trials included performance-based outcome measures such as eye tracking, memory testing, and computer-base testing. Each project additionally focused on EEG-specific outcomes employing high density EEG study pre- and post-treatment in both studies.

Results: In our single-dose challenge study, acamprosate, minocycline, and lovastatin dosing was not associated with any consistent changes in EEG signal. In 17 adolescents and adults (11 males and 6 females; mean age 26.3 years; range 16-43 years), baclofen (single 30mg dose) was associated with significant reduction in frequency gamma band EEG activity at rest. Increased gamma band activity is a known pathological EEG feature associated with FXS. In a FMRP-driven subgroup analysis, males who were Southern Blot and PCR diagnosed with a fully methylated full FMR1 mutation but who expressed trace levels of FMRP in blood showed a significant reduction in gamma band activity that correlated with improvement in social gaze on an eye tracking measure. This positive association between EEG and clinical change was not noted in females or males expressing zero FMRP in blood. Overall, a significant association was noted between FMRP level and gamma band EEG change with lower FMRP levels associated with greater reduction (rescue) of gamma band activity. In our study of BAER-101 in 13 adults with FXS, low dose (5mg BID) drug was associated improvement in memory as measured by the Repeatable Battery of Neuropsychiatric Status (RBANS) in males only. No significant BAER-101 associated improvement in EEG measures were noted across all patients. Our FMRP-driven subgroup analysis noted that males expressing zero FMRP in blood had low-dose BAER-101 associated improvement in social withdrawal as measured by the Aberrant Behavior Checklist (ABC), an opposite directional change compared to males expressing some FMRP. Additionally, this zero FMRP male subgroup showed a relevant correlation between gamma band power change and memory improvement with gamma band reduction being associated with more improvement in memory as measured by the RBANS.

Conclusions: We have noted early evidence that FMRP status in fragile X syndrome (FXS) may represent a disease/disorder continuous molecular measure that impacts small molecule treatment response. The ability to effectively identify molecular patient subgroups based on peripheral FMRP analysis holds promise for use in larger Phase II and III studies to match patient groups to particular drugs under study. We believe FMRP levels will support future small molecule studies in FXS and potentially enable personalized medicine approaches in this field. We need to gather data from larger patient groups to potentially confirm our initial results that baclofen use is associated with a strongest positive signal in males expressing trace levels of FMRP and BAER-101 use is associated with a positive signal in males expressing zero FMRP.

Keywords: Fragile X Syndrome, Clinical Trial Methodology, EEG

Disclosures: Forge Therapeutics, Impel, Scioto Bioscience, Stalicla: Consultant (Self)

P119. Stress-Induced Plasticity of a Novel CRH GABA Projection Disrupts Reward Behaviors

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Background: Disrupted operation of reward circuits is thought to underlie several emotional disorders including depression and drug abuse, disorders commonly arising after early-life stress. Yet, how early-life adversities (ELA) impact the functional maturation of reward circuitries to promote disease remains unclear. 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 behaviors, including the basolateral amygdala (BLA). The BLA mediates associative learning for aversive and appetitive stimuli, and stimulation of glutamatergic projections from the BLA to NAc promotes appetitive behaviors. Here, we identified a novel projection that expresses the stress neuropeptide corticotropin-releasing hormone (CRH) to connect the basolateral amygdala (BLA) and nucleus accumbens (NAc). In the NAc, CRH + axon terminals modulate reward and motivational behaviors. Here, we identify the role of this CRH + BLA-NAc projection during reward in naïve and ELA mice.

Methods: Pairing viral-genetic approaches with CRH-IRES-Cre mice and Cre-dependent viruses, we identified CRH + BLA projections to the NAc. To determine the function of this novel CRH + BLA-NAc projection we used chemo-, optogenetic and electrophysiology strategies in control (CTL) and ELA mice. In these mice, excitatory or inhibitory Cre-dependent DREADDs and optogenetic viruses were injected into BLA, followed by medial NAc shell targeted microinjections of CNO or light activation. In behavior, we tested the function of this pathway using reward, and non-reward tasks.

Results: Male ELA mice have reduced preference for sucrose, palatable food, and a sex-cue, compared with CTLs. Viral-genetic tracing combined with electrophysiology identified a novel GABAergic projection that co-expresses the stress neuropeptide CRH from the BLA to the medial NAc shell. In freely behaving mice, exciting this projection using chemo- and optogenetic techniques reduced preference for sucrose, palatable food, and a sex-cue, but did not alter non-reward-mediating tasks. In adult ELA mice, chemogenetic inhibition of the GABAergic CRH + BLA-NAc projection rescued these reward behaviors.

Conclusions: Here, we identify a novel GABAergic CRH + BLA-NAc projection and establish its role in mediating the effects of stress on reward behavior. These discoveries provide potential selective targets for prevention and intervention in the disruption of such behavior that accompanies several psychopathologies.

Keywords: Basolateral Amygdala, Nucleus Accumbens, CRH, Early Life Stress, GABA

Disclosure: Nothing to disclose.

P120. Quantitative Sensorimotor Traits as Familial Endophenotypes Associated With Autism Spectrum Disorder (ASD)

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Background: Sensorimotor impairments, including reduced feedforward and feedback control of motor behaviors, are common in autism spectrum disorder (ASD) and have been documented in unaffected first-degree relatives. These findings suggest sensorimotor traits may serve as endophenotypes useful for understanding heritable risk pathways associated with ASD. To determine the extent to which sensorimotor traits were familial in ASD, sensorimotor behaviors were examined across a cohort of family trios that included individuals with ASD (probands) and each of their biological parents. We also tested multiple separate motor behaviors (rapid and sustained) across multiple effector systems (hand and eye).

Methods: Fifty-eight individuals with ASD (probands; ages 5-17 years), 109 parents (ASD parents; ages 29-54 years), and 89 neurotypical (NT) control participants matched on age, sex, and nonverbal IQ completed manual and oculomotor tests. To assess feedforward control processes, participants completed tests of rapid precision gripping (manual motor) and visually guided saccades (VGS; oculomotor). During the rapid gripping test, participants viewed a static red/green target bar and were instructed to press the load cells as quickly as possible when the red target bar turned green so that a white force bar reached the level of the green target bar set to 15, 45, or 75% of their maximum force. The accuracy of initial force output was examined. During the VGS test, participants fixated a central cross-hair and made reactive saccades to suddenly appearing peripheral targets at + 12 or 24 deg. The accuracy of saccades was examined. To assess sensory feedback control of motor behavior, participants completed sustained precision gripping (manual) and smooth pursuit eye movement tests (oculomotor). The sustained gripping test was similar to the rapid grip test, but participants were instructed to maintain a constant force at the level of the target bar for 8 sec. The variability of sustained grip force was examined. During the smooth pursuit test, participants tracked targets moving from 0 to + 15 deg at varying velocities (2-30 deg/s). Smooth pursuit gain, or the ratio of pursuit velocity relative to target velocity, was examined. We also conducted subgroup analyses comparing families with at least one parent showing broader autism phenotypic traits (BAP+) and those with two parents without BAP traits (BAP-).

Results: During the rapid precision grip and VGS tests, probands showed reduced accuracy relative to NT controls. These differences were specific to probands with BAP- parents. BAP- parents also showed reduced saccade accuracy relative to BAP + parents and NT controls. Saccade accuracy was interrelated among family trios. During the sustained precision grip and smooth pursuit eye movement tests, probands showed performance deficits relative to NT controls, including increased variability during gripping and reduced gain during smooth pursuit. Increased force variability was more severe in probands with BAP + parents than those with BAP- parents. BAP- parents showed increased precision grip force variability relative to BAP + parents and controls.

Conclusions: Findings that rapid sensorimotor behaviors are selectively impacted in BAP- probands and parents suggest they may reflect polygenic liabilities independent of familial autistic traits. Sustained sensorimotor behaviors were affected in BAP + probands and BAP- parents implicating overlapping or additive polygenic risk with core autism traits. These findings are consistent with prior research indicating sensorimotor impairments may serve as useful, quantitative endophenotypes reflecting inherited polygenic risk for ASD. Results also indicate that separate sensorimotor behaviors associated with distinct neurophysiological processes may represent unique pathways of familial risk for ASD.

Keywords: Autism, Sensorimotor, Endophenotypes

Disclosure: Nothing to disclose.

P121. Identifying Cerebral Cortex Indices That Predict Autistic Symptom Severity

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Background: Abnormal cortical anatomy is among the significant features of the neuropathology in autism spectrum disorders (ASD). Several cortical regions have been identified to be altered in individuals with ASD. However, which cortical index can be correlated with clinical severity of autistic symptoms is inconclusive. This study aims to identify the cortical components that can better explain the overall severity of ASD.

Methods: We recruited 122 patients with ASD and 118 typically-developing controls (TDC). All the participants underwent brain MRI assessment. Cortical thickness was analyzed by using FreeSurfer software with 74 automatic parcellations. Autistic symptom severity was measured by Social Responsiveness Scale (SRS), Social Communication Questionnaire (SCQ), and Autism Spectrum Quotient (AQ). We adopted principal component analysis on the cortical thickness and cortical volumes of cerebral cortex to identify the main components of the cortical indices, and examined their correlations with autistic symptom severity.

Results: In principal component analysis, there were 9 components for white matter volume, 3 for cortical volume, and 6 for cortical thickness. To correlate these factors with autistic traits, we found that none of the 9 white matter components were significantly correlated with AQ or SRS total scores. For cortical volume components, we found that the second component was correlated with AQ and SRS total scores. The correlation coefficients were 0.27 for AQ total scores, 0.37 for SRS total scores. Both are low-level correlations. As for cortical thickness component, the first three components were correlated with AQ total scores. The correlation coefficients were ranging from 0.28 to .45, with the highest correlation on factor 3. For SRS, factor 2, 3, 4 were correlated with total scores, correlation coefficients ranged from 0.24 to 0.34, the highest one on factor 3, too. The top one is the third component, to the level of medium correlation.

We further examined other cortical thickness parameters to see whether that can better correlate with ASD severity. We adopted the other two cortical parcellation instead of FreeSurfer. Using 7 network built based on the intrinsic functional connectivity proposed by Yeo, the factor 1 and 3 were correlated with AQ and SRS, correlation coefficients were as high as 0.39, a bit lower than those in DKT40 cortical thickness components that reaches 0.45. Finally, regarding the BA principal components, the first two factors were correlated with both AQ and SRS. The correlations between factor 1 and AQ or SRS can be as high as 0.7.

Conclusions: This study found a major component of cortical thickness that was significantly correlated with autistic symptom severity. Autistic severity is associated with the principal components of cortical thickness and volume, but not with those of white matter volumes. Autistic severity is also associated with the principal components based on function connectivity. The

highest correlations were among the sensory and motor area components based on BA area. Our findings warrant further validation.

Keywords: Autism, Cerebral Cortex, ASD Core Symptoms

Disclosure: Nothing to disclose.

P122. Differential Symptom Response to 12-Week Mixed Amphetamine Salts in ADHD Youth With Versus Without Familial Risk for Bipolar I Disorder: Associations With Polyunsatutared Fatty Acid Biostatus

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Background: The initial onset of bipolar I disorder (BD) frequently occurs during the peripubertal period, and is commonly preceded by attention deficit/hyperactivity disorder (ADHD). Prospective studies have shown that ADHD increases risk for developing mood disorders including BD, and is associated with an earlier age at onset of mood symptoms. However, vulnerability factors associated with the risk of developing BD in youth with ADHD remain poorly understood. Having a first-degree relative with BD robustly increases risk for BD in offspring, and youth with a first-degree BD relative exhibit higher rates of ADHD and more severe ADHD symptoms. Additionally, retrospective studies suggest that antecedent stimulant exposure may precipitate or exacerbate manic symptoms and accelerate the onset of mania in a subset of individuals. Lastly, BD youth exhibit deficits in omega-3 polyunsaturated fatty acids (n-3 PUFA) including docosahexaenoic acid (DHA), and developmental DHA insufficiency in rodents alters dopamine neurotransmission and response to psychostimulants. To investigate these potential risk factors, this study compared the effects of 12-week psychostimulant treatment in ADHD youth with ('high-risk', HR) and without ('low-risk', LR) a first-degree relative with BD. It was hypothesized that HR youth would exhibit greater increases in symptoms previously found to precede and predict the initial onset of BD, including manic symptoms and parent-reported ratings of dysregulation, compared with LR youth, and that higher n-3 PUFA biostatus would be negatively correlated with these changes.

Methods: ADHD youth (ages 10-18 years) with ('high-risk', HR) and without ('low-risk', LR) a first-degree relative with BD were enrolled. LR youth received 12-week open-label mixed amphetamine salts-extended release (MAS-XR), and HR youth were randomized to MAS-XR or placebo (PBO). All ADHD patients met DSM-5 criteria for ADHD (any type), had no exposure to psychostimulants for at least 3 months prior to enrollment, and had no comorbid mood, conduct, eating, or psychotic disorders. Clinician ratings of ADHD (ADHD-rating scale, ADHD-RS), mania (Young Mania Rating Scale, YMRS), depression (Children's Depression Rating Scale-Revised, CDRS-R), global functioning (Children's Global Assessment Scale, CGAS), and global symptom severity (Clinical Global Impression-Severity Scale, CGI-S) were performed, and parents completed the Child Behavior Checklist (CBCL), at baseline and week 12. Baseline red blood cell (RBC) membrane PUFA (DHA, eicosapentaenoic acid [EPA] + DHA, arachidonic acid, AA) levels were determined by gas chromatography. Group x time interactions in baseline-endpoint change scores were calculated, and correlations between symptom change scores with baseline fatty acid levels were performed.

Results: A total of $n = 96$ (HR: $n = 47$; LR: $n = 49$) ADHD youth (mean age: 13.9 ± 2.5 years) were enrolled (LR-MAS: $n = 49$; HR-MAS: $n = 31$; HR-PBO: $n = 16$). There were no significant baseline group differences in age, sex, race, pubertal status, body mass index, or prior exposure to psychostimulants. The HR-MAS group had lower baseline RBC levels of DHA ($p = 0.01$) and EPA + DHA ($p = 0.007$), but not AA ($p = 0.91$), compared with LR-MAS but not HR-PBO. Following 12-week MAS-XR treatment (median MAS-XR dose: LR: 17.7 ± 5.2 vs HR: 15.8 ± 5.7, $p = 0.12$), the LR-MAS group exhibited significantly greater reductions in ADHD-RS total scores ($p = 0.015$) and inattentive subscale scores ($p = 0.0001$) compared with HR-MAS. A greater percentage of LR-MAS achieved remission (ADHD-RS endpoint score <18) compared with HR-MAS (92% vs. 61%, $p = 0.0001$). LR-MAS youth also exhibited significantly greater improvements in overall illness severity (CGI-S, $p = 0.0001$) and global functioning (CGAS, $p = 0.0008$) compared with HR-MAS, and there was a trend for greater reductions in CBCL-dysregulation profile subscale score ($p = 0.08$). For all ratings, there were no significant differences in baseline-endpoint change in HR-MAS versus HR-PBO groups, and no significant group differences were observed for changes in YMRS and CDRS total scores. Among all ADHD subjects ($n = 96$), higher baseline RBC DHA levels were associated with greater improvements in global functioning scores ($p = 0.04$), and higher AA levels were associated with greater reductions in depression ($p = 0.02$), CBCL total score ($p = 0.02$) and CBCL externalization ($p = 0.03$), internalization ($p = 0.04$), and dysregulation ($p = 0.01$) subscale scores. In HR-MAS and LR-MAS groups, AA levels were differentially associated with changes in CGI-S (group interaction, $p = 0.028$), ADHD-RS total score ($p = 0.040$), and ADHD-RS hyperactivity/impulsivity subscale score ($p = 0.02$).

Conclusions: Following 12-week MAS-XR treatment, ADHD youth without a BD family history exhibit greater reductions in inattention symptoms, and greater improvements in overall illness severity and global functioning, compared with ADHD youth with a BD family history. ADHD youth with a BD family history exhibited lower DHA biostatus compared with ADHD youth without a BD family history, and both DHA and AA were associated with different symptom changes. These findings suggest that ADHD youth with a BD family history may require alternative or adjunctive treatments for inattention and global functioning, and PUFA associations with symptom changes warrant further investigation.

Keywords: Bipolar Disorder, ADHD, Psychostimulants, Prodrome

Disclosures: Myriad, Medscape: Advisory Board(Self), Alkermes, Janssen: Consultant (Self), Alkermes, Lundbeck, Janssen, Allergan, Shire: Contracted Research (Self),

P123. Community Sample Data-Derived Communication Profiles and Early Autistic Phenotypes

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Background: Delays in language development are one of the earliest features associated with Autism Spectrum Disorder (ASD) (Tager-Flusberg, 2016); often, observations of atypical communication abilities serve as a tocsin for parents to seek neurodevelopmental evaluations for their child (Richards et al., 2016). Autistic children pronounce their first words and phrases later than their peers (Roemer et al., 2019, Charman et al., 2003) and delays in non-verbal communication (e.g., gestures) associate with impaired verbal communication - even before language development (Colgan et al., 2006, Luyster et al., 2008, Mitchell et al., 2006). Further, receptive and expressive language skills associate negatively with restricted and repetitive behaviors in both autistic

(Ray-Subramanian and Ellis Weismer, 2012) and neurotypical (NT) children (Larkin et al., 2017) with improvements in language skills predicting symptom reduction. Yet, little is known about the role of early verbal and non-verbal language development on sensory-motor and communication trajectories in ASD and NT samples.

In the present analysis, we first used the MacArthur Bates-Communication Development Inventory (MB-CDI): Words and Gestures (Infant Form) (Fenson et al., 1994) to delineate data-driven, early communication profiles in a community sample. The MB-CDI infant form is a widely-used and validated measure designed and normed for use with 8- to 18-month old and older, developmentally-delayed children; caregivers are asked to rate their child's abilities on several components of language development, yielding raw scores and percentile rankings for expressive (Words Produced; WP) and receptive (Words Understood; WU, Phrases Understood; PhU) language, as well as a child's use of action and gestures (Total Gestures; TG). In an effort to explore the informative potential of these novel profiles, we then examined associations between these groupings with data collected concurrently using the Video-Referenced Rating of Reciprocal Social Behavior (vrRSB; 18-30 month version), a parent-report measure that has demonstrated strong reliability quantifying autistic traits in community samples (Marrus et al., 2018, Marrus et al., 2015, Marrus et al., 2020) and the Repetitive Behavior Scales – Early Childhood Supplement (RBS-EC) (Wolff et al., 2016, Sifre et al., 2021), a parent-report measure of restrictive and repetitive behaviors for children (8 months - 8 years).

Methods: Survey measures (MB-CDI: WG, vrRSB, RBS-EC, demographics) were collected online from parents of N = 904 (47.9% Female), community-ascertained children (Mean Age = 18.08 ± 0.53 months) during the baseline phase of a larger, descriptive longitudinal study. Applying data-driven, two-step cluster (TSC) analysis with MB-CDI percentile rankings (WP, WU, PhU, TG), we employed a log-likelihood distance measure, Akaike's Information Criteria (AIC) clustering criterion, and a maximum of 15 clusters. Silhouette coefficients of cohesion and separation, along with membership variables of each cluster solution, were used to derive parsimonious profile TSC groupings. Multiple general linear models (GLMs) were used to examine associations between MB-CDI data-derived TSC groups, sex, age, vrRSB, and RBS-EC scores.

Results: TSC analysis with percentile rankings for TG/WP variables yielded a four-cluster solution with a good silhouette measure of cohesion and separation coefficient (0.62): 1. LowWP/LowTG (N = 390); 2. HighWP/LowTG (N = 139); 3. LowWP/HighTG (N = 243); 4. HighWP/HighTG (N = 132). A similar four-cluster solution was found for WU/WP in the good cluster quality range (coefficient = 0.56): 1. LowWU/LowWP (N = 354); 2. LowWU/HighWP (N = 233); 3. HighWU/LowWP (N = 170); 4. HighWU/HighWP (N = 147). TSC analysis with PhU alone yielded a three-cluster solution (coefficient = 0.74): 1. LowPhU (N = 385); 2. MidPhU (N = 362); HighPhu (N = 157).

Covarying for age, GLM analyses demonstrated discriminant grouping by profile cluster membership and sex for ASD characteristics on vrRSB subscale and composite scores ($p < 0.01$) as well as mean frequency and interference subscale and composite scores on the RBS-EC ($p < 0.05$). Broadly, clusters with lower MB-CDI percentile rankings on all included variables (e.g., LowWP/LowTG, LowWU/LowWP) associated with higher vrRSB and RBS-EC scores (i.e., increased risk for ASD). However, clustering also suggested distinct profiles for individuals showing asymmetrical development of verbal and non-verbal communication skills (e.g., LowWP/HighTG, HighWU/LowWP) that may provide further insight into the clinical heterogeneity of ASD.

Conclusions: Emergent ASD features. Of particular interest, infant siblings of autistic children at heightened risk for an ASD diagnosis (18.7% recurrence rate) demonstrate substantial variability in their language and gesture development (Ozonoff et al., 2011, Iverson et al., 2018) suggesting that associations between expressive and receptive verbal abilities and gestures should be examined in the broader population to capture heterogeneity in communicative development. Our community sample data will inform efforts to characterize early markers of ASD and focus early therapeutic targets for more tailored interventions.

Keywords: Autism, Communication, Language Delay, Cluster Analysis, Community Detection

Disclosure: Nothing to disclose.

P124. Local Network Architecture and Attention Problems in Children With Concussion

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Background: Attention problems are common after concussion in children. Short superficial white matter (SWM) fibers in the brain are particularly vulnerable to concussion in children due to their protracted myelination and location at the grey-white matter interface beneath the cortex. The objectives of this study are to describe alterations in the SWM in children with concussion, and their impact on network community structure and attention.

Methods: Male and female children age 9-10 years with concussion (N = 339) were matched to children without concussion (N = 339) from the Adolescent Brain Cognitive Development Study. Particle filtering tractography was applied to multishell diffusion MRI data to generate matrices weighted by four measures from the tensor model (FA, MD, AD, RD) and two from fiber orientation distribution functions obtained from Constrained Spherical Deconvolution (AFD, NuFO). SWM was derived via length thresholding (<85mm), while measures of local community (modularity, mean clustering coefficient) were calculated from the complete matrices. Attention was assessed with the CBCL attention problem scale. Differences between the concussion and control group and relationship between measures were assessed with linear mixed effects models.

Results: Children with concussion had more clinically significant attention problem scores ($p = 4.9x10\text{-}6$). Concussed children had elevated FA, MD, RD, AFD and NuFO ($ps<2.0x10\text{-}8$), lower AD ($p = 2.0x10\text{-}16$) in SWM as well as lower network modularity ($p = 2.0x10\text{-}16$) and mean clustering coefficient ($p = 2.0x10\text{-}16$) compared to controls. Interactions between SWM measures and age showed that in younger children with concussion altered SWM measures were associated with more attention problems.

Conclusions: Maturation of SWM fibers and local network structure may be altered by concussion and impair attention.

Keywords: Concussion, Attention, Diffusion MRI, Brain Networks, White Matter Microstructure

Disclosure: Nothing to disclose.

P125. Predicting Longitudinal Trajectories of Child Psychopathology With Explainable Neural Network Ensembles: An Adolescent Brain and Cognitive Development Study Analysis

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Background: Early identification of children with behavioral and psychopathological problems is of critical importance to clinicians

and families alike. Although predictive modeling methods such as machine learning (ML) have been used to identify predictive factors, to date most of this work has been cross-sectional, i.e., using baseline predictors to predict baseline symptoms. Here we use an explainable, multimodal, ensemble ML approach using a large dataset (the Adolescent Brain and Cognitive Development (ABCD) Study), in which we use baseline features to predict the emergence of "Persistent" (vs. "Absent/Remitting") Child Behavior Checklist (CBCL) scale scores (Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Rule-Breaking Behavior, Aggressive Behavior, Internalizing, Externalizing, Total Problems) a two-year follow-up period.

Methods: Demographic, environmental, neurocognitive, and brain imaging data were trained in separate neural network-based models and combined in an ensemble to predict Persistent CBCL scores (defined as a CBCL t-score of ≥ 65 on at least two timepoints (baseline/one-year/two-year follow-ups) vs. Absent/Remitting (defined as a CBCL t-score of ≥ 64 on at least two timepoints). Neuroimaging features were reduced by principal components analysis (PCA) prior to inclusion, with separate PCAs performed for each modality (volumetric structural MRI, diffusion tensor imaging, resting state fMRI, task fMRI for each contrast of interest). Site was also included as a predictor. Because the sample was highly imbalanced (~95% were Absent/Remitting for all CBCL scales), the minority class (Persistent) was upsampled and loss function weights were modified such that incorrectly predicting the minority class resulted in greater loss. Learner performance was validated by k-fold cross validation. The explainable artificial intelligence (XAI) integrated gradient method was used to identify the most salient predictors.

Results: Out of a sample size of 7902 with complete data, 378 were defined as Persistent and 7524 as Absent/Remitting on average across CBCL scores. Mean model accuracy across all scales was 79% (95% C.I. 78%-80%), including 83% accuracy (95% C.I. 82%-84%) for Total Problems, 83% accuracy for Externalizing broadband score (95% CI 81%-85%), and 74% accuracy for Internalizing broadband score (95% C.I. 73%-75%). Predictive accuracies for 2-year trajectories of specific syndromes ranged from 70% (for Somatic Complaints) to 90% (for Rule Breaking Behavior). XAI suggested Reasoning scores on the WISC-V battery, School Risk and Protective Factors Questionnaire School Involvement and Environment Scores, family income, and sleep problems were the five most salient predictors of syndrome trajectories.

Conclusions: These results suggest that machine learning using baseline data can predict 2-year trajectories of CBCL scores in children. Cognitive reasoning, school problems, family income levels, and issues with sleep may be particularly effective predictors of clinically significant trajectories.

Keywords: Developmental Psychopathology, Machine Learning, explainable-AI, ABCD Study, Sleep Disturbances

Disclosure: Nothing to disclose.

P126. Efficiency of Evidence Accumulation as a Potential Mediator of the Relation Between Neural Response to Cognitive Demand and ADHD Symptoms in Youth

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Background: Efficiency of evidence accumulation (EEA), a cognitive mechanism formally defined in computational models that drives people's cognitive performance across many contexts, has recently been found to be impaired in several psychopathologies, and most clearly in childhood Attention-Deficit/Hyperactivity Disorder (ADHD). Although emerging findings suggest that EEA may be supported by the frontoparietal network and related neural systems that respond to external task demands, there is currently a dearth of research testing whether EEA plausibly mediates relations between the functioning of these neural systems and psychopathology. In the Adolescent Brain Cognitive Development (ABCD) study sample, we investigated relations between individual differences in neural responses to cognitive demand on the n-back task, which is known to engage frontoparietal and other task-positive networks while suppressing networks associated with off-task processing (e.g., default mode network), and individuals' EEA. We then evaluate whether EEA is a potential mediator of the relationship between neural responses to cognitive demand and ADHD symptoms measured one year later by both parent- and teacher-report.

Methods: We focused on a subsample of youth from ABCD Release 4.0 (n = 2347) who 1) had n-back neuroimaging data from the baseline timepoint that passed quality control checks, and 2) had valid parent and teacher reports of ADHD symptoms at the year-1 follow-up visit. EEA was measured by fitting the diffusion decision model (DDM) to data from the n-back task and data from a separate task completed in the neuroimaging session, the stop-signal paradigm ("go" trials only), using Bayesian estimation within the Dynamic Models of Choice R suite. We created multivariate "expression scores" for individuals' neural responses to cognitive demand by taking voxel-wise parameter estimates for the group-level 2-back versus 0-back contrast – which robustly activates task-positive networks and deactivates task-negative networks – and multiplying these estimates by each individual's voxel-wise parameters for the same contrast. Hence, higher values of such scores indicate that an individual displays a greater expression of the normative pattern of neural responses to cognitive demand. ADHD symptoms were measured dimensionally by fitting a bifactor model to parent- and teacher-report data from the 1-year follow-up and estimating scores for the general factor that represents shared variance across the reporters and across home and school contexts. We first tested bivariate relations between the neural expression scores, EEA, and ADHD symptoms. We then tested formal mediation models in which EEA mediates the relation between the neural expression scores and future ADHD symptoms. All inferential models were estimated in MPlus with clustered bootstrapping used to estimate standard errors while accounting for the nesting of participants within families and ABCD study sites.

Results: Expression scores for neural responses to cognitive demand on the n-back task showed a strong positive relation with EEA on the same task (standardized B = 0.58, p < .001) as well as a smaller relation with EEA on the stop-signal task (B = 0.19, p < .001). Expression scores were negatively related to ADHD symptoms (B = -0.16, p < .001). EEA was related to future ADHD symptoms regardless of whether it was measured on the n-back task (B = -0.17, p < .001) or stop-signal task (B = -0.16, p < .001). The mediation model involving EEA from the n-back displayed an effect of the expression score on EEA (B = 0.58, p < .001), an effect of EEA on future ADHD symptoms (B = -0.12, p < .001), and a significant total indirect effect involving mediation by EEA (B = -0.07, p < .001). A remaining direct effect of the expression score on ADHD symptoms was also detected (B = -0.09, p = .001). Despite the smaller bivariate relation between the neural expression scores and EEA measured on the stop-signal task, all of the above relations remained significant in a second mediation analysis involving EEA on the stop-signal rather than the n-back, suggesting that this mediation effect is not specific to n-back performance.

Conclusions: In a subsample from the ABCD study, we found that neural responses to cognitive demand on the n-back – which

reflect activation of frontoparietal and other task-positive networks and deactivation of the default mode network – show strong relations to EEA on this task as well as more general relations to EEA measured on a separate stop-signal task. Furthermore, we find that EEA partially mediates the relation between neural responses to cognitive demand and future ADHD symptoms measured across parent and teacher reports and that this mediation effect is robust to whether EEA is measured on the same task as the neural responses. These findings both establish neural responses to cognitive demand as a key neural correlate of individual differences in EEA in childhood and provide the first formal test of whether EEA plausibly mediates the relationship between neural functioning and ADHD symptoms.

Keywords: Attention Deficit Hyperactivity Disorder, Computational Cognitive Neuroscience, Computational Psychiatry, Frontoparietal Network

Disclosure: Nothing to disclose.

P127. Combined Effects of Gestational Flame Retardants and Immune Activation on Behavior in Mice

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Background: Evidence suggesting a role for environmental factors contributing to the origin of several neurodevelopmental disorders has accumulated in recent years, and increasingly points to a possible interaction between genetic vulnerability and the environment in the generation of autism and schizophrenia. Indeed, epidemiological studies have consistently found that maternal infection and chemicals in the environment, on their own or in conjunction with genetic factors, increase the risk for ASD. Animal models of maternal infection have determined that the maternal immune activation (MIA) is a key factor in the neurodevelopmental alterations observed in offspring. In addition to exposure to infectious agents, there is increasing evidence that exposure to environmental toxins may contribute to ASD risk. Polychlorinated biphenyls (PCBs, banned since 1976) and polybrominated diphenyl ethers (PBDEs, used as fire retardants) have been accumulating in the environment for decades. Mouse models have demonstrated that exposure to these compounds produce alterations in neurodevelopment leading to changes in synaptic plasticity, and behavioral alterations. Because of the adverse effects observed in humans and the toxicity observed in animal models, it has become an increasing concern that PBDE chemicals contribute adversely to neurodevelopment. Our previous studies showed that prenatal poly(I:C) exposure impaired probabilistic reversal learning, decreased social approach in offspring and produced long term effects on the genes involved in glutamine neurotransmission, mTOR signaling and potassium ion channel activity. This study tested if gestational and early prenatal exposure to PBDE chemicals found in flame retardants will exacerbate the effects of maternal immune activation on behavioral phenotypes linked to neurodevelopmental disorders.

Methods: After completing a dose response of BDE-47 in a previous experiment, we concluded that a 0.03 mg/kg/day dose of BDE-47 was well tolerated in dams. Female C57BL6/J mice (8 weeks of age; Jackson Lab, Bar Harbor, ME) were exposed to 0.03 mg/kg BDE-47 in corn oil via a cornflake for 4 weeks prior to mating and then during gestation through weaning (~10 weeks total). A separate group of female mice received a cornflake with corn oil only. After timed-matings pregnant females were exposed to either Poly(I:C) (20 mg/kg) or saline on E12.5. Offspring were tested in a behavioral battery with relevance to neurodevelopmental disorders.

Results: Overall, litter survival was low in the experiment. The effects was more pronounced in BDE-exposed litters, with litter survival being ~ 35%, compared to Vehicle-exposed litters (~58%). BDE-exposed female mice showed increased locomotor activity compared to vehicle-exposed mice [$F(1,46)=5.98$, $p < 0.05$] with the effects slightly more pronounced in the combined BDE-PolyI:C group. There were no differences in locomotor activity in male mice as a result of BDE or PolyI:C exposure; however, male BDE-exposed mice showed increased Rearing behavior [main effect of BDE; $p < 0.05$]. When mice were tested in the social approach task at 7 weeks old, no differences in social behavior were observed. Because our previous studies showed that male mice are more sensitive to social approach deficits in the MIA model, we re-tested male mice in the social approach task at 6-7 months old. BDE had no effect on social approach; however, male offspring of dams treated with PolyI:C on E12.5 did not spend siginificantly more time investigating the novel mouse vs an empty cup (NS). This resulted in a trend for Vehicle- PolyI:C mice to show decreased percent time with the stranger mouse. Mice were then tested for sensitivity to the dopamine agonist, amphetamine. Female mice exposed to PolyI:C during gestation showed decreased sensitivity to the locomotor-stimulating effects of amphetamine [main effect of PolyI:C; $F(1,37)=4.66$, $p < 0.05$] with the effect primarily being driven by the Vehicle-PolyI:C group [block x gestational exposure x PolyI:C interaction; $F(8,296)=2.30$, $p < 0.05$]. A different pattern of effects was observed in male mice, with BDE-exposed male mice showing decreased sensitivity to amphetamine-induced locomotor activity [$p < 0.05$].

Conclusions: Overall, BDE-exposed mice showed alterations in locomotor activity, investigatory behavior, and sensitivity to amphetamine in a sex-specific manner. Whereas, gestational exposure to PolyI:C produced a mild social approach deficit in male mice and altered response to amphetamine in female mice. Interestingly, there were no significant interactive effects between gestational exposure to BDE-47 and PolyI:C. One limitations of the study is the overall low litter survival.

Keywords: Maternal Immune Activation, Flame Retardant, Neurodevelopmental Disorders

Disclosure: Nothing to disclose.

P128. Functional Analysis and Pharmacological Screening in Zebrafish Mutants of ASD Risk Genes

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Background: Autism spectrum disorders (ASDs) are a group of complex neurodevelopmental disorders that affect social behavior and communication, characterized by the presence of repetitive, restrictive behaviors. Pharmacological interventions that target the core deficits in ASD are still lacking, mainly due to its complex biology and clinical heterogeneity. Whole-exome sequencing has revealed "high confidence" risk genes associated with ASD (hcASD), which are advancing our understanding of the biology of ASDs. In this context, the zebrafish has become an optimal vertebrate model for genetic studies due to its fast development, transparency of its embryos, availability of efficient methods for generating mutant lines, and tractability for high-throughput analyses.

Methods: Using CRISPR/Cas9 technology, we have generated zebrafish mutant lines of ten hcASD genes. To investigate how gene disruption alters basic sensory processing and rest-waking activity, we have established a novel behavioral high-throughput in vivo system, which was used across the different hcASD zebrafish mutant lines. Zebrafish mutant larvae at 5-7 days-post-fertilization are placed in a 96-well plate where responses to lights on and off stimuli and rest-wake behavior are tracked.

Results: These analyses generated a unique behavior fingerprint for each mutant. In parallel, this system was used to screen wild-type zebrafish larvae treated with over 750 FDA-approved drugs to identify their effects on these behaviors, generating a drug-behavior fingerprint. By comparing the effect of the drug-behavior profile to mutant behavioral fingerprints, we were able to identify several dysregulated pathways in mutants and potential suppressors of hcASD behavioral phenotypes. More specifically, using linear mixed model (LMM) analysis, we identified the most significant anti-correlating pharmacological compounds for each zebrafish mutant line. These drugs include compounds representing a range of mechanisms, including estrogenic compounds, anti-inflammatory, and anti-cancer drugs. Next, we exposed mutants to select anti-correlating compounds to identify suppressors of mutant behavioral phenotypes.

Conclusions: The discovery of pharmacological suppressors of these phenotypes has the potential to identify drug candidates for further investigation in ASDs.

Keywords: Autism, Zebrafish, Drug Discovery - New Approaches, High Throughput Screening

Disclosure: Nothing to disclose.

P129. Adverse Childhood Experience and Grey Matter Volume: Results From a Pilot Sample of Puerto Rican Children in New York State

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Background: Increased exposure to adverse childhood experiences (ACEs) is a well-established risk factor for a wide range of negative mental health outcomes (Dube et al, 2003; Dube et al, 2006; Campbell et al, 2016; Shin et al, 2018; Hughes et al, 2017; Leza et al, 2021). ACEs have also been found to relate to changes in brain structure and function (e.g. Teicher et al, 2016; Philips et al, 2014), leading some to theorize that ACE-related mental health risks could be driven by ACE-related neural alterations (Puetz et al, 2015; Edalati et al, 2015).

In line with this perspective, some authors have suggested that low or moderate stress early in life can prepare organisms for future stressful experiences, shaping the brain and behavior to adapt to the environment (Oshri et al, 2020). Indeed, studies in rodents and primates have found that exposure to stress early in life is associated with greater resilience to later novel stressors (Gapp et al, 2014; Parker et al, 2004; Parker et al 2004). Investigations into ACE-related neurodevelopmental alterations have yielded results consistent with this viewpoint. For example, studies have found lower grey matter volumes (GMV) in visual brain regions in adults who witnessed violence as children, and increased GMV in adults who experienced parental verbal abuse (Teicher et al, 2016; Tomoda et al, 2011; Tomoda et al, 2012).

The current pilot results are an early look from a study examining the impact of ACEs on neurodevelopment in children aged 10-14 whose families have participated in the Boricua Youth Study (BYS) in New York, New York. The BYS is an intergenerational, representatively sampled cohort of Puerto Rican families, originally recruited from the South Bronx, New York, and San Juan, Puerto Rico. BYS families have been followed for 20+ years, with a focus on mental health outcomes. Here, in preliminary data, we examined the relationship between ACE-exposure and GMV in children of the original BYS cohort using non-negative matrix factorization (NMF), a constrained dimension reduction technique designed to yield sparse components. In this way, we limit the number of statistical comparisons without sacrificing the interpretability of our results.

Methods: Sixteen children (ages 10 to 14; 9 female) participated in a study at the New York State Psychiatric Institute, New York, NY. ACEs were measured using the CDC Kaiser scale (Fellitti et al, 1998). All participants underwent MR imaging during which T1-weighted images were acquired. Cortical GMVs were extracted from these data using the FreeSurfer pipeline, version 7.2.0.

NMF was applied to the resulting cortical GMV data matrix. When applied to brain morphometry data, matrix factorization techniques decompose the dataset into a set of components, or clusters of brain regions, with a set of subject-specific weights for each component. Each subject's data can then be reconstructed by taking a weighted sum of the components. Therefore, the subject-specific weights serve as a low-dimensional representation of the original dataset, reducing the number of subsequent statistical tests. NMF enforces a non-negativity constraint on both the weights and the components. This leads to sparse components, each concentrated on only a few brain regions. Thus, relationships between subject-level component weights and exposures of interest can be more easily interpreted.

Prior to dimension reduction, all volumes were divided by their standard deviation to promote interpretability of the resulting components. The NMF algorithm was initialized using non-negative double singular value decomposition in order to encourage sparsity and was run with coordinate descent under a Frobenius norm loss using the scikit-learn package until the default numerical tolerance was reached. Sixteen components were used for this decomposition allowing for near perfect data reconstruction while significantly reducing the dimensionality of the data. Following NMF, the relationship between GMV data and ACE exposure was examined through a series of linear models. Each model used subject-level ACEs to predict the reduced dataset (i.e., the weights). These models were fit using the python statsmodels package, version 0.13.2.

Results: In this preliminary sample, ACEs ranged from 0 – 2 (mean total ACEs = 1.06, SD = 0.77). A component primarily concentrated about the left supramarginal gyrus, right lateral occipital cortex, and bilateral precentral cortex showed a significant, positive, relationship with ACEs (beta = 1.01, $p = 0.043$), indicating a positive correlation between greater ACE-exposure and increased GMV in these regions.

Conclusions: In these pilot analyses of preliminary data, we found exposure to ACEs was in the low range (0-2 ACEs). Within this lower range of ACEs, without correction for multiple comparisons, greater exposure to ACEs was associated with increased GMV in brain regions including the left supramarginal gyrus, right lateral occipital cortex, and bilateral precentral cortex. Given that increased GMV in left supramarginal gyrus in early childhood has been associated with improvements in cognitive control (Berger, 2022), it is possible that ACE-related increases in GMV in this region are a sign of neurocognitive adaptations to environmental stressors. Future analyses in a larger sample can further test these findings, as well as associations between GMV and task performance, to determine if ACE-related structural brain changes relate to cognitive task performance.

This research was funded by NIDA: K08DA049913

Keywords: Adverse Childhood Experiences (ACE), Grey Matter Morphometry, Neurodevelopment

Disclosure: Nothing to disclose.

P130. Functional and Physiological Neurobiomarkers of Vicarious Threat Extinction Learning in Parent-Child Dyads

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Background: A critical aspect of socioemotional development during childhood is the learning and unlearning of fear associations from one's environment, much of which occurs while observing a parent or caregiver. While mechanisms of vicarious threat extinction have been explored in animal models and adults, the neurobiological substrates of this formative learning process during childhood remains largely unexplored.

Methods: In this study, we investigated whole-brain activation during day vicarious threat extinction using 27 parent-child dyads (youth ages 10-14). To characterize how previously learned threat associations can be modified or extinguished through parent observation, dyads completed a threat extinction paradigm where acquisition consisted of viewing visual stimuli with (CS+) and without (CS-) partial reinforcement of electrodermal stimulation (ES). During vicarious extinction, youth observed a video of their parent undergoing direct extinction, during which exposure to the CS- and CS + was not paired with ES (CS + vic). The parent completed the behavioral paradigm during simultaneous recording of skin conductance response, while youth completed all phases while undergoing functional magnetic resonance imaging and skin conductance recording. To estimate stimulus specific patterns in whole-brain activation, scans during vicarious extinction training were preprocessed using FMRIPREP and results comparing activation during the CS + vic and CS- presentation following cluster-size thresholding are presented (voxelwise threshold $p < 0.0001$). Finally, parent-child physiological synchrony was estimated using cross-recurrence quantification analysis (CRQA).

Results: During vicarious extinction training, youth exhibited significantly decreased activation in the bilateral posterior insular cortex (pIC) while watching their parent view the CS + vic, as compared to watching their parent view the CS- (left, $k = 423$, pcorr<0.01; right, $k = 447$, pcorr<0.01). Differential activation in the right pIC during the CS + vic was further positively correlated with parent-child autonomic synchrony during the task ($r = 0.502$, $p = 0.034$). Interestingly, parent-child synchrony measures were not predictive of pIC activation while observing the CS- ($r = -0.222$, $p = 0.374$).

Conclusions: These results first demonstrate novel biomarkers of the transmission of threat and safety learning in parent-child dyads during a critical period of adolescence and suggests a potential mechanism of this transmission via parent-child autonomic synchrony. Differential activation in the bilateral pIC while viewing a parents' exposure to previously learned aversive stimuli provides additional support for the critical role of the pIC in extinction memory consolidation and may further be involved in decoding parent modeling of threat responses to aversive stimuli.

Keywords: Functional MRI (fMRI), Parent - Child Dyads, Extinction Learning

Disclosure: Nothing to disclose.

P131. Developmental Patterns and Cognitive Relevance of Intrinsic Neural Timescales in Humans

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Background: Many neuropsychiatric disorders are thought to have a developmental origin given their typical early onset and substantial genetic risk. As such, it is imperative to describe the normative developmental trajectories of neuroimaging phenotypes to contextualize neurodevelopmental alterations in individuals who develop a neuropsychiatric disorder. One such neuroimaging phenotype is the resting-state functional MRI measure of intrinsic neural timescale (INT). INT reflects the time window of neural integration and is thought to reflect the strength of recurrent excitation in cortical microcircuits—an index of excitation-inhibition balance (E/I) critical for cognition. We, and others, have recently reported reduced INT in patients with schizophrenia, but it is not clear how these reductions relate to neurodevelopment.

Importantly, E/I is affected in a range of neuropsychiatric disorders and recurrent excitation plays a critical role in cognitive functions such as working memory. Persistent activity in higher-order cortical circuits, including dorsolateral prefrontal cortex, has long been thought to be a key substrate for maintaining information over delay periods in working-memory tasks. Synthetic biophysical models of canonical microcircuits, even in their simplest form, can themselves perform working-memory tasks, recapitulating primate behavior and electrophysiology. Also consistent with these models is the observation that single-neuron INT positively correlates with working-memory performance, although this has yet to be tested with non-invasive INT measures in humans.

Here, we used two large-scale neuroimaging studies from the Human Connectome Project (Development [HCP-D] and Young Adult [HCP-YA]) to characterize age-related developmental patterns of INT throughout the brain and to test the hypothesized link between resting-state INT and working-memory performance.

Methods: Resting-state fMRI data were collected from two publicly available datasets ($n = 1601$; 55% female): 1) HCP-D ($n = 591$, 6–21 years old); 2) HCP-YA ($n = 1010$, 22–37 years old). INT maps were estimated as previously described (Wengler et al., eLife, 2020). Briefly, the autocorrelation function of the fMRI signal at each grayordinate was estimated and the sum of the autocorrelation coefficients during the initial positive period was calculated. INT maps were parcellated using the HCP-MMP-v1.0 atlas and averaged across hemispheres for a total of 188 parcels. INT maps were harmonized using ComBat. Developmental curves were calculated for each parcel using general linear models: INT = B0 + B1*age + B2*age^2 + B3*sex + B4*motion. Inflection points were calculated for each parcel and hierarchical effects evaluated by correlation; hierarchical rank was determined by ranking INT values from the HCP-YA group-averaged INT map. The relationship with cognition was evaluated in the HCP-YA sample ($n = 781$, excluding subjects with worse performance on 0-back than 2-back) by partial correlation between N-back performance and the average INT in parcels significantly activated by the N-back task—2nd-level results for the HCP-YA sample determined 47 a priori parcels (contrast: 2-back>0-back)—controlling for motion (mean framewise displacement). Permutation tests were used to determine statistical significance and correct for multiple comparisons.

Results: Nearly all parcels showed significant age effects characterized by an inverted-U shape with an increase in INT across early childhood/adolescence and a decrease in INT in adulthood (age: 180/188 parcels positive effect, $P < 0.05$, P_FWE,-permutation = 0.001; age2: 182/188 parcels negative effect, $P < 0.05$, P_FWE,permutation = 0.001). Inflection points (INT peaks of fitted quadratic functions) ranged from ~21–36 years of age and occurred later in higher-order brain regions (correlation between inflection point and hierarchical rank: $r = 0.75$, P_permutation = 0.001). A sex-stratified analysis demonstrated that females had later inflection points than males (mean difference = 4.20 years, $t = 18.6$, P_permutation = 0.001). Results were

robust to controls for motion, including in a motion-matched subgroup (n = 1343) and a subgroup with more stringent motion exclusion-criteria (n = 690).

Subjects with better cognitive performance (as indexed by 2-back minus 0-back accuracy on the N-back task) had longer INT in brain regions which are activated by the task (r_partial = -0.09, P_permutation = 0.012; i.e., less difference between 2-back and 0-back accuracy). This was driven by subjects with longer INT having better performance during the 2-back condition requiring working memory (r_partial = 0.12, P_permutation = 0.001).

Conclusions: Our results describe the age-related developmental patterns of INT across pre- and post-pubertal development, showing that almost all brain regions exhibit an inverted-U-shaped pattern across the studied age range (5–37 years). Higher-order brain regions (e.g., association cortex) reach their developmental peak (longest INT value) later in life than lower-order brain regions (e.g., sensory cortex). In addition, we showed that subjects who have longer INT—measured at rest—performed better on an N-back task. These results confirm the theoretical link between temporal integration windows at rest (i.e., INT) and a subject's ability to maintain information in working memory, supporting the potential value of INT for studying developmental cognitive dysfunction in neuropsychiatric illness.

Keywords: Intrinsic Neural Timescale, Brain Development, Cognition, Working Memory, Magnetic Resonance Imaging

Disclosure: Nothing to disclose.

P132. Sex-Dependent Effect of Adolescent Stress on Adult Affective Dysregulation and PV/SST Content in the pLPFC

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Background: Stress is a socio-environmental risk factor for the development of psychiatric disorders, with the age of exposure potentially determining the later outcome. Early life adversity, such as during adolescence, has a significant impact on the development of neuropathological states in which females respond differently to stressors compared to males. Adolescence is a critical period for maturation of the medial prefrontal cortex (mPFC) and it is also when sex differences in PFC structure begin to emerge. Dysfunction of GABAergic network within the prelimbic portion of PFC (plPFC) during development increases vulnerability to adult affective dysregulation. However, the precise neurobiological mechanisms/circuits that contribute to differential sex responsivity to adolescent stress are understudied. Here we investigated the impact of stress exposure during adolescence on adult susceptibility to anhedonia, helplessness and dopaminergic activity in both sexes.

Methods: Male Sprague-Dawley rats were subjected to a combination of footshock/restraint stress during adolescence (postnatal day 31-40). The rats were tested for sucrose preference (PD70), learned helplessness (PD72-73; Day 1, inescapable footshock; Day 2 escape session) and single-unit extracellular electrophysiology recording of VTA DA neurons (>PD78; four days after LH day 2). Independent group of animals were submitted or not to stress and perfused at PD31, PD41, PD51, and PD75 for posterior immunohistochemistry analysis of PV/SST content in the plPFC. All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Results: Adolescent stress decreased the sucrose preference (n = 4 each group, $t6 = 4.41$, $p < 0.05$, t-test) and increased the % of helplessness behavior only in males (Naïve 35.71%, 9 nonhelplessness, 5 helplessness; Stress 75%, 4 nonhelplessness, 12 helplessness, Chi-square=30.79, $p < 0.05$). No significant effect was found regarding sucrose preference or helplessness behavior in all female groups (Sucrose preference, n = 4 naïve, n = 5 stress, $t7 = 0.21$, $p > 0.05$, t-test; Helplessness; Naïve 46.15%, 7 nonhelplessness, 6 helplessness, Stress 43.75%, 9 nonhelplessness, 7 helplessness, Chi-square=0.0022, $p > 0.05$). Moreover, a decreased number of spontaneously active DA neurons was observed in rats showing helpless behavior across all groups exposed to LH. However, adolescent stress increased the number of DA neurons when the rats were not exposed to LH (Behavior, $F5,37 = 24.29$, ANOVA, $p < 0.05$). This is similar to data showing that chronic mild stress attenuates the hyperdopaminergic state in MAM rats. Adolescent stress increased the PV (n = 4 each group, Age x Condition $F2,18 = 3.85$, Two-way ANOVA, $p < 0.05$) and SST (n = 4 each group, Age x Condition $F2,18 = 4.49$; Two-way ANOVA, $p < 0.05$) expression in the plPFC at PD51 only in males.

Conclusions: Our findings indicate that stress during adolescence increases negative affect behavior, such as decreased sucrose preference and increased helplessness, along with down-regulation of dopaminergic activity in the VTA only in males. Males have an increased expression of GABAergic markers PV and SST at PD51. Females are resilient to adolescent stress at all behavioral and physiological markers. Therefore, a sex-dependent predisposition of early life adverse events that impair plPFC activity may enhance susceptibility to affective disturbance in adulthood.

Keywords: Adolescence, Sex Difference, Early Life Stress, Dopamine, Affective Behavior

Disclosure: Nothing to disclose.

P133. Maturation of Nucleus Accumbens Synaptic Transmission Signals a Sensitive Period for the Rescue of Social Deficits in a Mouse Model of Autism Spectrum Disorders

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Background: Social behaviour emerges early in development, a time marked by the onset of neurodevelopmental disorders featuring social deficits, including autism spectrum disorders (ASD). Although deficits in social interaction and communication are at the core of the clinical diagnosis of ASD, very little is known about their neural correlates at the time of clinical onset of the disorders. The nucleus accumbens (NAc), a brain region extensively implicated in social behaviour, undergoes synaptic, cellular and molecular alterations in early life, and is particularly affected in ASD mouse models.

Methods: Male and female C57BL/6J and BTBR T + Itpr3tf/J mice were bred in house and tested in a three-chamber social interaction or social memory tests at postnatal day (P)30 (n = 27, 32). Slice electrophysiology of nucleus accumbens medium spiny neurons was conducted as in our previous work (Arruda-Carvalho et al., J Neuroscience 2017) at P4, P6, P8, P15, P21 and P30 (n = 10-22 cells/group) examining spontaneous excitatory and inhibitory transmission. Rapamycin (0.5mg/kg) intraperitoneal injections took place daily from either P4-P8 (n = 12, 14) or P60-P64 (n = 9,10) in BTBR mice, which were tested for social interaction at P30 and P86, respectively.

Results: To explore a link between the maturation of the NAc and neurodevelopmental deficits in social behavior, we compared age-dependent changes in spontaneous synaptic transmission in NAc shell medium spiny neurons between the highly social C57BL/6J mouse strain and the idiopathic ASD mouse model BTBR

T + Itpr3tf/J, which show deficits in social interaction (Two-way RM ANOVA, significant effect of chamber F1.536, $90.61 = 38.79$, $p < 0.0001$, and chamber x strain interaction F2, $118 = 16.71$, $p < 0.0001$) at postnatal day (P) 4, P6, P8, P15, P21 and P30. We found changes in spontaneous excitation and inhibition started as early as P4, highlighting a potential critical period in the maturation of NAc which could modulate the efficacy of interventions aimed at NAc-dependent behavior. To test this possibility, we injected BTBR mice in either early life (P4-P8) or adulthood (P60-P64) with the mTOR1 antagonist rapamycin, a well-established rescue intervention for ASD-like behavior. We found that rapamycin treatment rescued social interaction deficits in BTBR mice when injected in infancy (Two-way RM ANOVA, significant effect of chamber F2, $48 = 22.42$, $p < 0.0001$, and chamber x treatment interaction F2, $48 = 9.436$, $p < 0.0003$), but not in adulthood (Two-way RM ANOVA, significant effect of chamber F2, $34 = 7.904$, $p = 0.0015$ only).

Conclusions: In this study, we found age-dependent changes in the maturation of spontaneous excitatory and inhibitory transmission within and between strains, with BTBR mice showing overall increased spontaneous inhibition and decreased excitation at timepoints as early as P4. Given the timeline of these changes, we hypothesized that early developmental timepoints might encompass a critical period for the efficacy of rescue manipulations correlating with improved outcomes. Consistent with this, rapamycin treatment in infancy, but not adulthood, reversed the social interaction deficits in BTBR mice. Studying brain regions involved in the pathophysiology of neurodevelopmental disorders at clinically-relevant timepoints may offer novel insight into the timing and targets of therapeutic interventions to maximize positive outcomes.

Keywords: Social and Behavioral Deficits, Nucleus Accumbens, Brain Development, Autism Spectrum Disorder

Disclosure: Nothing to disclose.

P134. Single-Nucleus Transcriptomic Analysis of the Prefrontal Cortex During Development and Early-Life Stress

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Background: The brain contains interconnected circuits which are neither completed at birth or invariant across life. This neuronal plasticity is essential for life-long adaptive features like continuous learning and memory. However, this plasticity, especially when associated with severe adverse factors during early-life, can lead to the derailment of normative brain development and contribute to the etiology of behavioral deficits and psychiatric disorders. One of the most influential environmental factors during early-life is parental/caregiver care. Childhood adversity has been estimated to account for a significant percentage of adult-onset mental health disorders. In extreme cases of childhood adversity, institutional rearing where the infants were deprived of caregiver contact, cognitive deficits and dysregulated prefrontal cortex (PFC) function were found. In this study we hypothesize that early-life adversity, in the form of maternal separation, leads to long-lasting changes in the transcriptome of specific PFC cell populations leading to a dysregulation of PFC function.

Methods: Here, we use a mouse model of maternal separation. The brains of pups and adults of normal reared or maternal separated (MS) animals were collected. In one set, we performed single-nucleus RNAseq with hashing to contrast the PFC transcriptome of these groups. In a separate cohort, we recorded whole slice voltage dye (VSD) responses to explore how changes in receptors identified by snRNAseq altered PFC responses.

Results: Using snRNAseq, as expected, we observed that the changes between ages were much more accentuated than changes between treatments. We also observed that the proportion of oligodendrocytes in adult-MS animals was similar to infant animals. Furthermore, we found that most of the differential gene expression between MS and standard reared animals was in interneurons, affecting pathways related to GABAergic, glutamatergic, and serotonergic functions. Using VSD we observed that adult-MS responses to GABAergic and serotonergic agonists were similar to the responses of more immature normal-reared animals.

Conclusions: This study suggests that MS leads to an immature PFC that may be linked to the behavioral deficits observed in animals and humans exposed to poor early-life care.

Keywords: RNAseq, Prefrontal Cortex, Early-life Stress

Disclosure: Nothing to disclose.

P135. Effects of Juvenile Ketamine and/or Psychological Stress Exposure on Spatial Memory Performance in Adult Mice

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Background: Ketamine is currently being used for the management of treatment resistant depression in adolescent patients. However, the possible long-term effects of ketamine exposure during adolescence have not been thoroughly assessed. Thus, we examined whether repeated exposure to concomitant ketamine and/or psychological stress, during the adolescent stage of development, results in long-lasting spatial memory alterations in male and female C57BL/6 mice ($N = 36$; 9 per group, $N = 44$; 11 per group, respectively).

Methods: Male and female postnatal day (PD)-35 mice underwent 10 days of vicarious defeat stress (VDS; a form of psychological stress) with or without ketamine exposure (20 mg/kg; PD35-44). Once mice reached adulthood (PD70) separate groups were assessed for spatial memory performance adopting a water maze task.

Results: We found that singular pre-exposure to ketamine or VDS increased the latency (sec) to locate the escape platform in adult male, but not female, mice – revealing that ketamine, like psychological stress, induces an enduring spatial memory impairment in males only. However, history of concomitant ketamine and VDS prevented spatial memory impairment in adulthood.

Conclusions: Together, our findings suggest that ketamine, as a prophylactic treatment for adolescent psychological stress-induced illnesses, does not lead to long-term changes in spatial memory. However, juvenile recreational ketamine-use, like psychological stress history, results in an enduring spatial memory deficit in a male-specific manner.

Keywords: (R,S)-Ketamine, Adolescent, Spatial Memory

Disclosure: Nothing to disclose.

P136. Prepregnancy High BMI is Associated With Sex-Specific Alteration in Maternal and Fetal Heart Rate Parameters and Differential Placental DNA Methylation With Later Effects on Mother-Reported Infant Temperament

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Background: Increased rates of obesity in general world-wide is also paralleled by increased BMI during pregnancy, which is known to impact pregnancy and birth outcomes. Recent research also indicates that early in-utero factors associated with maternal obesity could affect the long-term health trajectories of individuals including metabolic and neurobehavioral outcomes that are believed to be mediated by an underlying molecular fingerprint set during these early developmental periods by epigenetic mechanisms. High BMI is also associated with altered stress and autonomic nervous system physiology in individuals, which could contribute to these outcomes. Studies indicate that the developing fetus could present early markers of programming effects of altered autonomic nervous system to different maternal environmental exposures through heart rate (HR) measurements and a marker of future child neurobehavioral functioning. The placenta is an integral organ and a key mediator of environmental exposures affecting the developmental programming of the fetus leading to long lasting health impacts. Sex-specific effects of maternal obesity have been reported in the literature; however, no studies have examined its effects sex-specific on fetal neurobehavioral and infant temperament outcomes and the underlying differential placental DNA methylation. In this study we aim to examine the impact of maternal pre-pregnancy BMI on stress related psychological and physiological variables in pregnant women and fetal neurodevelopment by measuring fetal heart rate and later infant temperament. In addition, we investigate the epigenetic modification of genes in the placenta in response to high pre-pregnancy BMI.

Methods: Pregnant women (n = 176) answered the Perceived Stress Scale (PSS) questionnaire and gave saliva samples at gestation weeks (GA) 12-22, 23-28, and 34-36, and attended fetal laboratory assessment sessions in GA 23-28, and 34-36 to obtain maternal and fetal HR at baseline and in response to maternal cognitive challenge. Placentae near the fetal surface were collected at birth and DNA methylation was assessed using Illumina array (n = 147). At a 4-month postpartum visit (n = 75), mothers completed the Infant Behavioral Questionnaire to assess infant temperament.

Results: We found no association between BMI and diurnal salivary cortisol or PSS. However, only women with high BMI had higher HR, lower HR variability in both temporal and frequency domains. These effects were only exhibited in women pregnant with female and not male fetuses. Moreover, we found similar sex-specific differential effect of high BMI on fetal HR across gestation, with female fetuses of high BMI women exhibiting significantly lower HRV in the 3rd trimester. Interestingly, these sex-specific effects continued into infancy with female infants of high BMI women reported to have lower scores on orienting/regulation dimension of temperament, including soothability. Preliminary differential DNA methylation analysis revealed 4 differentially methylated probes, three of which were on the chromosome 12 and one on chromosome 10. Only the probe on the 10th chromosome was associated with an annotated gene, H2AFY2, and located in the 5'UTR. This probe was hypomethylated. DMR analysis also revealed a region in chromosome 10 in H2AFY2 gene, which codes for macroH2A2, a core histone from the H2A. macroH2A2 is known as an epigenetic repressor associated with X chromosome inactivation and transcriptionally supressed regions genome wide. DNA methylation analysis stratified by sex did not reveal any significant association.

Conclusions: Our results indicate that maternal pre-pregnancy high BMI impacts on maternal HR parameters are only observed in women pregnant with females and these effects are mirrored in their female fetuses. These sex-specific effects continued into infancy and were associated with neurobehavioral outcomes related to infant temperament. Maternal pre-pregnancy high BMI left a molecular signature on placental DNA, with reduced methylation in the promoter of a gene coding a histone variant that is associated with transcription repression, which could impact the transcriptional landscape of the placenta and fetal development.

Keywords: Obesity, Sex-specific Effects, Infant Behavior, DNA Methylation, Fetal Neurobehavior

Disclosure: Nothing to disclose.

P137. Relationships Between Adrenarche-Associated Hormone Concentrations and Reward-Related Neural Processing in Prepubertal Children

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Background: The pubertal transition involves significant physical, social, emotional, and cognitive changes that are linked to greater propensity to engage in risky behaviors, and is timed with the development of several neuropsychiatric disorders. These puberty-related behavioral changes, such as increased impulsivity and risky choices, have been attributed, at least in part, to the effects of emerging pubertal hormones (adrenal, gonadal, and growth hormones) on reward-related brain regions, including the prefrontal cortex, ventral striatum and subcortical limbic regions. However, it has been challenging for experimental approaches to disambiguate the neurotrophic effects of adrenal androgens from those of gonadal steroids. Adrenarche, marked by increased secretion of adrenal androgens including dehydroepiandrosterone sulfate (DHEAS), is the earliest stage of pubertal development, usually starting between 7 and 8 years of age, and precedes gonadarche (i.e., surges in the gonadal hormones estradiol and testosterone). Here, using a reward paradigm with fMRI, we examined the relationships between DHEAS serum concentrations and reward-related brain activation in healthy, typically-developing boys and girls who were carefully documented to be prepubertal and, thus, prior to increases in gonadal hormones (i.e., gonadarche).

Methods: Ninety typically developing, prepubertal children (as ascertained by clinician physical examination; 37 girls, mean age=8.6 + -0.3y; 53 boys, mean age=8.6 + -0.3) completed three runs of a modified monetary incentive delay task in a 3T MRI scanner. Data were derived from the first, baseline prepubertal visit of participants in a larger longitudinal study. The reward paradigm involved trials of low and high reward probability with anticipated levels of difficulty, along with control trials without monetary gain. Serum samples of DHEAS and testosterone were assayed using liquid chromatography-tandem mass-spectrometry, and DHEAS concentrations were log transformed for analysis. Serum estradiol was assayed using a chemiluminescence assay. Two separate whole-brain, voxel-wise multivariate model analyses using AFNI's 3dMVM were run to test for correlations between DHEAS and activation during anticipation of reward and activation during receipt of reward (reward gain). Log-transformed DHEAS was and included as a continuous variable while controlling for sex. Post-hoc Spearman's correlations were run in R to determine correlation directionality.

Results: Both plasma estradiol and testosterone were at prepubertal concentrations, and no significant sex differences were observed in age or serum DHEAS (p's > 0.6). Among participants, DHEAS ranged from 0.15 mcg/mL to 1.63 mcg/mL (the DHEAS biochemical threshold for onset of adrenarche is reported to be ≥ 0.4 mcg/mL.) During reward gain, log-transformed DHEAS

concentrations were positively correlated with left hippocampal and right ventromedial prefrontal cortex (vmPFC) activation (pFDR<0.05). In contrast, negative correlations were observed during reward anticipation between log-transformed DHEAS and activations in bilateral dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex, orbitofrontal cortex, and caudate (pFDR<0.05).

Conclusions: These neurofunctional/DHEAS relationships were found in prepubertal children prior to increases of the gonadarche-related hormones estradiol and testosterone, suggesting that adrenal androgens are associated with and may influence the function of key neural systems related to reward processing. Future directions will involve longitudinal analyses to better understand the maturation of these reward-related brain systems, beginning with this prepubertal baseline sample and following through various pubertal stages to disentangle the effects of the relevant endocrinological events (e.g. gonadarche) on brain development.

Keywords: Adrenarche, Puberty, Children and Adolescents, Brain Development

Disclosure: Nothing to disclose.

P138. Prenatal Exposure to Early Life Adversity and Neonatal Brain Connectivity on Early Executive Function at Age 2 Years

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Background: Childhood exposure to early life adversity (poverty and psychosocial stressors) alters brain structural and functional development in frontal, parietal, and temporal regions involved in the neural networks of executive function (EF). Aberrant brain development and related problems in EF increases risks for psychiatric disorders including attention deficit hyperactivity disorder and anxiety disorders. However, whether associations between early life adversity and brain development begin prenatally to alter emerging EF is unknown, which may inform the timing of early preventative interventions. Maternal attunement to infant mental states (Maternal Mind-Mindedness, MMM) is one aspect of the early caregiving environment that supports EF and brain connectivity in older children. The extent that MMM buffers the adverse effects of early life adversity to shape the neural underpinnings of emerging EF is unclear.

Methods: This longitudinal study focuses on 399 infant-mother dyads oversampled for poverty exposure, identified from the March of Dimes Research Center at Washington University in St. Louis. During pregnancy, mothers completed background measures analyzed with confirmatory factor analysis which resulted in two latent factors: Social Disadvantage (education level, health insurance status, income-to-needs ratio [INR], area deprivation index, nutritional intake) and Psychosocial Stress (depression symptoms, perceived stress, racial discrimination, stressful/traumatic life events). At birth, neonates (56% male, 44% female) underwent resting state functional ($n = 319$) and diffusion ($n = 303$) MRI scans on a Prisma 3T scanner. Key resting state networks included cingulo-opercular (CO), frontoparietal (FPN), ventral (VAN) and dorsal attention (DAN), and default mode (DMN) networks. Key white matter tracts included the cingulum, uncinate, and inferior fronto-occipital fasciculus (IFOF). At the 1-year follow-up, MMM was observed during a parent-child interaction task ($n = 232$ coded). At age 2 years, the Minnesota Executive Function Scale (MEFS) task was used to assess EF outcomes ($n = 125$ assessed to date) and INR was collected again as a measure of postnatal disadvantage. Prenatal Social Disadvantage and Psychosocial Stress factors were related to neonatal brain connectivity measures using multiple regression adjusted for covariates (age at scan, preterm birth [<37 weeks gestation], sex). Adversity variables, brain connectivity measures, and MMM ratings were fitted to MEFS scores using multiple regression and moderation analyses adjusted for covariates.

Results: Prenatal exposure to Social Disadvantage was independently related to aberrant neonatal CO ($\beta = -.15$) and VAN-FPN ($\beta = .13$) network connectivity and right dorsal cingulum ($\beta = -.17$), bilateral inferior cingulum ($\beta = -.20$) and left IFOF ($\beta = -.17$) microstructure (all $p \leq .03$). Psychosocial Stress was independently related to CO-DMN connectivity ($\beta = .16$, $p = .007$) and left uncinate microstructure ($\beta = .14$, $p = .01$). Social Disadvantage and aberrant FPN-CO, IFOF, and cingulum connectivity predicted poorer EF at age 2 years (all $p \leq .03$). Maternal Psychosocial Stress was not independently related to EF. Infants born preterm had poorer EF outcomes at age 2 years than term-born infants ($t = 1.98$, $p = .05$) but there was no difference in EF by sex ($t = .0.12$, $p = .90$). At follow-up, year 1 MMM was positively associated with EF at age 2 years ($\beta = .24$, $p = .02$). Moderation analysis suggested that prenatal INR was associated with worse EF outcomes in the lower MMM exposure group whereas this association was reduced in the higher MMM exposure group (interaction term $B = -.06$, $p = .03$, total model $R2 = .19$). Although pre- and postnatal INR were highly correlated ($r = .92$, $p < .001$), there was no interaction between postnatal INR and MMM on EF ($p = .07$). Moderation analysis also showed that infants exposed to higher MMM had stronger neonatal cingulum connectivity-EF outcome relationships than infants exposed to lower MMM (interaction term $B = -.4.56$, $p = .03$, total model $R2 = .28$). There was no interaction between MMM and either FPN-CO or IFOF connectivity on EF (all $p > .05$).

Conclusions: Integrating multi-modal neuroimaging, observational, and behavioral data elucidates the pathways by which EF delays emerge in early childhood. Prenatal exposure to poverty and altered neonatal brain connectivity increase risks of poorer EF outcomes. MMM may buffer the effects of poverty exposure and enhance brain-EF relationships, making MMM an important intervention target to support brain-EF development.

Keywords: Prenatal Programming, Resting State fMRI, Diffusion MRI, Executive Function

Disclosure: Nothing to disclose.

P139. Maternal Inflammation During Pregnancy is Associated With ADHD in Children at Age 10: Results From the COPSYCH Study

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Background: Increasing evidence support that maternal inflammation during pregnancy affects early neurodevelopment, however the evidence for long-lasting risks for aberrant neurodevelopment are scarce and based on preclinical data and human observational register studies.

In the current prospective birth cohort, COPSYCH, we investigated associations between early life inflammation in terms of maternal inflammation during pregnancy and psychopathological

clinical outcomes in the children at age 10. We aim to repeat the analyses using the inflammatory status from the children at 6 months of age as the independent variable. Moreover, will corroborate clinical findings using diffusion weighted imaging parameters.

Methods: COPSYCH is a translational research alliance between CNSR (Center for Neuropsychiatric Schizophrenia Research) and COPSAC (Copenhagen Studies of Asthma in Childhood) (ClinicalTrials.gov: NCT00798226 and NCT00856947).

COPSYCH is established on the population based COPSAC2010 cohort consisting of 700 unselected mother-child pairs, who have been followed prospectively since pregnancy week 24.

At 10-years of age the children have completed a 2-day visit comprising of a thorough examination of neurodevelopment including categorical and dimensional psychopathological examinations (Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS) and various validated rating scales) and neurocognitive tests as well as a 3 Tesla cerebral MRI scan including diffusion tensor imaging (DTI) sequences. Longitudinally measures of the inflammatory marker high sensitivity CRP (HS-CRP) as well as comprehensive data on potential confounders were obtained from the COPSAC2010-database.

Logistic regression and multiple linear regression were used to estimate odds ratios (OR) for psychopathological outcomes. Statistical analyses were conducted using multiple linear regression for continuous outcomes (symptoms scores and ratings scales). The independent variable, HS-CRP, was log 2-transformed to ease interpretation. Logistic regression was used for binary outcomes (ADHD yes/no).

Sample size: The COPSYCH2010 cohort at baseline, $N = 700$. Follow up rate at the current 10 years follow-up was $N = 592$ (86 %). Complete datasets after adjusting: $N = 567$.

The results were checked for interaction of gender and if present the analyzes were stratified by sex. There was no interaction of sex on diagnosis level, but it was significant for symptom scores and rating scales, indicating that the effect of HS-CRP was driven by the boys (likely due to statistical power). HS-CRP vs K-SADS symptom score ADHD: estimate 0.40, interaction of sex, $p = 0.01$. Boys: estimate 0.60, $p < 0.001$. Girls: estimate: 0.16, $p = 0.07$. ADHD-RS and CBCL DSM 5 ADHD scale showing similar results. Statistical significance was set at $p < 0.05$. Statistical analyzes were calculated using R-software.

Results: Out of the 700 children in the birth cohort, 592 children underwent the COPSYCH visit at age 10 (84.6%). Sixty-five, (10.9% fulfilled a research diagnosis for ADHD (16 girls (25%) and 49 (75%) boys). Maternal HS-CRP in pregnancy week 24 (mean 11.4 mg/l) was significantly associated with K-SADS diagnosis of ADHD, OR 1.48 (1.22-1.88), $p < 0.001$. The association was preserved at symptom level as well as across validated questionnaires (ADHD-RS and CBCL-DSM5 ADHD scale).

Exploratory analyses revealed that the association was driven by the inattentive type of ADHD (i.e. ADD), OR 1.65 (1.24-2.07), $p < 0.001$ over the conventional ADHD symptomatology including hyperactivity, impulsivity, and inattention OR 1.23 (0.96-1.59), $p = 0.1$.

No significant associations with HS-CRP were revealed for the other neurodevelopmental disorders including autism spectrum Disorder, Tourette's syndrome, or tic disorder.

Conclusions: The results add clinical data to the growing evidence of the importance of prenatal early life exposures including maternal inflammation. Identifying inflammation as an important marker will provide a potential target for future increased awareness and prevention during pregnancy, ultimately improving neurodevelopmental trajectories in children.

Keywords: Maternal Inflammation, ADHD, Birth Cohort

Disclosures: Boehringer Ingelheim: Contracted Research (Self), Lundbeck: Honoraria (Self)

P140. Cortical and Subcortical Structural Differences in ADHD Youth With and Without a Family History of Bipolar I Disorder: A Cross-Sectional Morphometric Comparison

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Background: Attention deficit/hyperactivity disorder (ADHD) commonly precedes the onset of bipolar I disorder (BD), and prospective studies have found that prodromal ADHD significantly increases risk for developing mood disorders including BD. Moreover, the prevalence rates of ADHD in youth with BD are substantially higher than the general population, particularly in pre-pubescent children. Additionally, having a first-degree relative with BD robustly increases the risk of developing BD, and youth with a first-degree BD relative exhibit higher rates of ADHD and more severe ADHD symptoms. While these findings suggest that ADHD in conjunction with familial risk for BD may represent a different and more severe illness that confers greater risk for developing BD, associated neuropathogenic mechanisms remain poorly understood. The initial onset of BD frequently occurs during the peripubertal period which is associated with linear increases in frontal cortex gray matter expansion and connectivity with subcortical regions implicated in emotional regulation. Previous structural MRI studies have observed cortical and subcortical gray matter structural abnormalities in youth with BD or ADHD compared with healthy developing youth. However, the majority of these studies did not control for BD familial risk, ADHD comorbidity, and/or psychostimulant exposure. In the present cross-sectional study, we investigated regional cortical and subcortical morphometrics in psychostimulant-free ADHD youth with and without a first-degree relative with BD and a typically developing control group. We additionally evaluated relationships between morphometrics and relevant symptom measures.

Methods: ADHD youth (ages 10-18 years) with ('high-risk', HR) and without ('low-risk', LR) a first-degree relative with BD and typically developing healthy controls (HC) with no personal or family history of a DSM-5 Axis I psychiatric disorder were enrolled. All ADHD youth met DSM-5 criteria for ADHD (any type), had no exposure to psychostimulants for at least 3 months prior to enrollment, and had no comorbid mood, conduct, eating, or psychotic disorders. Clinician ratings of ADHD (ADHD-rating scale, ADHD-RS), mania (Young Mania Rating Scale, YMRS), depression (Children's Depression Rating Scale-Revised, CDRS-R), global functioning (Children's Global Assessment Scale, CGAS), and global symptom severity (Clinical Global Impression-Severity Scale, CGI-S) were performed, and parents completed the Child Behavior Checklist (CBCL). High-resolution 3D T1-weighted images were acquired using a Philips 3.0 T MR scanner. FreeSurfer image analysis was used to obtain measures of cortical thickness, surface area, as well as the volumes of 14 subcortical structures. Group differences were evaluated using a general linear model with the age and sex as covariates. Post hoc permutation tests evaluated pair-wise group differences (FDR corrected $p < 0.05$). Partial correlations were computed for symptom severity measures and all significant structural differences in LR and HR groups, and interaction effects were tested by using linear mixed effects models with age and sex as covariates (FDR corrected $p < 0.05$).

Results: A total of $n = 142$ adolescents (mean age: 14.16 ± 2.54 years, 35.9% female) were included in the analysis (HC, $n = 48$; low-risk, $n = 49$; high-risk, $n = 45$). No significant group differences were observed for age, sex, handedness, or prior psychostimulant exposure in the ADHD groups. Compared with the LR group, the HR group had higher ADHD-RS hyperactivity/impulsivity subscale

scores ($p = 0.03$), YMRS ($p = 0.004$), and CGI-S ($p = 0.01$) total scores, and higher CBCL total score ($p = 0.002$) and internalization ($p = 0.01$), externalization ($p = 0.002$), and dysregulation ($p = 0.047$) subscale scores. For subcortical volumes, no significant differences were found between LR and HC groups. The HR group exhibited smaller bilateral thalamus, caudate nucleus, and hippocampus volumes compared with the LR group as well as the HC group with the exception of the right caudate. For cortical surface area, the LR group exhibited a larger right parsopercularis compared with the HC group. In contrast, the HR group exhibited smaller left postcentral, bilateral inferior parietal, right precuneus, left temporal pole, right fusiform, and left rostro anterior cingulate cortices compared with the HC group, and smaller bilateral lateral orbitofrontal, left superior frontal, left postcentral, bilateral inferior parietal, right precuneus, left temporal pole, right fusiform, and right parahippocampal cortices compared with the LR group. Among LR and HR ADHD subjects ($n = 94$), ADHD-RS inattention subscale scores were positively correlated with left lateral orbitofrontal surface area ($p < 0.0001$), and YMRS total scores were inversely correlated with left inferior parietal surface area ($p < 0.0001$) and right precuneus surface area ($p < 0.0001$).

Conclusions: Psychostimulant-free ADHD youth with a BD family history exhibit a more severe clinical profile, including greater mania and dysregulation symptoms, and more extensive cortical and subcortical volume abnormalities compared with ADHD youth without a BD family history and healthy youth. These findings suggest that BD family history in conjunction with ADHD is associated with a more pervasive disruption of peripubertal neurodevelopmental trajectories that distinguish it from ADHD alone.

Keywords: Bipolar Disorder, ADHD, Brain Imaging, Prodrome

Disclosure: Nothing to disclose.

P141. Family History of Alcohol and/or Substance Use Problems and Frontal Cortical Thickness From 9-10 to 11-12 Years of Age: A Longitudinal Analysis of the Adolescent Brain Cognitive Development (ABCD) Study

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Background: Individuals with a family history of alcohol or substance use problems (FH+) are themselves at risk for increased use, misuse, and eventual development of alcohol and/or substance use disorders (AUD/SUD). Having a FH + has also been associated with impaired development of prefrontal brain regions, particularly those related to executive functioning (EF) and impulsivity. Lower EF and high impulsivity are also unique contributors to alcohol/substance-related problems and AUD/SUD.6–12 Prior cross-sectional studies showed that FH + adolescents exhibited thinner frontal cortices including aspects of the inferior frontal gyrus (e.g., pars triangularis), as well as lateral and medial orbital frontal cortices. Children with FH + (ages 9-10) had lower whole-brain cortical thickness, including thinner cortices in the left precentral and paracentral lobules, and greater surface area in the right precentral lobule compared to children with FH-. Little is known about changes in cortical thickness as a function of FH + during early adolescence – a critical period for brain maturation. Most studies to date have been cross-sectional and/or include broad age ranges that do not allow sufficient focus on the beginning of adolescence. Our aim was to examine frontal cerebral cortical thickness trajectories among 9–12-year-old preadolescents with a positive (FH+), versus negative (FH-), family history of alcohol/substance use problems.

Methods: Data from the ABCD Data Release 4.0 including structural MRI measures at two time points (baseline [$n = 11,878$] and 2-year follow-up [$n = 6,571$]). FH + ($n = 2,861$) was defined as having ≥1 biological parents and/or ≥2 biological grandparents with a history of alcohol/substance use-related problems (e.g., alcohol/substance use-related separation/divorce, being laid off/fired related to alcohol/substance use problems; arrests/DUIs; alcohol/substance harmed health; in an alcohol/substance treatment program; caused arguments or were drunk/intoxicated a lot). Individuals with no parents or grandparents with a history of alcohol/substance use problems were classified as FH- ($n = 7,017$). Individuals with only one grandparent with a FH of alcohol/substance problems ($n = 1,595$) or with missing data on all parents and grandparents were excluded ($n = 405$). Our primary outcome was cortical thickness within 11 frontal regions (caudal middle frontal, frontal pole, lateral orbital and medial orbital frontal, paracentral, pars orbitalis, pars opercularis, pars triangularis, precentral, superior frontal, and rostral middle frontal), based on the Desikan-Killiany cortical parcellation atlas.

Statistical Analyses

A linear mixed-effects model was fit to test associations between FH + and frontal cortical regions addressing the 11 frontal cortical regions in both hemispheres, while adjusting for the fixed effects of age, sex, race/ethnicity, parental marital status, household income, prenatal exposure to alcohol, drugs, and tobacco, and intracranial total volume, and the random effects of family relationships (e.g., siblings), MRI scanner and participant ID as random effects. To examine the extent to which age-related change in cortical thickness was qualified by FH, we also added an interaction term for FH, timepoint (i.e., baseline and 2-year follow-up) and frontal cortical regions. We also ran pairwise comparisons examining differences in specific frontal cortical regions between FH + and FH- at each timepoint. Analyses were conducted using the 'lme4'and 'emmeans' packages in R.

Results: In this sample, 24.9% of the preadolescents had a FH + , and 61.2% a FH- (13.9% with only 1 grandparent with a FH of alcohol/substance use were not included). Having a FH + was associated with more pronounced reductions in frontal thickness in the 2-year follow-up compared to baseline (FH + , β -0.10, $p < 0.001$; FH- β -0.08, $p < 0.001$; overall FH x time interaction, $p = 0.003$). Given the significant overall associations, we next examined thickness measures in each frontal region separately. At baseline, FH + (vs. FH-) was associated with reduced paracentral (β-left -0.11 $p = 0.001$) and precentral (β-left -0.08 $p = 0.001$), and thicker medial orbital frontal cortices (β-left 0.07 $p = 0.004$; β-right 0.05 $p = 0.04$). At the two year follow-up, FH + continued to be associated with thinner paracentral (β-left: -0.14 $p < 0.001$, β-right: -0.08 $p = 0.008$), precentral (β-left -0.11 $p < 0.001$, β-right -0.12 $p < 0.001$), superior frontal (β-left -0.08 $p = 0.01$) and rostral middle frontal cortices (β-left -0.07 $p = 0.02$), and thicker frontal pole (β-right 0.06 $p = 0.04$).

Conclusions: Whereas previous studies have documented cross-sectional associations between brain structure and FH of alcohol or substance use problems, preliminary results from the present longitudinal study indicate that FH + is associated with altered cortical thickness development in frontal areas (particularly within pre- and paracentral regions). Specifically, when comparing FH + and FH- youths, having FH + exhibited differences in frontal cortical thickness at 9-10 years of age. Further, these differences in cortical structure increased in magnitude during early adolescence (ages 11-12), at which time structural alterations in other regions (including areas implicated in emotion regulation, such as the superior and middle frontal cortices) are also detected. Future research may examine any dose-dependent (i.e., number of relatives) and specific substance effects of FH + on adolescents' brain development.

Keywords: Adolescent Brain Cognitive Development Study, Cortical Development, Cortical Thickness

Disclosure: Nothing to disclose.

P142. Altered EEG Coherence in Adolescents With Recent Suicidal Behaviors

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Background: Several studies have attempted to characterize suicidality using Quantitative EEG measures. There is limited prior work focused on EEG power and coherence. Most of these studies were examined adults. No prior study has investigated EEG coherence in adolescents with suicidal ideations and behaviors.

Methods: Thirty adolescents aged 13-18 years admitted to an inpatient psychiatry unit with suicidal ideations and suicidal behaviors (assessed with the Columbia-Suicide Severity Rating Scale (C-SSRS)) and thirty with no prior psychiatric diagnoses were recruited. All participants underwent diagnostic assessments and resting EEG recordings. Three patients dropped out before completing EEG recordings. The inpatient group was further divided into 2 sub-groups, those with suicidal ideations ($n = 9$) or suicidal behaviors ($n = 18$) based on the C-SSRS assessment. ANCOVA models were used to test if intra-hemispheric coherence differed amongst the groups controlled for age and gender. Bonferroni correction was used for post-hoc comparisons.

Results: Patients with suicidal behaviors (SB) had higher delta ($p = 0.024$), alpha($p = 0.024$) and beta coherence ($p = 0.006$) over right PFC (Fp2-F4) compared to patients with suicidal ideations (SI) only. The SB group also had higher delta power ($p = 0.027$) over left parieto-occipital cortex (P7-01) compared to the SI only group. Over right temporo-parietal (T8-P8) and centroparietal cortex (C4-P4), the SB group had lower theta ($p = 0.036$), alpha ($p = 0.045$) coherence compared to healthy controls. Depression severity (assessed with the Children's Depression Rating Scale Revised) did not differ between SB and SI only groups ($p > 0.05$).

Conclusions: Patients with SB had higher intra-hemispheric coherence within the right prefrontal cortex for both lower and higher frequencies except theta coherence but had decreased coherence in temporo-parietal centroparietal cortex. These results may suggest altered default mode network and inhibitory mechanisms in patients with recent suicidal behaviors as compared to patients with suicidal ideations. Given the paucity of research in this area, further research is needed to replicate the results in larger sample sizes.

Keywords: EEG, Adolescent, Suicide, Suicidal Behavior, Suicidal Ideation

Disclosures: Neuronetics, Magventure: Other Financial or Material Support (Self), Engrail Therapeutics, Sunovion, Myriad Neuroscience, Procter and Gamble Company: Advisory Board (Self)

P143. The Interrelationship Between Myelination, Chronotype, and Stress in Adolescents at High Risk for Psychiatric Disorders

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Background: Chronotype characterizes a tendency towards morningness or eveningness (e.g., larks versus night-owls). The relevance of chronotype to the development of depression, diabetes, alcohol use disorder, cannabis use disorder, and obesity has been increasingly established through associational studies. Chronotype is developmentally regulated, and shows the greatest amount of change in adolescence, precisely the time when many psychiatric disorders are first identified and important changes in brain myelination occur. However, whether chronotype or eveningness plays a role in the development of some of these disorders has been much less explored and investigated.

A number of studies have investigated the relationship between trauma and chronotype, and found an association between childhood trauma and a chronotype preference towards eveningness suggestive of mediation by emotional dysregulation. However, the relationship between stressful life events and chronotype has not been explored in as much depth.

Some genome-wide studies have most prominently related chronotype to the anterior cingulate as a potential neuroanatomical phenotype that could be related to chronotype or eveningness. Increased activation of the anterior cingulate has been observed during suppression of negative emotions when contrasted with maintaining negative emotions. To properly function, the brain needs myelin, an electrically insulating structure that surrounds axonal fibers. It has been shown based on fractional anisotropy imaging that poor sleep quality or short sleep duration are associated with lower myelin content in the human neo-cortex. Intracortical myelin in the anterior orbitofrontal cortex mediates the association between poor sleep and negative emotionality, which can mechanistically explain how poor sleep influences affective behavior. Diffusion tensor imaging, such as fractional anisotropy is sensitive to degree of myelination of anatomical structures like the anterior cingulate.

Methods: The Adolescent Brain Cognitive Development (ABCD) study offers a large sample of approximately 11,000 at high risk adolescents followed over a 10-year period with data on chronotype, neuroimaging, and stressful life events. This study tracks biological and behavioral development from adolescence into young adulthood. We used a mediation model to examine the relationship between chronotype, fractional anisotropy of the anterior cingulate, and stressful life events using the Munich Chronotype Questionnaire (MCTQ) and the Life Events Scale. In our comprehensive final model, we included race, sex, and socioeconomic status as covariates to control for. All analyses were conducted in R using lavaan.

Results: We found that cross-sectional data suggest a potential relationship between chronotype and myelination of the anterior cingulate mediated by stressful life events. Lesser myelination of the anterior cingulate was associated with more eveningness.

Conclusions: Our work provides a new look into the interrelationship between chronotype, myelination, and stressful life events in the ABCD study. Understanding the role of myelination in stress in those predisposed towards eveningness is useful because it may improve our understanding of the biological mechanisms that contribute to stress, and lead to better prevention and treatment efforts using interventions such as increased lifestyle regularity and daytime light exposure.

Keywords: Chronotype, Myelination, Adolescence, ABCD, Stress

Disclosure: Nothing to disclose.

P144. Examining the Impact of Food Insecurity on White Matter in Children and Adolescents: An ABCD Study

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Background: Adolescence is a critical phase of life between late childhood and adulthood that is characterized as the second

greatest and most rapid period of development after infancy. As a result, adolescence has been recognized as a critical period of increased vulnerability, during which exposure to environmental and social stressors, in addition to the impact of pubertal hormones or other life events, can have long-standing functional and structural impacts.

Food insecurity is a growing public health concern in the United States and around the globe. Food insecurity is defined as a lack of means to achieve reliable access to a sufficient quantity of food necessary to maintain a healthy diet. In the US, it is estimated that approximately 14.8% of households suffer from food insecurity, with around 7% of those affected with low or very low food security being children (6.1 million children). While food insecurity does not necessarily equate to extreme states of hunger, starvation, or malnourishment, it can lead to inconsistent or limited access to healthy foods and nutrients. Previous reports show that very young children are often protected from the effects of food insecurity, however, adolescents are often at a greater risk. This lack of access to sufficient resources and diet-derived nutrients may be detrimental during this critical period of brain development, particularly for the brain's white matter, which is actively maturing during adolescence.

White matter is comprised of a lipid-rich substance, called myelin, that surrounds neuronal axons to provide insulation and increase the rate at which electrical impulses are carried along the axon. The creation and maintenance of myelin is governed by oligodendrocytes, which require an array of resources, such as iron, fatty acids, and assorted vitamins such as Vitamin D and Vitamin K, the concentrations of which are principally dependent on food intake. As a result, developing white matter may be particularly susceptible to the potential lack of resources incurred by the inconsistent or limited access to nutritious foods which occur in food insecure homes. However, to date there have been no neuroimaging studies which aim to investigate whether food insecurity influences or impacts white matter development in children and adolescents.

In this study, we will utilize extant data from the Adolescent Brain and Cognitive Development (ABCD) study to evaluate the extent to which food insecurity can impact overall white matter health in children and adolescents aged 9 to 15 years. More importantly, given the complex interplay between food insecurity and other known factors that have an impact on brain development (i.e., socioeconomic status, stress, childhood trauma, etc.), we plan to utilize a machine learning approach to determine whether there is a link between food insecurity and alterations in white matter health, independent of the impacts of other environmental, financial, or social stressors.

Methods: Of the 11,877 individuals included in the ABCD baseline sample, 954 parents (8%) responded to having had an instance of food insecurity in the last twelve months. We will employ propensity score matching using K-nearest neighbors to construct comparable groups of children/adolescents with food insecurity (FI) matched with respect to age, sex, race, ethnicity, intelligence quotient, and, if feasible, years of parental education and/or socioeconomic status, to children/adolescents without food insecurity (noFI).

For the imaging data for those subjects included in our identified groups, we will utilize the diffusion MRI imaging data that has already been pre-processed and harmonized across the 21 imaging sites as part of an on-going NIMH-funded effort led by Dr. Rathi. Whole-brain two tensor tractography has been performed and quality controlled. An unsupervised fiber clustering atlas (whitematteranalysis) has been applied and will be utilized to extract whole brain average fractional anisotropy (FA) for each participant in this sample.

Results: The statistical analyses in this study are presently ongoing. We will train a machine learning model to determine whether the matched FI cohort exhibits differences in whole brain average FA from the non-FI cohort. To understand the covariance structure of the variables used to train the model, we will employ an interpretability approach which will enable mediation analysis and ranking of the variables based on the learnt weights. This will enable us to understand the relative importance of food insecurity on white matter health in children/adolescents, independent of the effects of other confounds, such as socioeconomic status.

Conclusions: To our knowledge, this will be the first study to determine whether there is an association between food insecurity and white matter microstructure in children and adolescents. This study will serve as a first step toward furthering our understanding of the role access to healthy and nutritious foods may play on brain development.

Keywords: White Matter Development, ABCD, Food Insecurity

Disclosure: Nothing to disclose.

P145. Family History of Substance Use Disorder and Parental Impulsivity are Differentially Associated With Neural Responses During Risky Decision-Making

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Background: Risky decision-making is associated with the development of substance use behaviors during adolescence. Although prior work has investigated risky decision-making in adolescents at familial high risk for developing substance use disorders (SUDs), little research has controlled for the presence of co-morbid externalizing disorders (EDs), another SUD risk factor. Additionally, few studies have investigated the role of parental impulsivity in offspring neurobiology associated with risky decision-making. The purpose of this study was to address these gaps in the literature by (i) investigating differences in neural functioning during risky decision-making in children with externalizing disorders with and without family histories of SUD; and (ii) investigate the relationship between parental impulsivity and child neural functioning during risky decision-making. Based on prior work in decision-making and neurobiology of children at risk for SUDs, we hypothesized that children with co-morbid familial histories of SUD and EDs would show greater responsiveness to negative outcomes of risky decisions within anterior insula cortex (aIC), rostral anterior cingulate cortex (ACC) and lateral orbitofrontal cortex (OFC) compared to both psychiatric controls and healthy controls, and that these differences would be related to parental impulsivity.

Methods: One-hundred twenty-five children (28 healthy controls, 47 psychiatric controls (PC) with EDs without a familial history of SUD, and 50 high-risk (HR) children with co-morbid EDs with a familial history of SUD) participated in the Balloon Analogue Risk Task (BART) while undergoing functional magnetic resonance imaging. Impulsivity for parents and children was measured using the UPPS-P Impulsive Behavior Scale. AIC, rostral ACC, and lateral OFC were defined as structural regions of interest (ROIs) using the Freesurfer parcellations from the Desikan-Killiany atlas. A 3 (Group: HC, PC, HR)-by-2 (Laterality: Left, Right)-by-3 (Region: lOFC, rACC, aIC)-by-2 (BART Choice: Inflate, Win) repeated measures ANCOVA was conducted on the BOLD response data modulated by probability of balloon explosion from these ROIs. The covariate of interest in this ANCOVA was parental impulsivity; this analysis was also repeated with child impulsivity included as a covariate of no interest.

Results: We found that individuals in the psychiatric control group showed greater activation, as chances of balloon explosion

increased, while making choices, relative to the healthy control and high-risk groups in the rostral ACC and lateral OFC. We also found a positive association between greater activation and parental impulsivity in these regions. However, within rostral ACC, this relationship was moderated by group, such that there was a positive relationship between activation and parental impulsivity in the HC group, but an inverse relationship in the HR group. These relationships remained significant when controlling for child impulsivity.

Conclusions: These findings suggest that there are key differences in the neurobiology underlying risky decision-making in individuals with EDs with and without a familial history of SUD. These data also indicate that the association between parental impulsivity and rostral ACC activity during risky decision-making may be a marker of future risk for SUDs. We propose that development of biomarkers of SUD risk (based on these findings) would provide further insight into determining level of risk for SUDs across different populations. However, future work regarding the roles of how altered rostral ACC and lateral OFC activity may confer risk for SUDs and following high-risk samples longitudinally is needed.

Keywords: Addiction Comorbidity, Alcohol Use Disorder and Drug Addiction, Impulsivity

Disclosure: Nothing to disclose.

P146. Neural Network Functional Interactions Influence White Matter-Emotional Behavior Relationships in Infants

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Background: Low levels of positive emotionality (PE) and high levels of negative emotionality (NE) in childhood signal risk for subsequent behavioral and emotional problems. Elucidating the neural basis of these temperamental features in infancy can identify objective biomarkers of risk that reflect underlying pathophysiological processes. Our goal was to determine how functional interactions among large-scale neural networks supporting emotional regulation influence white matter microstructural-emotional behavior relationships in 3-month-old infants. We hypothesized that microstructural-emotional behavior relationships will be differentially mediated or suppressed by underlying resting-state functional connectivity, particularly between Central Executive Network (CEN) and Default Mode Network (DMN) structures.

Methods: This sample comprised caregiver-infant dyads [52 infants (42% female, mean age at scan=15.10 weeks) and their primary caregiver (98% biological mother)], with neuroimaging of the infant and assessments of infant emotional behavior at 3 months. Infant white matter and functional connectivity were assessed by diffusion-weighted imaging/tractography and resting-state magnetic resonance imaging (MRI) during natural, non-sedated sleep. The Infant Behavior Questionnaire-R provided measures of infant PE and NE. Following significant white matter-emotional behavior relationships, multimodal analyses were performed using whole-brain voxelwise mediation.

Results: Results revealed that greater cingulum bundle tract volume was significantly associated with lower infant PE (ß = -0.263, $p = 0.031$); however, a pattern of lower functional connectivity between CEN and DMN structures suppressed (i.e., reduced) this otherwise negative relationship. Greater uncinate fasciculus tract volume was significantly associated with lower infant NE (ß = -0.296, $p = 0.022$); however, lower orbitofrontal cortex (OFC)-amygdala functional connectivity (supporting emotional regulation), suppressed this otherwise negative relationship, while greater OFC-CEN functional connectivity (supporting executive control over emotion) mediated (i.e., facilitated) this relationship.

Conclusions: Functional interactions among neural networks have an important influence on white matter microstructural-emotional behavior relationships in infancy. These relationships can elucidate neural mechanisms contributing to future behavioral and emotional problems in childhood.

Keywords: Infant Brain, Infant Emotionality, Multimodal Neuroimaging, Diffusion Weighted Imaging, Resting State Functional Connectivity

Disclosure: Nothing to disclose.

P147. Prenatal Neighborhood Crime Exposure and Neonatal Frontolimbic Connectivity are Associated With Socioemotional Functioning at 12 Months

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Background: Growing up in a neighborhood with high crime rates is associated with sleep disturbances, mental health problems, and altered brain function. Our previous study found that prenatal exposure to neighborhood crime was associated with reduced frontolimbic connectivity in the neonatal brain, partially mediated by maternal psychosocial stress (Brady et al. 2022). It is unknown whether prenatal crime exposure and subsequent neonatal brain changes lead to altered behavior in infancy or beyond. It is plausible that prenatal crime exposure relates to later behavior, as studies in middle childhood and adolescence demonstrate a link between living in a high-crime neighborhood and mental health problems. We hypothesize that prenatal crime exposure will be related to poorer socioemotional outcomes, mediated by both maternal psychosocial stress and neonatal frontolimbic connectivity.

Methods: The study utilized a longitudinal cohort of 399 women recruited during pregnancy as part of the eLABE study at Washington University. Prenatal addresses were collected for each mother and coded by census block group. Crime data for each block group was obtained from Applied Geographic Solution's CrimeRisk Database. Violent crimes (e.g., murder, rape, aggravated assault, robbery) and property crimes (e.g., burglary, larceny, vehicle theft, arson) were examined. In the neonatal period (mean postmenstrual age at scan=41 weeks), 319 non-sedated infants (male=55%, mean gestational age=38 weeks) were scanned using a resting-state functional MRI sequence (TR = 800 ms, TE = 37 ms, Voxel size=2.0×2.0×2.0 mm3, MB = 8) on a Prisma 3T scanner and had at least 10 minutes of high-quality resting-state data after pre- and post-processing, including stringent motion correction. At one year of age, the mothers of 293 infants completed the Infant-Toddler Socioemotional Assessment (ITSEA), which contains information about four domains of behavior: externalizing, internalizing, dysregulation, and competence. Pearson correlations were used to examine the relationship of block-tract group crime to the ITSEA t-scores. Linear models probed the relationship between the significant frontolimbic connections in Brady et al. 2022 (i.e. Thalamus-Default Mode Network (DMN), Thalamus-anterior Frontoparietal Network (aFPN), Amygdala-Hippocampus, and Amygdala-DMN) and relevant ITSEA t-scores,

controlled for gestational age at birth and scan. Results were corrected for multiple comparisons using an FDR procedure. Structural equation models (SEMs) were created to test whether maternal psychosocial stress and neonatal neuroimaging mediated the relationship between prenatal crime exposure and ITSEA t-scores. To assess the specificity of exposure to crime compared to other forms of adversity, a composite measure of disadvantage experienced during pregnancy (income-to-needs, Area Deprivation Index, education, insurance status, and Healthy Eating Index) was included.

Results: The results of this study demonstrated that prenatal exposure to high levels of neighborhood violent crime was related to higher externalizing ($q < .001$), internalizing ($q = .03$) and dysregulation ($q < .001$) t-scores, but not competence t-scores ($q = .31$) at age 1 year. Living in an area with high property crime during pregnancy was also related to higher externalizing ($q = .001$) and dysregulation ($q < .001$) t-scores, but not internalizing ($q = .06$) or competence t-scores ($q = .81$). All of these relationships continued to be significant after the addition of disadvantage to the models, except violent crime's relationship with internalizing symptoms. Frontolimbic connections in the neonatal brain, specifically the Thalamus-DMN ($q = .05$; $q = .005$), Thalamus-aFPN ($q = .03$; $q = .004$), and Amygdala-DMN ($q = .02$; $q = .005$), were related to externalizing t-scores and dysregulation t-scores, respectively. Amygdala-Hippocampus connectivity was not related to externalizing or dysregulation t-scores. In SEM models predicting externalizing symptoms from violent crime, the direct relationship with violent crime remained significant, but mediation was non-significant. In SEM models predicting dysregulation symptoms, the indirect path through maternal psychosocial stress and Thalamus-aFPN mediated the relationship between adversity and dysregulation symptoms. Additionally, there were direct relationships between Thalamus-DMN and Amygdala-DMN and dysregulation scores. For property crime, SEM models showed that the direct relationships with crime and disadvantage both independently predicted externalizing and dysregulation symptoms.

Conclusions: These findings provide evidence that exposure to a high crime environment in utero is associated with poorer socioemotional processing in the externalizing and dysregulation domains at age 1 year. Crime seemed to be the most important predictor for externalizing symptoms whereas frontolimbic connectivity seemed to be most important for dysregulation symptoms. A limitation of this analysis is that it does not account for crime exposure during the first year, but this is a planned future direction of the study. Nevertheless, the current findings suggest that in utero exposure to crime and neonatal brain may affect socioemotional functioning. Given the effect of prenatal crime exposure, this study reiterates the need to reduce neighborhood rates of crime, as well as promote the development of frontolimbic connectivity.

Keywords: Pregnancy, Neighborhood Crime, Functional MRI (fMRI), Stress and Adversity, Infant Behavior

Disclosure: Nothing to disclose.

P148. High-Throughput Functional Analysis of Autism Genes in Zebrafish Identifies Convergent Pathways

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Background: Whole-exome sequencing studies in autism spectrum disorders (ASDs) have led to the identification of a rapidly growing list of high confidence (hcASD) risk genes. These genes converge on biologically relevant mechanisms, including mid-fetal glutamatergic projection neurons, chromatin modification, and synaptic function, providing new insights into the biology underlying ASDs. At the same time, understanding how loss of function of hcASD genes affects specific cell types and the establishment of neural circuits in a developing vertebrate brain remains a central challenge.

Methods: Here, we leverage the unique advantages of zebrafish as a high-throughput, in vivo system to analyze the function of 10 hcASD genes in parallel at the behavioral, structural, and circuit levels. First, we generated mutants of the zebrafish orthologs of 10 hcASD genes using CRISPR/Cas9. These genes span a range of biological functions, including gene expression regulation (CHD8, CUL3, KDM5B, POGZ, TBR1), neuronal communication (CNTNAP2, SCN1A/SCN2A, GRIN2B), and the cytoskeleton (DYRK1A, KATNAL2). Second, to determine how gene loss of function affects basic behaviors, we performed high-throughput automated assays of visual-startle and sleep-wake behavior. Third, we developed a novel computational pipeline to analyze whole-brain structure and activity across all mutant lines in parallel. Fourth, to identify overlapping molecular pathways, we performed whole-brain RNA-seq in three mutants with the most robust behavioral and brain activity phenotypes.

Results: We establish a novel high-throughput pipeline for the parallel functional analysis of 10 hcASD genes in zebrafish and identify multiple points of convergence and divergence across risk genes at the behavioral, structural, circuit, and molecular levels. First, we identify unique behavioral fingerprints affecting arousal and sensory responsiveness across hcASD gene mutants. Second, we identify the forebrain as a significant contributor to brain size differences across mutants. Third, using whole-brain activity mapping, we find that regions involved in sensory-motor control show altered baseline brain activity and identify 22 out of 149 regions in the zebrafish brain with significant differences in baseline brain activity in more than half of mutants. Fourth, RNA-seq reveals the conservation of ASD gene-associated pathways, including neurogenesis and FMRP targets, and implicates novel cellular mechanisms in a subset of mutants.

Conclusions: High-throughput parallel in vivo analysis of 10 ASD risk genes in zebrafish identifies convergent phenotypes affecting brain structure and baseline activity, and uncovers shared molecular and cellular pathways in a subset of mutants.

Keywords: Autism Spectrum Disorder, Genetics, Zebrafish, Neurodevelopment

Disclosure: Nothing to disclose.

P149. Multidimensional Neuropsychiatric Phenotypes in Children With Noonan Syndrome

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Background: The Ras-mitogen-activated protein kinase (Ras-MAPK) pathway is central to neurodevelopment. Noonan syndrome (NS), the most common disorder associated with Ras-MAPK pathogenic variants, is associated with highly variable neuropsychiatric outcomes. We address limitations in previous research with a focus on prepubertal children, comparison to typically developing (TD) children, comprehensive neuropsychiatric evaluation and controlling for overall cognitive abilities

Methods: Forty-five children with NS (8.48 + /-2.13 years; 29 female) and 40 TD children (8.75 + /-1.96 years; 22 female) were evaluated with objective parent report and psychiatric interview measures

Results: Children with NS demonstrated higher levels of attention-deficit/hyperactivity disorder (ADHD) (attention, hyperactivity, and inhibition), autism spectrum disorder (ASD) (maintaining social relationships, behavioral rigidity, and sensory sensitivity) relative to TD children, all p's<0.05 and η2 effect sizes were in the large range (>0.14). Children with NS also demonstrated higher levels of oppositional defiant disorder (ODD) (aggression) symptoms relative to TD children (all p's<0.05, η2 effect sizes were in the medium range). Forty- eight percent of children with NS met the criteria for ADHD, 19% for ODD, and 25% demonstrated clinically significant symptoms seen in children with ASD.

Conclusions: Children with NS are at increased risk for a spectrum of ADHD, ASD, and ODD associated symptoms. Over 50% of children fulfilled criteria for at least one of the tested clinical diagnoses. In contrast to the currently accepted categorical diagnostic approach, a dimensional approach reveals significant ASD symptoms in NS.

Keywords: ADHD, Neurodevelopment, Noonan Syndrome, ASD

Disclosure: Nothing to disclose.

P150. Using an Optimized Fragile Messenger Ribonucleoprotein (FMRP) Assay to Predict Disease Severity in Fragile X Syndrome

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Background: The fragile X messenger ribonucleoprotein 1 (FMR1) gene-specific protein product, FMRP, is critical for brain development. In fragile X syndrome (FXS), FMRP levels are markedly reduced, if not absent. Previous studies have demonstrated the relationship between FMRP levels and general cognitive functioning; however, due to limitations of available assays, FMRP was not able to be reliably and reproducibly detected at extremely low values, preventing the examination of clinical relationships across the entire spectrum of individuals with FXS. Identifying these relationships may be critical to planning clinical care and understanding development over time.

Methods: A sample of 65 individuals with FXS (> 200 CGG repeats; 67% male), aged 8-45 years, completed a battery of psychosocial, neurocognitive, and electrophysiological measures. Participants whole blood samples were obtained via venipuncture and analyzed using our validated Luminex-based immunoassay to determine FMRP levels. Pearson correlations were conducted to examine the relationship between FMRP levels and clinical phenotypes.

Results: In both males and females with FXS, higher FMRP levels were associated with higher IQ scores (m: $r2 = .20$, p-.01; f: $r2 = .24$, $p = .02$), consistent with prior findings. Of note, full mutation, fully methylated males who produce little to no FMRP showed a leftward shifted (mean=25, standard deviation=15) but otherwise normal distribution of IQ scores (skewness = .15, kurtosis = -.82). In males only, higher FMRP levels were associated with less severe stereotyped behavior ($r2 = .12$, $p = .04$) and irritability ($r2 = .10$, $p = .07$). In females only, higher FMRP levels unexpectedly were associated with more severe social avoidance ($r2 = .40$, $p = .04$) and depressive symptoms ($r2 = .58$, $p = .01$). FMRP levels were not significant related to any executive function variables in males or females with FXS. However, lower levels of FMRP were associated with increased relative gamma power, a neurophysiological biomarker of neural hyperexcitability, across FXS patients ($r2 = .14$, $p = .04$).

Conclusions: Together, our optimized FMRP assay provides new evidence of the link between FMRP expression and clinical and neurophysiological phenotypes in FXS, and these relationships, importantly, may differ by biological sex. In addition, our findings also offer a paradigm-shifting role of FMRP in cognitive function in full mutation males: FMRP drives the leftward shift in IQ, but does not account for the remaining variance in cognition in full mutation males with FXS. This is in contrast to ours and others' findings indicating FMRP has a dose-dependent effect on IQ in mosaic males and full mutation females who express higher levels of protein. There is much future work to better understand the mechanism by which deficient or absent FMRP leads to clinical phenotype. Still our novel work is a critical step towards establishing molecular markers of disease severity in FXS. Ultimately, this foundational work is paving the pathway towards the development of individualized "precision medicine".

Keywords: Fragile X Syndrome, FMRP, Biomarker

Disclosure: Nothing to disclose.

P151. Genomic Architecture and Genetic Risk for Autism Spectrum Disorder in Individuals of Latin American Ancestry

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Background: Autism spectrum disorder (ASD) is characterized by deficits in social communication and the presence of restricted interests and/or repetitive behaviors. Risk for ASD is complex but largely genetic, with both common and rare genetic variation contributing to risk. While common variation accounts for most genetic liability for ASD, rare variation, often de novo, accounts for substantial individual liability.

In anticipation of both the tremendous impact and consequential challenges that high-throughput sequencing would have on ASD genomics, the Autism Sequencing Consortium (ASC) was formed in 2010 and recently completed an analysis of 65,000 exomes (Fu et al. 2022, in press), which included samples from ASC sites as well as SPARK data (SPARK Consortium 2018). In Fu et al, the ASC reported 71 genes associated with ASD at a false discovery rate (FDR) ≤ 0.001; a threshold that approximates exome-wide significance ($p < 2.5e\text{-}6$), and 185 genes at FDR ≤ 0.05. Analysis of the SPARK dataset identified 60 largely overlapping genes with exome-wide significance (Zhou et al. 2022, in press).

Despite the success of these large-scale genetic analyses, the question of whether the genetic architecture of ASD differs across ancestral populations remains. Notably, there has been no systematic effort to investigate the genetic architecture of ASD in Latina/Latino populations (hereafter Latinx), the largest minority in the United States.

We have established the Genomics of Autism in Latinx Ancestries (GALA) Consortium to develop a large prospective cohort to examine the role of genetic and environmental factors in ASD across ancestral backgrounds and collect genetic materials for large-scale analyses. Our first aim was to explore whether the rare damaging burden signal is similar across ancestral backgrounds. In parallel, we are analyzing existing and emerging GALA and other Latinx samples for ASD gene discovery.

Methods: The GALA Consortium has collected ca. 1,000 ASD Latinx participants, largely as trios from South and Central America and/or the USA. Collection of an additional 1,600 Latinx ASD trios is ongoing. All sites will use a similar strategy to screen and phenotype probands. All samples will be subject to genotyping and whole exome sequencing (WES). Single Nucleotide Polymorphism (SNP) data will be used to estimate SNP-based heritability at various levels of ancestry. Rare de novo and inherited single nucleotide variation (SNV), and insertion deletion

variation (indel) will be identified from WES. Chromosomal microarray and WES data will identify potentially damaging copy number variation (CNV). With these data, we will first test whether the genomic architecture of ASD, as reflected by (a) the exonic de novo rate, (b) mutation burden in highly conserved genes (pLI ≥ 0.995), and (c) polygenic risk, differs between Latinx and non-Latinx populations. Accounting for ancestry, we will then use the TADA (Transmitted and de novo Association) framework to identify Latinx ASD genes impacted by rare variation, integrating de novo and inherited variation from trios and case-control data, incorporating SNV, indel, and CNV. SNP data from GALA will be contributed to the PGC-ASD analyses to facilitate greater power for cross-ancestry GWAS.

Results: We first determined whether the genomic architecture and ASD genes impacted by rare deleterious variation will differ across ancestries. We hypothesize that they will not. To examine this, we took all de novo mutations in Fu et al. and examined rates as a function of ancestry. We looked at highly conserved genes and all remaining genes. We observed that the signal is overwhelmingly found in the most conserved genes across all ancestries. Thus, when separating the samples by genetically defined ancestry, results were similar across groups. We are now analyzing WES data from the existing and emerging GALA samples, together with Latinx samples from SPARK. Power for gene discovery can be estimated based on these results and it is reasonable to estimate at least 25 ASD genes in the full GALA Latinx samples at a strict FDR < 0.05 and more at more lenient thresholds. Genetic findings will be compared with those in European samples and if, as we predict, the genes overlap, we will carry out joint analysis across multiple ancestries. First results from GALA are already implicating known ASD genes, consistent with shared molecular risk architecture. Analyses are ongoing and results from all analyses will be presented.

Conclusions: Genetic variation that harbors strong impact on neurodevelopment will overwhelmingly be found in highly conserved genes; those depauperate of deleterious variation in very large, heterogeneous databases. Hence, we hypothesized that the rare mutational burden in conserved genes will not differ across ancestries and that there will be extensive overlap between ASD genes identified. Our analyses are consistent with this hypothesis and support continued ASD gene discovery in Latinx and other populations. Biology, here reflected in genes that are intolerant to deleterious variation due to critical function and dosage sensitivity, is universal and not impacted by "local" genetic processes.

Going forward, we can carry out genotype-phenotype correlations in Latinx samples and explore the role of access to care on phenotype and genomic architecture. As we transition from studying rare deleterious variants to standing, inherited variation, improved methods for integrating data across populations will be needed.

Keywords: ASD, Rare Genetic Variation, Multi-Ancestry, Copy Number Variation, Gene Discovery

Disclosure: Nothing to disclose.

P152. The Role of Fragile X Messenger Ribonucleoprotein (FMRP) in Neural Response To and Discrimination of Speech Sounds in Children With Fragile X Syndrome

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Background: Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability (ID), resulting from the silencing and inactivation of the FMR1 gene with resultant loss of fragile X messenger ribonucleoprotein (FMRP) expression leading to widespread changes in brain structure and function. While delays in language are almost universal in those with FXS, there is limited research examining auditory and language processing, particularly in early life and in males. And few have examined the relationship between FMRP and functional brain response to language in FXS. Here we examine the relationship between FMRP levels and neural activation to speech and nonspeech sounds using fNIRS.

Methods: Participants. Current sample includes 38 full-mutation FXS (diagnosis determined via genetic testing) and 15 typically developing control subjects. 18 of 38 participants with FXS also had blood drawn to assess FMRP levels.

fNIRS. All fNIRS data were collected using a custom NIRx NIRScout (NIRx Medical Technologies, Brooklyn, NY) machine with lasers at wavelengths of 685 and 830nm and a 7.8125 Hz sampling frequency with an auditory 2x2x4 montage designed to cover temporal lobe areas bilaterally including the primary auditory cortex. Data was collected using NIRStar acquisition software with auditory stimuli programmed and presented using NIRStim software.

Auditory Task. The experimental design was a passive listening task lasting approximately 20 minutes (variable due to jitter, actual time was 1,208 seconds plus or minus up to 68 seconds of jitter). The auditory task consisted of a modified version of the Stories paradigm created by Cincinnati MR Imaging of Neurodevelopment (C-MIND) (Schmithorst, Holland, and Plante, 2006; Sroka et al., 2015). Clips of 5 narrative stories for young children read by female voice, interspersed with control nonspeech tones frequency-matched to human speech (200-4000Hz center frequencies, 0.5-2.0Hz duration broadband sweep).

FMRP. FMRP was measured using an optimized luminex-based assay (Boggs et al. 2022) which is able to detect a wide range of FMRP levels, including very low protein levels. Blood processing has been completed on 11 of 18 FXS subjects to date, with additional subjects to be added for final analysis.

Data Analysis. fNIRS data were then processed using the Homer3 toolbox (https://github.com/BUNPC/Homer3). Activation patterns to speech and nonspeech stimuli were examined in relation FMRP levels in participants with FXS.

Results: Children with FXS showed activation for speech and nonspeech sounds in language regions. Controls showed activation for speech and neural differentiation between the speech and nonspeech stimuli. FMRP demonstrated a non-significant relationship to neural activation patterns in early analyses. Additional analyses will be completed on the complete cohort including examination of the relationship between FMRP and language lateralization. Descriptive statistics will be used to characterize males versus females with FXS, however small sample size will limit further subgroup analysis.

Conclusions: Effective biomarkers for language impairment and development in FXS will require understanding the mechanisms that lead to impairment, which will, in turn, require functional neuroimaging methods like fNIRS. Optimally, it will include linking neural changes to both phenotypical presentation and molecular etiology. This work provides early examination of the relationship between peripheral FMRP and neural activation patterns in FXS.

Keywords: Language Delay, Fragile X Syndrome, fNIRS

Disclosure: Nothing to disclose.

P153. A Multimodal Approach to Investigating Theta Burst Stimulation and Auditory/Language Processing: Piloting an Autism Spectrum Disorder Study

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Background: Challenges in language processing are a central characteristic of Autism Spectrum Disorder (ASD), a complex heterogeneous neurodevelopmental disorder that also includes restricted and repetitive behaviors and deficits in social interaction. Previous literature suggests dysfunctional γ-aminobutyric acid (GABA) transmission may underlie auditory and language processing deficits in individuals with ASD. With current advancements in neuroimaging and neurophysiology technologies, researchers can undertake the challenge of clinical investigations studying GABAergic and other neurochemical transmission, as well as structural and functional connectivity associated with the pathophysiology of auditory and language processing deficits.

The pilot phase of this study, presented herein, serves multiple purposes: (1) to confirm feasibility and tolerability of study procedures, (2) to develop and test the performance of novel processing and analysis pipelines, (3) to establish the relationship between baseline local neurochemical concentrations in Wernicke's area, structural and functional network connectivity, and magnetoencephalography (MEG) indices of auditory and language processing, and (4) to investigate the impact of single session, individually targeted, continuous theta burst stimulation (cTBS) on the abovementioned outcomes in 20 adult non-clinical volunteers. Individualized stimulation targets are determined by task-related peak activation within the left posterior superior temporal cortex (pSTC) during the Auditory Description Decision Task (ADDT). We utilize magnetic resonance spectroscopy (MRS) to quantify neurochemical concentrations (including GABA, glutamate, glutamine, n-acetyl aspartate (NAA), and creatine) within the targeted region. Diffusion-weighted imaging (DWI) is collected to investigate structural connectivity. Functional MRI (fMRI) is acquired to evaluate activation and functional connectivity at rest and during the language task and MEG is conducted to understand the temporal aspects of auditory processing. These measures together provide a comprehensive, overarching picture of brain mechanisms underlying language and auditory processing.

Methods: Twenty non-clinical participants (5 males, 23.15 ± 0.97 years) completed MEG, MRI, and MRS at baseline, thereafter two cTBS visits, a minimum of a week apart, were conducted. During cTBS visits MEG (MEG + TMS visit), MRI, and MRS (MRI + MRS + TMS visit) data were collected prior to and post-stimulation.

MEG data was collected using a CTF 275 whole head MEG (1200Hz sampling rate), while participants listened to validated auditory stimuli designed to probe evoked power and inter-trial coherence in the gamma frequency band, as well as local and long-range network functioning (as measured by phase-amplitude coupling between the alpha and gamma frequency bands and the latency of evoked fields such as the M100, and MMF). All MRI and MRS scans were acquired using a General Electric (GE) 3T MRI scanner. A 15.6cm3 voxel centered on the individually defined target was used for spectroscopy. A single cTBS session comprised of 200 3-pulse 50Hz bursts with an inter-burst interval of 200ms, totaling 40s. Stimulation was applied at 80% of active motor threshold.

Results: As of submission, all 20 participants have been enrolled. No serious adverse events have been reported; indeed, all volunteers have tolerated all procedures. The first objective, to confirm feasibility and tolerability of study procedures has been met. Secondly, processing and analysis pipelines are being developed and tested. The language processing task used for TMS targeting reliably activated ($p \leq 0.05$) a defined region within pSTC for each participant. An acute effect of the cTBS on the MEG measures has been noted as well. Data analysis is ongoing. Results of the inferential statistics will be presented at the annual conference.

Conclusions: We report both baseline and pre-post cTBS Phase I data investigating the relationship between local neurochemical transmission, structural and functional network connectivity, and MEG indices associated with auditory and language processing networks in 20 adult non-clinical volunteers. In addition to observing the impact of cTBS on auditory and language processing, this phase serves as a proof of feasibility and tolerability of study procedures and enables the development of multimodal imaging analysis pipelines for the main phase of the study, which will include 86 adolescents (age 14-17) with ASD.

Keywords: Human Neuroimaging, Multimodal Neuroimaging, Repetitive Transcranial Magnetic Stimulation (rTMS), Autism Spectrum Disorder, Auditory Processing

Disclosure: Nothing to disclose.

P154. Development of a Novel qEEG Based Diagnostic Classifier for Adolescent Depression

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Background: The early recognition, accurate diagnosis and prognosis determination in youth with Major Depressive Disorder (MDD) is challenging. Imprecise nosology and overlapping presentations are particularly difficult in childhood (Mendelson and Tandon, 2016). As a result, there is an unmet need for evidence-based biomarkers in childhood psychiatric diagnoses.

We previously reported in a pilot biomarker study that quantitative electroencephalogram (qEEG) showed a difference in coherence (one measure of resting connectivity) between an outpatient sample of youth with Major Depressive Disorder (MDD) and healthy controls (HC). Adolescents with MDD showed a pattern of decreased coherence when compared with HC. We have developed predictive model, or diagnostic classifier, in a pilot sample of adolescents with MDD, using EEG measures of coherence, power and cross correlation.

We report on a new inpatient sample of youth with MDD and suicidality and measures of connectivity as measured by qEEG compared to HC. In addition, we report on new findings of the use of this predictive model in this inpatient sample of youth with MDD and suicidal ideation.

Methods: The initial diagnostic classifier was developed in an outpatient sample of youth with MDD. 21 youth, age 14-17 (F = 17 M = 4) with MDD and 14 healthy controls, age 14-17 (F = 10 M = 4) received a one-time resting EEG. The Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID), the Children's Depression Rating Scale (CDRS), the Snaith–Hamilton Pleasure Scale (SHAPS), and the Pediatric Anxiety Rating Scale (PARS), were given.

QEEG in four frequency bands (alpha, beta, theta, and delta) was collected, and coherence, cross-correlation, and power data streams obtained. A two-stage analytical framework was then used to develop the final logistic regression model which was then evaluated via a receiver-operating characteristic curve (ROC) analysis.

The validation sample consisted of a group of 27 MDD youth, age 13-18 (F = 21 M = 6) admitted to an inpatient child psychiatric unit for acute suicidality and 29 HC, age 13-18 (F = 16 M = 13). This cohort were given the MINI-KID, the CDRS and the PARS. This cohort of youth had resting EEG data collected in addition to task based data. The diagnostic classifier was validated in the resting EEG data collected in this sample. Coherence, cross-correlation and power data streams were obtained in the same four frequency bands.

Results: Within the initial training data set of outpatient youth with MDD, 6 qEEG dyads (all coherence) had significant predictive

values. The top 10% of predictive markers used to develop the classifier included coherence at the following dyads:F3-C3 (alpha frequency(fr)), P3-O1 (theta fr), F3-C3 (theta fr), F3-C3 (beta fr), CZ-PZ (beta fr), Fp2-F4 (theta fr), F3-C3 (delta fr), T8-P8 (beta fr), P3-O1 (alpha fr), Fp2-F4 (alpha fr), and power at the following dyads: C3-P3 (alpha fr), T7-P7 (theta fr), F7-T7 (theta fr), Fp1-F7 (alpha fr), F8-T8 (theta fr), P8-O2 (theta fr), F3-C3 (alpha fr), C4-P4 (delta fr), C4-P4 (theta fr), P7-O1 (theta fr), P4-O2 (beta fr), T8-P8 (theta fr). Within the final biomarkers, just 4 predictors, including F3-C3 (R frontal) alpha coherence, P3-O1 (R parietal) theta coherence, CZ-PZ (central) beta coherence, and P8-O2 (L parietal occipital) theta power were used in the final model, which yielded an ROC area of 0.8226.

In the validation sample set of youth with MDD and suicidality, the model that included the top 10% of classifiers yielded an ROC of 0.8122. The final model from the training data set included 4 variables, one of which, CZ-PZ, was not available in the validation sample. This model with only the 3 variables common to both data sets, yielded an ROC 0.5668. In the validation sample of youth with MDD and suicidality, 7 qEEG dyads were statistically significantly different in MDD youth than in HC ($p < 0.05$): T8-P8 coherence (beta fr), T8-P8 coherence (theta fr), T7-P7 coherence (beta fr), P3-O1 power (theta fr), F8-T8 power (alpha fr), T8-P8 coherence (alpha fr); E5_E60 cross correlation.

Conclusions: In these two samples of youth with MDD, qEEG as a diagnostic biomarker continues to show promise. A classifier developed in a training set of youth with MDD also showed robust predictive value in a validation sample of youth with MDD. The expanded classifier, including a broader range of dyads, performed more robustly than the narrow, 4 item classifier. Both sets of MDD participants show differences in measures of connectivity in frontal regions, consistent with changes in the default mode network in depressed youth.

Future longitudinal studies are warranted, especially in youth prior to the initiation of treatment. In addition, studies investigating how medications, severity of anxiety, and anhedonia impact qEEG results are indicated. Studies investigating the difference between MDD youth with and without suicidality are also indicated in the future based on this preliminary research.

Keywords: Adolescent Depression, Biomarker, EEG Connectivity

Disclosure: Nothing to disclose.

P155. Baseline Alterations and Acute Effects of Escitalopram on Amygdala Functional Connectivity in Youth at High Risk for Bipolar Disorder

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Background: Youth with a family history of bipolar disorder, especially those who present with depression and/or anxiety symptoms, are at high risk for developing bipolar disorder and disrupted functional connectivity (FC) within the prefrontal-amygdala emotional network. Antidepressants are commonly used to treat depression and anxiety in youth. However, the neural effects of antidepressant exposure in these high-risk youth remain unknown. Our aims were 1) to compare resting state FC in depressed and/or anxious youth at high risk for developing bipolar disorder and healthy comparison youth and 2) to compare early changes in resting state FC in these high-risk youth following treatment with escitalopram and psychotherapy vs. placebo and psychotherapy.

Methods: We conducted a two-site, double-blind, placebo-controlled trial of escitalopram in youth (12-17 years old) with depression and/or anxiety and a first-degree relative with bipolar I disorder (BD). At baseline, 121 high-risk youth and 55 healthy controls (HC) without any first- or second-degree relative with a mood or psychotic disorder were recruited. High-risk youth were then randomized to receive psychotherapy plus escitalopram ($n = 77$) or psychotherapy plus placebo ($n = 44$) for up to 16 weeks.

A 3-Tesla scanner with an 8-channel phased-array head coil was used at each site to acquire resting-state functional and anatomical images at baseline in high-risk and HC youth, and at 4-weeks after treatment initiation in high-risk youth. Two 5-minute functional runs used TR = 2000 ms and slice thickness=3 mm. Image analysis was performed using SPM, CONN, and MATLAB. Preprocessing steps included discarding the initial ten volumes, slice-timing, realignment, structural segmentation and normalization, functional normalization, outlier detection, and smoothing. Denoising was then performed, which applied linear regression of confounders, band-pass filtering, and linear detrending to remove unwanted motion, physiological, and other artificial effects from the BOLD signal. Next, the FC map between the amygdala and voxels of the whole brain was generated for each youth, which was then taken to two second-level statistical analyses: 1) High-risk vs. HC t-tests of amygdala-based FC with age, sex and site as covariates using thresholds $p < 0.005$ at voxel level and $p < 0.05$ at cluster level with False discovery rate (FDR) correction and 2) treatment (escitalopram or placebo)-by-time (baseline or week 4) interactions using a full-factorial analysis with age, sex, and site as covariates, and using the same thresholds as the baseline analyses. Connectivity values were extracted from clusters with significant treatment-by-time interactions. Post-hoc analyses were performed to calculate the direction of FC changes from baseline to week 4 in each treatment group.

Results: Baseline differences in amygdala-based FC between high-risk and HC youth:

The baseline analyses included one hundred and six high-risk youth and 46 age- and sex-matched HC with usable data. Compared to HC, high-risk youth had decreased connectivity between left amygdala and left ventral prefrontal cortex extending into insula and superior temporal cortex (peak coordinate: x = -46, y = 16, z = -16; cluster size=122; t = 4.78; FDR-corrected p = 0.036), and decreased connectivity between the right amygdala and bilateral dorsal anterior cingulate cortex (peak coordinate: x = -4, y = 26, z = 14; cluster size=217; t = 5.48; FDR-corrected p = 0.002) and right dorsolateral prefrontal cortex (peak coordinate: x = 22, y = 52, z = 14; cluster size=129; t = 4.45; FDR-corrected p = 0.014) as compared to HC.

Escitalopram effect on amygdala-based FC in high-risk youth:

Eighty-four high-risk youth with usable baseline and week 4 data were included; 50 of them received escitalopram and 34 received placebo. Significant treatment-by-time interactions were found between right amygdala and bilateral supplementary motor cortex (peak coordinate: x = -2, y = -26, z = 62; cluster size=264; F = 20.18; FDR-corrected p = 0.011), left lateral occipital cortex including left fusiform gyrus (peak coordinate: x = -44, y = -78, z = 10; cluster size=424; F = 16.97; FDR-corrected p = 0.001) and right occipital fusiform gyrus (peak coordinate: x = 32, y = -64, z = -6; cluster size=197; F = 15.72; FDR-corrected p = 0.032). Post-hoc analyses showed these amygdala FCs significantly decreased in youth treated with escitalopram relative to youth who received placebo.

Conclusions: We found that depressed and/or anxious youth at high-risk for bipolar disorder had decreased amygdala connectivity in regions that subserve emotion perception and regulation relative to healthy individuals. These findings suggest that brain functional connectivity within and between emotion processing and regulation networks are disrupted in youth at high risk for bipolar disorder. These alterations may serve as potential targets for early intervention in this population. Additionally, amygdala

connectivity with sensory and motor cortices decreased significantly in high-risk youth after four weeks of treatment with escitalopram and psychotherapy relative to those receiving four-weeks of placebo and psychotherapy. Further analyses will examine whether the significant effects of escitalopram on the interface among emotional, visual and motor networks are associated with subsequent behavioral and mood changes resulting from antidepressant treatment in this population.

Keywords: Bipolar Disorder, High Risk, Functional Connectivity

Disclosures: Myriad, Medscape: Advisory Board (Self), Alkermes: Consultant, Contracted Research (Self), Lundbeck, Janssen: Contracted Research (Self), Janssen: Consultant (Self), Allergan, Shire: Contracted Research (Self)

P156. Brain Development Trajectories in Adolescents With and Without Non-Suicidal Self-Injury

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Background: Adolescence is a critical period for brain development, and is also notable for the onset of non-suicidal self-injury (NSSI), a risk factor for future suicide attempts. Examination of brain developmental processes associated with the onset and course of NSSI during adolescence promises to advance understanding of risk mechanisms and to guide intervention strategies.

Methods: 164 adolescents ages 12-16 years (assigned female at birth) with and without a history of NSSI were enrolled in a longitudinal study that involved annual clinical and neuroimaging assessments for three years. NSSI frequency and severity was evaluated using the Self-Injurious Thoughts and Behaviors Interview. Brain imaging included a structural scan, a 12-minute resting-state fMRI scan and a 6-minute task fMRI scan to measure threat-related response in which participants matched emotion (fearful, angry) faces or neutral shapes. Functional data were parcellated using the Glasser atlas for cortical regions and the Harvard-Oxford atlas for subcortical regions. Given the relevance of the Sustained Threat domain to NSSI, following our prior work indicating a relationship between NSSI severity and threat-related circuitry at baseline (Başgöze et al., 2021), brain metrics for longitudinal analysis included amygdala activation in response to threat, and resting state functional connectivity (RSFC) between bilateral amygdala and ventromedial PFC (vmPFC). In addition to the standard methods of calculating RSFC, to further explore neural connectivity and allow examination of the direction of neural connections, we used causal discovery analysis to identify causal relationships (edges) among regions across the brain. Since very few participants were found to have causal edges involving the amygdala, these analyses focused on connections amongst key frontal regions implicated in emotion regulation – bilateral medial and dorsolateral frontal cortex (mPFC and dlPFC)– as well as the default mode network (DMN), which has been implicated in depression and self-related processing. For the task data, we focused on the activation in the emotion>shape contrast in bilateral amygdala. For longitudinal analyses, we first evaluated developmental associations on this set of brain metrics across adolescence in this NSSI-enriched sample by conducting separate regression analyses for each brain metric using generalized estimating equations (GEEs) to estimate the association of baseline age and change in age from baseline on connectivity strength. Second, to evaluate the impact of NSSI behavior on brain development, we added a term for baseline NSSI group and the interaction between baseline NSSI group (none, Mild [1-4 lifetime episodes], Moderate [more than 5 episodes and frequency of at least once per month], and Severe [more than 5 lifetime episodes and frequency of more than once per month]) and change in age from baseline. Results were considered statistically significant if the two-sided p-value was less than 0.05. P-values were not adjusted for multiple comparisons.

Results: Usable clinical and imaging data was available for 140 participants at Time 1, 87 participants at Time 2, and 39 participants at Time 3. For the analyses examining overall developmental trajectories in this sample, baseline age and change in age from baseline were positively associated with the brain metrics ($p < 0.05$) in the models for right amygdala activation to threat and the causal edge from DMN to left dlPFC. When examining group by time effects, the trajectory of change over time for the Severe NSSI group differed significantly from the No NSSI group for causal edges DMN to left mPFC and right mPFC to DMN. The trajectory of change over time for the Moderate NSSI group differed significantly from the No NSSI group for causal edges DMN to right mPFC (which also had a main effect of age), and right mPFC to right dlPFC. When examining NSSI severity group without a significant interaction with change in age, the Mild NSSI group had significantly weaker connections than the No NSSI group in the edges from left dlPFC to DMN and from left mPFC to left dlPFC. Additionally, the Severe NSSI group had significantly lower RSFC from the amygdala to vmPFC (which also had a main effect for age) compared to the No NSSI Group.

Conclusions: We report evidence for developmental change in key circuits relevant to emotion regulation and self-related processing across mid-adolescence. Severity of NSSI in adolescence is associated with neural signatures, some of which are present across time and some which manifest as change over time, characterizing distinct neurodevelopmental trajectories in key circuits. Understanding these patterns is critical for conceptualizing and testing novel treatments designed to restore healthy neurodevelopment.

Keywords: Brain Development, Adolescence, Non-Suicidal Self-injury (NSSI)

Disclosure: Nothing to disclose.

P157. Developmental Change in Human Substantia Nigra: Preliminary Observations Early and Late in Life Assessed With Neuromelanin-Sensitive MRI

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Background: In recent years neuromelanin-sensitive MRI (NM-MRI) has been applied to assay the integrity and function of human catecholamine systems in vivo. To optimize use of the method it is important to account for the known accumulation of neuromelanin (NM) in the substantia nigra (SN) throughout the lifespan, which has been shown to have a large impact on the signal. This is relevant not only in late life, where the method has often been applied in the context of neurodegenerative illness, but also early in life, where the method allows the rare opportunity to directly assay dopamine system function in the context of pediatric disorders. While prior work has indicated the SN signal may follow an inverted-u pattern, peaking in middle age and declining slightly late in life, this work did not examine the SN in anatomical detail, a level of analysis that may be necessary to optimize the use of the method for mechanistic studies or as a biomarker.

Methods: NM-MRI images were collected at two separate locations using the same turbo spin echo sequence. Sample 1 was

collected at the uOttawa Institute of Mental Health Research and included individuals aged from age 7-30 years (n = 27) and from 52-82 years (n = 22). Sample 2 was collected at the Montreal Neurological Institute and individuals aged over 52 years old, all of whom had longitudinal data with 1-2 annual follow-up scans (n = 68). NM-MRI signal in the SN was examined using a pipeline that brings NM-MRI images to standardized space and performs intensity normalization relative to a reference region near the SN with low NM content, the crus cerebri. In Sample 1, voxelwise robust linear regression analyses were performed within the SN to predict NM-MRI signal based on age and sex. In Sample 2, linear regressions were performed for each participant's longitudinal data to determine the slope of change in NM-MRI signal over time for all SN voxels, followed by 1-sample t-tests on these slopes. Permutation tests were applied for significance testing.

Results: In order to consider developmental change in the SN both early and late in life, we divided Sample 1 into individuals younger than 30 and older than 52 years old. In the younger group, the whole SN showed a significant increase in signal with age ($R = 0.63$, $p = 0.0005$, partial correlation controlling for sex) and voxelwise analysis found a large set of voxels within the central SN that increased with age (1000 of 1879 voxels thresholded at $p < 0.05$, corrected $p < 0.001$, permutation test). The annual increase in signal here was equal to 0.24% of the reference region signal. Almost no SN voxels showed decreasing signal over this range of age (4 voxels). In the older individuals, a set of 172 voxels in central SN showed increasing signal with age (annual change=0.19%) and a set of 112 voxels in peripheral SN showed decreasing signal with age (annual change = -0.21%).

When examining change in SN signal longitudinally over time in a separate sample collected at a different institute (Sample 2, with a similar age range to the older group from Sample 1), there was a large cluster of voxels where signal decreased over time (thresholded at $p < 0.05$, 515 voxels, corrected $p < 0.0001$, permutation test, median annual change across subjects = -0.40%) and a small cluster of voxels where signal increased over time at this threshold (9 voxels, median annual change across subjects=0.34%). Examining the spatial overlap between SN voxels where signal changed with age (Sample 1, older group) and SN voxels where signal changed over time (Sample 2) there was significantly more overlap than would be expected by chance both for increasing signal voxels (corrected $p = 0.007$, permutation test) and decreasing signal voxels (corrected $p < 0.0001$).

Conclusions: We found evidence that the NM-MRI signal increases at a relatively rapid rate throughout the SN in early life. Even into later life, signal in a portion of the central core of the SN continues to increase while the size of the SN appears to shrink, perhaps due to loss of dopamine cells during normal aging. These findings provide rare insight into the development of the human dopamine system. They should support the use of NM-MRI as a biomarker by enabling anatomically detailed age-specific norms for signal within the SN. This is particularly relevant as the method may have key applications in both youth and aging populations (e.g. in pediatric disorders and neurodegenerative disorders). These findings are consistent with NM contributing significantly to the contrast given the known tendency of NM to accumulate linearly throughout the lifespan. In future, with an expanded and longitudinal sample, we will develop a model of developmental change in NM-MRI signal across the full lifespan and we will investigate the impact of other clinical and demographic factors that may need to be considered to optimize potential use of the method as a biomarker.

Keywords: Dopamine, Brain Development, Neuromelanin-Sensitive MRI, Healthy Individuals, Substantia Nigra

Disclosure: Terran Biosciences: Patent,Contracted Research (Self)

P158. Sex Differences in the Microbiome-Gut-Brain Axis are Time-Of-Day Dependent

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Background: Circadian rhythms orchestrate a wide range of homeostatic processes, including metabolism, immune function, and the bidirectional communication between the gut and brain. Emerging evidence points to the gut microbiome and its metabolites as a novel class of entraintment signals, but the precise molecular mechanisms by which diurnal rhythms in microbial metabolites contribute to these processes remains unknown. Moreover, circadian rhythms are influenced by sex, suggesting that microbiome-gut-brain axis function is sex-specific and influenced by time-of-day.

Methods: To examine the hypothesis that sex differences in the microbiome-gut-brain axis are time-of-day dependent, we leveraged a novel systems biology approach by combining longitudinal analyses with dietary manipulation, metabolomics, bulk and single-cell transcriptomics of gut and brain, single-cell immunophenotyping, and machine learning. We harvested tissues from C57Bl/6J and BALB/c male and female mice every 4 hours across the day-night cycle.

Results: Bulk transcriptomic analyses of the intestinal tract revealed that transcriptional signatures were sex-specific and dependent on time-of-day (FDR < 0.05). Diurnal gene expression patterns in the gut were synchronized with the production and peripheral availability of microbial-derived short-chain fatty acids (SCFAs). Integrated single-cell immunophenotyping, metabolomics and transcriptomics revealed that time-of-day shifts in the peripheral availability of SCFAs were associated modifications to circulating immune composition and transcriptional signatures in key neural regions controlling whole-body metabolism, including the arcuate nucleus of the hypothalamus. These sex-specific time-of-day patterns were abolished in germ-free mice, suggesting that an intact gut microbiome is necessary for the synchronization of sex differences across the microbiome-gut-brain axis ($p < 0.05$ for all comparisons). Further, biosynthesis of SCFAs requires availability of soluble fibers in the diet. Thus, to determine whether dietary availability of soluble fibers and subsequent availability of SCFAs is a necessary time-of-day sex differences in the microbiome-gut-brain axis, mice were fed a refined high-fat low-fiber diet (HFD) or a grain-based chow (GBC) control for six weeks. Time-of-day effects on SCFA availability were disrupted in mice consuming HFD, suggesting that dietary availability of soluble fiber is essential is necessary for daily oscillations in cecal weights and SCFA abundance.

Conclusions: Our results show that synchronization of diurnal feeding patterns with availability of key nutrients control sex differences in the microbiome-gut-brain axis and regulate host immunity and neuroendocrine function. Our findings motivate exploration of the host circadian clock and microbial circadian rhythmicity and highlight the need to consider sex and timing of sample collection in neuroscience experiments.

Keywords: Microbiota-Gut-Brain Axis, Immunity and Neurodevelopment by Sex, Immunometabolism, Neurometabolism, Brain Regulation of Metabolism

Disclosure: Nothing to disclose.

P159. Social Deficits Induced by Combined Air Pollution and Maternal Stress are Prevented by Microbial or Dopaminergic Intervention

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Background: Epidemiological data suggest that perinatal exposure to environmental toxicants, such as air pollution, is associated with risk for autism spectrum disorders (ASD). Psychosocial stressors may activate the maternal immune system, increasing sensitivity to these toxicants. Still, the mechanisms by which this synergism occurs have yet to be elucidated. ASD is a male-biased disorder and is defined by impairments in social interaction/communication and repetitive/restrictive behaviors. Importantly, the gut-brain axis has also been strongly implicated in ASD. Using a novel mouse model of combined prenatal diesel exhaust particles (DEP) and maternal stress (MS), we found that male offspring display reduced sociability, changes in the gut microbiome, and altered microglia and dopaminergic tone following DEP/MS.

Methods: First, we aimed to test whether restoring control-typical gut microbiota could rescue social behavior following DEP/MS via a cross-fostering procedure. DEP/MS-exposed male and female pups were fostered on the day of birth to either a DEP/MS or a CON-exposed dam. Social behavior was assessed during adolescence. Second, we used a chemogenetic approach to test whether activation of the dopamine system could rescue social behavior during adolescence. DAT-Cre+ CON or DEP/MS males received stereotaxic microinjection of a control virus or an excitatory DREADD receptor virus into the ventral tegmental area. Clozapine-N-oxide was administered prior to behavioral testing. Finally, we conducted RNA sequencing of isolated microglia from CON and DEP/MS male offspring and compared gene expression patterns to those of germ-free mice, LPS-challenged mice, and across development (based on previously published datasets) using rank-rank hypergeometric overlap (RRHO) analysis.

Results: We found that cross-fostering of DEP/MS-exposed male pups to CON dams on the day of birth prevented decreases in sociability ($t(1,13)=3.334$, $p < 0.01$). Second, we observed that DREADD activation of VTA dopamine neurons restored social preference in DEP/MS-exposed male offspring ($F(2,26) = 4.253$; $p < 0.05$). Finally, we found that microglial gene expression patterns in DEP/MS-exposed males corresponded with gene expression signatures in germ-free mice. Interestingly, this was opposite of comparisons between DEP/MS-exposed males and males that were acutely treated with LPS.

Conclusions: These results suggest that intervening at the level of either the gut microbiome (perinatally) or the dopamine system (acutely during adolescence) is sufficient to rescue social functioning following prenatal DEP/MS exposure in male offspring. Furthermore, our findings suggest DEP/MS induces a microglial phenotype that is similar to that of germ-free mice, potentially one that is immature and less immunocompetent.

Keywords: Autism, Air Pollution, Microglia, Dopamine, microbiome

Disclosure: Nothing to disclose.

P160. Investigating Developmental Microglial-Parvalbumin Interneuron Interactions in Microglial-MyD88 Deficient Mice

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Background: Parvalbumin+ interneurons (PVIs) are fast-spiking, GABAergic cells critical for coordinating firing activity to maintain normal brain function. Developing PVIs are susceptible to inflammation, making them a common cell type impacted in disorders such as schizophrenia, autism spectrum disorders, and major depressive disorder. Decades of studies also point to the impact of perinatal inflammation on the function of microglia, the resident immune cells of the brain, and their roles in disease-relevant synaptic alterations and behaviors in mice. Importantly, microglia interact closely with developing neurons, influencing their maturation and survival. Despite this, nearly all existing studies on microglia-neuron interactions in the early postnatal period focus only on excitatory neurons, with still very little known about microglia-interneuron interactions across development. We hypothesized that broad ablation of microglial inflammatory signaling would be protective for developing PVIs following an early life immune challenge. However, loss of microglial inflammatory signaling resulted in an increased density of PVIs across the male brain, suggesting a role for microglial-MyD88 dependent signaling in regulating PVI development in baseline conditions. Here, we aim to investigate the mechanisms linking microglial inflammatory signaling pathways and adult PVI density.

Methods: Our lab developed a mouse in which the toll-like receptor adaptor protein MyD88 (myeloid differentiation primary response 88) is ablated specifically from microglia (Cx3cr1-CreBT-MyD88f/f), effectively blunting microglial proinflammatory signaling. In adulthood, we characterized social and cognitive behaviors and PVIs across the brain by immunohistochemistry. To investigate the role of microglial-MyD88 signaling in very early PVI development, we used RNAscope to identify future PVIs prior to the expression of the parvalbumin protein. Finally, PVI synaptic density changes across wild-type development were determined by co-labeling of PVI-specific presynaptic label synaptotagmin-2 (Syt2) and postsynaptic label gephyrin, which was quantified using software developed by the Eroglu Lab at Duke University.

Results: We found that microglial-MyD88 loss had a significant effect on PVI density in the hippocampus ($F(1,37) = 5.54$, $p = 0.024$; $n = 6$-12/group) and frontal cortex of males ($F(1,10) = 10.82$, $p = 0.0082$; $n = 3$-5/group), but not females ($F(1,12) = 0.01774$, $p = 0.8963$; $n = 3$-5/group). Microglial-MyD88 genotype did not significantly impact the density of somatostatin (SST) interneurons, another abundant subtype of interneurons, in the frontal cortex of males ($F(1,13) = 1.578$, $p = 0.231$; $n = 3$-5/group) or females ($F(1,13) = 0.746$, $p = 0.404$; $n = 3$-5/group). Mice lacking microglial-MyD88 signaling have normal sociability at baseline (males: $F(1,33) = 0.321$, $p = 0.575$; females: $F(1,31) = 1.659$, $p = 0.207$; $n = 8$-10/group), but males show reduced spatial reference memory in a Y-maze task ($t(1,22) = 2.591$, $p = 0.0167$; $n = 12$/group). We next hypothesized that microglial-MyD88 loss impacts adult PVI density developmentally. Preliminary results suggest that microglial-MyD88 loss does not impact the early postnatal P0-P8 cell death period of PVI development in the male dorsal hippocampus ($F(1,11) = 0.1723$, $p = 0.6861$; $n = 2$-4/group). Wild-type characterization of male and female PVI cell density from P4-P42 revealed no sex differences ($F(1,32) = 0.2732$, $p = 0.6048$; $n = 2$-5/group), but males have an increased PVI synaptic density in the third postnatal week ($F(1,42) = 46.43$, $p < 0.0001$; $n = 4$-7/group), identifying a potential mechanism through which adult PVI density is regulated in a sex-specific manner. Ongoing work will characterize synaptic elimination by wild-type and MyD88-deficient microglia in males and females during the PVI synaptic pruning period to attempt to identify a sensitive temporal window of PVI density regulation in males.

Conclusions: Microglia regulate developing PVIs in the male frontal cortex and dorsal hippocampus at baseline in a MyD88-dependent manner, with consequences for adult cognition. The early postnatal PVI cell death period is not altered by loss of microglial-MyD88, suggesting microglial regulation of the adult

PVI population size happens after the first postnatal week. Future work will probe microglial phagocytosis of PVI synapses in the early adolescent period.

Keywords: Microglia, Neurodevelopment, Parvalbumin Interneurons, Toll-Like Receptors (TLRs), Cognition

Disclosure: Nothing to disclose.

P161. Early-Life Inflammation and Depression in Adolescents Born Extremely Preterm

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Background: Adolescent depression is a major public health problem, with an estimated lifetime prevalence of 14% for major depressive disorder (MDD) between the ages of 15-18 years. Approximately 84% of depressed adolescents later experience depression in adulthood. Despite devastating clinical consequences of adolescent MDD, its etiology and pathophysiology remain poorly understood.

Increasingly, data support a potential role for inflammation in the pathogenesis of depression, although it is not yet established whether inflammation is a cause, a correlate, and/or a consequence of MDD. A recent meta-analysis demonstrated that elevations of inflammatory biomarkers, specifically interleukin-6 (IL-6) and C-reactive protein (CRP), can predate the onset of depression. However, available prospective studies to date have focused primarily on adults and older adults, with the strongest associations between inflammation and depression observed in geriatric samples. Less is known about the longitudinal associations in pediatric samples and no study to date has examined the role of early-life inflammation (ELI) in subsequent depression. A systematic understanding of how ELI interacts with well-known risk and protective factors of depression is also lacking.

In the present study, we examined a longitudinal association between ELI assessed during the neonatal period and depression at age 15 years in a cohort of adolescents who were born extremely preterm. Extremely preterm neonates are at high risk for experiencing neonatal inflammation and for developing later psychiatric disorders, including adolescent depression. Therefore, extremely preterm born individuals are an ideal group for an examination of these associations. We hypothesized that: (1) ELI will be associated with an increased risk of depression at age 15 years, (2) childhood adversity, a well-documented risk factor of depression, will amplify the risk of ELI on depression, and (3) social support, a well-documented protective factor of depression, will attenuate the risk of ELI on depression.

Methods: The Extremely Low Gestational Age Newborns (ELGAN) Study is a multi-site longitudinal study of children born < 28 weeks of gestation. We measured six circulating inflammatory proteins (i.e., interleukin-6 [IL-6], tumor necrosis factor-alpha [TNF-α], intercellular adhesion molecule-1 [ICAM-1], interleukin-8 [IL-8], serum amyloid A [SAA], and C-reactive protein [CRP]) in 1121 extremely preterm infants on postnatal days 1, 7, and 14. These proteins were chosen given their consistent associations with structural and/or functional neurologic outcomes in previous ELGAN studies. For each protein, we defined intermittent/sustained systemic inflammation (ISSI) as having top quartile levels within its gestational age stratum on ≥ 2 days during the first 14 postnatal days. At age 15 years, 670 surviving adolescents (344 male and 326 female) were screened for depression using the Child Behavior Checklist (CBCL) and the PROMIS Depression scale and assessed for the diagnosis of MDD using the Mini-International Neuropsychiatric Interview for Children and Adolescents (MINI-KID). We used multiple logistic and linear regression models to examine the association between the number of proteins with ISSI (0, 1-2, or ≥ 3 elevations) and depression at 15 years. Childhood adversity, as measured by the Yale-Vermont Adversity Scale, and adolescent social support, as measured by the PROMIS Family and Peer Relationships scales, were examined as moderators. Analyses were stratified by adolescent sex and adjusted for confounders as determined by directed acyclic graphs, including familial history of depression and non-infectious initiators of neonatal inflammation (i.e., small for gestational age status, maternal low socioeconomic status, maternal prenatal tobacco exposure, maternal obesity, and maternal hypertensive disorders). Associations were expressed as odds ratios (OR) and 95% confidence intervals (CI). For continuous outcomes, effect sizes were expressed as regression coefficients that quantify the difference in depression T-scores for participants with and without the exposure.

Results: In our extremely preterm sample, elevated ELI protein levels were not significantly associated with the diagnosis of MDD (ISSI 1-2: OR = 1.30, 95% CI = 0.75-2.22; ISSI ≥ 3: OR = 0.89, 95% CI = 0.42-1.82) or CBCL/PROMIS depression scores at 15 years (ISSI 1-2: βs -0.88 to 0.01; ISSI ≥ 3: βs -1.64 to -1.17). As expected, childhood adversity was positively associated with the adolescent MDD diagnosis (OR = 1.13, 95% CI = 1.08-1.18) and CBCL/PROMIS depression scores (βs 0.21 to 0.49), while adolescent social support was negatively associated with the MDD diagnosis (OR = 0.91, 95% CI = 0.86-0.96) and depression scores (βs -0.38 to -0.69). However, the proposed moderators did not significantly interact with ELI to predict adolescent depression at age 15 years.

Conclusions: We did not observe significant associations between neonatal systemic inflammation and depression measured at 15 years of age. Future research is needed to determine whether individual levels of inflammatory proteins or timing of inflammation would be better predictors for the onset of depression.

Keywords: Inflammation, Neonatal, Adolescent Depression, Biomarker

Disclosure: Nothing to disclose.

P162. Carving Adversity at its Joints: Dimensions and Downstream Effects of Traumatic and Early Childhood Experiences (TRACES)

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Background: Worldwide, over one billion children are at risk of enduring the chronic, pervasive sequelae of experiences so noxious to be characterized as 'adversity' – circumstances or events threatening physical or psychological well-being. The constellate biological and psychological effects of adverse experiences presage chronic increases for risk of mental illness, poverty, addiction, criminality, morbidity, mortality, suicidality, and critically, perpetuating adversity onto others. Traumatic and early life experiences (TRACES; Weems et al., 2021) are the single greatest preventable risk factor for psychopathology and impose an annual economic cost to the United States ($748bn) exceeding the annual economic output of nine in ten nations around the globe.

Though decades of research evince the link between adversity and chronic dysfunction, the very nature of adversity as a concept remains a topic of heated debate – with competing models

emphasizing the importance of discrete features (Smith and Pollak, 2020) or bi-dimensional characteristics (McLaughlin et al., 2020) in capturing neurobehavioral sequelae. The current work proposes a unifying reconceptualization of adversity as a multidimensional concept comprised of clusters of thematically-linked events likely to exert common downstream effects. A novel data-driven approach is applied to youth-reported exposure to over 100 different experiences reflecting various forms of adversity known to perpetuate maladjustment. These data were reduced to dimensional TRACES components and considered as predictors of status and maturational change in presence of psychiatric symptoms and cognitive development.

Methods: Analyses drew data from the 11,876 youth (ages 9-10; 51% female) and their caregivers participating in baseline (Year 0) data collection as part of the Adolescent Brain Cognitive Development (ABCD) Study, 11,225 dyads repeating assessment in ABCD Year 1, 10,414 in ABCD Year 2, and 6,251 in Year 3. Fluid intelligence was operationalized as a latent factor derived from age-corrected standardized scores obtained from relevant tests completed in an abbreviated administration of the NIH Toolbox (Y0, Y2). Internalizing and externalizing problems were operationalized using summary scores from the parent-report ASEBA Child Behavior Checklist Categorical gathered at each wave of data collection (Y0-Y3). A comprehensive review of assessment instruments administered through Year 2 (Y2) identified 106 youth or parent-report questionnaire items capturing exposure to adverse experiences. For items presented at multiple waves, the most recent data were used, while scores from items repeated across caregiver and child reports were combined by taking the larger value. The resultant list included binary, ordinal, count, and continuous items. Categorical principal components analysis (CATPCA) can appropriately model each of these item types (in contrast to traditional PCA), is robust to non-normality, and reduces items to formative composites.

Latent means (fluid intelligence) and path estimates (externalizing, internalizing; effects of adversity components) reflecting change were derived from models holding measurement invariance across time. Component scores were computed and entered as covarying predictors in separate latent difference score models of baseline status and developmental change in internalizing and externalizing problems, as well as fluid intelligence.

Results: An eight-component solution exhibited the best fit to the data and the most intuitive interpretation and included dimensions reflecting caregiver problems (e.g., incarceration, addiction, mental illness), community violence, bullying/peer aggression, chronic pain, poverty, discrimination, family conflict, and interpersonal violence. On average, ABCD youth exhibited increases in fluid intelligence [$\alpha = 1.63$ ($SE = 0.18$), $Z = 9.01$, $p < .001$), no change in externalizing problems, and an annual decline in internalizing problems [$\beta = -0.11$ (0.016), $p < .001$]. All adversity components, except for bullying/peer aggression ($\beta = 0.01$, $p = .42$), were associated with greater externalizing problems at baseline assessment (all $p < .05$), while community violence predicted increasing externalizing problems across time [$\beta = 0.019$ (0.008), $p < .05$]. Caregiver problems, community violence, bullying/peer aggression, discrimination, and family conflict each predicted greater levels of internalizing problems at baseline (all $p < .001$), while caregiver problems ($p < .05$), community violence, chronic pain, discrimination, and family conflict were associated with increased internalizing problems across time.

Conclusions: The findings demonstrate the viability of an alternative conceptualization of traumatic and adverse childhood experiences, as well as the utility of considering the components of a dimensional model as predictors of altered maturational change in cognitive ability and psychiatric problems. Presented findings will additionally examine moderating effects of race, ethnicity, and sex at birth. In total, this work demonstrates the merits of a multidimensional model that may enhance our collective ability to understand adverse experiences by carving nature at its joints.

Keywords: Childhood Adversity, Childhood Psychiatric Symptoms, Statistical Methods, Adolescent Brain and Cognitive Development Study, Longitudinal Analysis

Disclosure: Nothing to disclose.

P163. Sensitivity to Time and Treatment Effects of New Short Forms of the PANSS in an Outpatient Pediatric Randomized Controlled Trial

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Background: Global regulatory initiatives have resulted in an increasing number of psychopharmacology trials in the pediatric age range. Among the challenges in ensuring valid and reliable data in such trials are developmental limitations in symptom description, the need to integrate and weight information from varied sources (including the patient, parents/caregivers and other informants, and the global shortage of child-trained clinical investigators (Busner, 2013; Farchione, 2013). Moreover, few efficacy measures have been developed and validated specifically for pediatric trials. As a result, measures designed for and validated in adults, such as the Positive and Negative Syndrome Scale (PANSS), are frequently used in adolescent schizophrenia trials. The PANSS is a complex 30-item measure that has been extensively studied and shown to pose ratings challenges even in the adult patients for whom it was designed (e.g., Daniel and Dries, 2013). Our group has developed a 10-item PANSS based on psychometric analyses of NIMH TEOSS study data. The 10-item PANSS was reliable and sensitive to treatment changes. In the present study we attempt to replicate the findings using an independent multi-site trial (Findling et al., in press).

Will a 10 item PANSS optimized for pediatric trials perform similarly to the 30 item PANSS in an independent placebo-controlled multi-site trial?

Methods: The 6-week, double-blind, parallel group, acute phase data from the Johnson and Johnson- sponsored completed, positive, paliperidone study (Singh et al., 2011) were accessed from the YODA secure data environment. The trial included 201 12-17 year olds randomly allocated to placebo or one of three fixed doses of paliperidone. Analyses were performed using the mirt, lavaan, sjstat, rstatix and psych packages in R, using the same syntax and methods as the prior analyses (Findling et al., in press), with mixed regressions using random intercepts and partial eta-squared as the effect size estimate for time, treatment, and time x treatment interaction effects.

Results: The 10-item vs. 30-item versions had similar average inter-item correlations (.25 and .25), as well as similar partial eta-squared values for time – .37 (.32 to .41) versus .41 (.36 to .45), treatment (all .00) and time x treatment (.007 versus .003 for the full length). IRT models indicated similar reliability as in the development sample, with good precision across a similar range of severity. LOCF analyses found separation from placebo using both the 10 and 30-item versions on multiple, identical arms; ANCOVAs controlling for PANSS at phase entry produced similar eta-squareds at subsequent weeks (largest difference = .005, favoring 10-item version).

Conclusions: The 10-item version of the PANSS replicated well in an independent, larger adolescent sample using double-blind

RCT data. The similar sensitivity to treatment effects is particularly promising given the substantial reduction in scale length and corresponding decreases in required rater training, interview length, and respondent burden.

Keywords: Child Psychopharmacology, Clinical Outcome Assessments, Pediatric Clinical Trials

Disclosure: Signant Health: Stock / Equity (Self)

P164. Ecological Momentary Assessment Identifies Frustration as a Central Node in Irritability in a Transdiagnostic Sample of Youth

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Background: Irritability, an increased proneness to anger relative to peers, is a transdiagnostic symptom commonly occurring with anxiety and other mood symptoms. However, little is known about the temporal and dynamic interplay between irritability-related clinical phenomena. Network analysis has the potential to improve our understanding of this complex organization of symptoms and related constructs. Unlike traditional conceptualizations of psychopathology, which posits that symptoms are manifestations of an underlying, latent cause or disorder, network approaches conceptualize disorders as systems of causally connected, interacting, and mutually-reinforcing symptoms (Borsboom and Cramer, 2013; Borsboom et al., 2021). Thus, network approaches are suitable to delineate the patterns of interrelations between irritability-related symptoms and behavioral, cognitive, and mood constructs. A better understanding of the temporal dynamics between irritability-related symptoms may inform targets and timing for interventions. Using network analysis with smartphone-based ecological momentary assessment (EMA) to increase ecological validity, this study examined how daily naturally-occurring irritability symptoms and other mood and anxiety symptoms are connected. This is the first study to use network analysis to investigate the symptomatology of irritability in naturalistic settings.

Methods: Sample included 152 youth ages 8–18 years (Age: $M \pm SD = 12.28 \pm 2.53$; 69.70% male; 65.80% White/Caucasian, 9.90% African American, 4.60% Asian American, 2.60% American Indian, 13,20% Multi-race, 3.90% unknown) across several diagnostic/phenotypic groups enriched for irritability symptoms. The groups included disruptive mood dysregulation disorder (DMDD; $n = 34$), subthreshold DMDD (i.e., those with impairing irritability but failed to meet the full DMDD criteria; $n = 22$), attention-deficit/hyperactivity disorder ($n = 35$), anxiety ($n = 28$), and healthy comparisons ($n = 33$). Participants completed EMA three times a day for seven days (i.e., 21 data points per participant). EMA items included irritability and related symptoms (grouchiness/crankiness, annoyance/anger, frustration, feelings of unfairness) as well as other mood and anxiety symptoms (worry, happiness, sadness, mood lability), sampling across different affective chronometries (momentary vs. longer-lasting symptoms). Irritability was also assessed using parent-, child-, and clinician-reports of the Affective Reactivity Index (ARI). Network analysis using the multilevel vector autoregressive (mlVAR) model with EMA data estimated three networks: (1) temporal network, (2) contemporaneous within-subject network, and (3) between-subjects network of symptoms. Additionally, to evaluate whether and how EMA measures of irritability and related symptoms/constructs were associated with rating scale-based "trait-like" measures of irritability, we examined the mean level and variability (i.e., SD and root mean square of successive differences [RMSSD]) of EMA-rated irritability (i.e., four items: grouchiness/crankiness, annoyance/anger, frustration, feelings of unfairness) across 21 data points and their correlations with golden standard clinician-, child-, and parent-reports of irritability (i.e., ARI).

Results: Frustration was identified as the most central node in the network of irritability-related symptoms/constructs (i.e., grouchiness, unfairness) and other mood and anxiety symptoms (i.e., mood changes, worry). Frustration at one timepoint also positively predicted mood changes in the next timepoint. Moreover, we found both differences and similarities in the within-subject and between-subject processes between irritability-related constructs/symptoms and other anxiety and mood symptoms. While anger/annoyance was positively related to unhappiness/sadness within individuals and within measurement occasions, anger/annoyance was positively related to unhappiness/sadness, mood lability, and worry between/across individuals. Finally, compared to variability (i.e., SD and RMSSD; $r's = .13–.41$), the mean level of EMA-indexed irritability-related constructs were more strongly associated with retrospective "trait-like" measures of irritability (i.e., ARI; $r's = .23–.56$).

Conclusions: This study advances our understanding of symptom-level and temporal dynamics of irritability. Frustration emerged as the most central node in the network of irritability-related symptoms/constructs and other mood and anxiety symptoms. Frustration also predicted increases in mood changes at the subsequent timepoint. These findings have potential clinical implications, suggesting that frustration may be a primary treatment target for youth with irritability. Future experimental work or clinical trials are needed to systematically manipulate irritability-related features (e.g., frustration, unfairness) to further understand the causal relations among clinical variables. For example, clinical trials can be designed to test the efficacy of interventions/treatments targeting frustration given its high centrality. If the causal effect of frustration on other mood symptoms is validated, with the use of EMA, interventions could be developed and delivered right when youth become frustrated, and before frustration influences other mood symptoms downstream, to intervene in a timely and maximally effective way.

Keywords: Pediatric Irritability, Frustration, Ecological Momentary Assessment, Network Analysis

Disclosure: Nothing to disclose.

P165. Interpersonal Functioning and Relationship to Self-Harm in Adolescents Engaging in Either Suicide Attempts or Non-Suicidal Self-Injury

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Background: Suicide is the second leading cause of death in children and young adults (Eaton et al., 2005). However, completed suicides represent just a portion of adolescents suffering from suicidal behavior. Nationwide, over 16% of high school students seriously consider suicide, and over 8% attempt suicide each year. Furthermore, non-suicidal self-injury (NSSI), defined as deliberate destruction of one's body tissue without intent to die, is nevertheless strongly associated with suicide attempts (SA) in adolescents (Scott et al., 2015; Stewart et al, 2017). Interpersonal dysfunction is among the most robust predictors of the development and maintenance of both NSSI and suicidal

attempts in this age group (King and Merchant, 2008), but it is a complex, multifaceted construct. The purpose of this study is to better understand what components of interpersonal functioning lead to NSSI vs. SA in adolescents. We hypothesized that higher levels of interpersonal sensitivity and deficits in social problem-solving strategies would be observed across both NSSI and SA groups, but not in healthy controls.

Methods: This IRB-approved study compared self-reported interpersonal sensitivity (Interpersonal Sensitivity Measure [IPSM]), social problem-solving strategies (Social Problem Solving Inventory [SPSI]), and suicidal ideation (measured by the Beck Suicide Scale [BSS]), among three mutually exclusive, relatively homogenous groups of adolescents (ages 13-17) who: (1) engaged in NSSI without history of SA ($n = 50$, Mage $= 15.35$, $SD = 1.34$, 82% female), (2) made a SA without history of NSSI ($n = 49$, Mage $= 15.80$, $SD = 1.23$, 63% female), and (3) typically developing controls (TDCs) without history of psychopathology ($n = 43$, Mage $= 15.46$, $SD = 1.30$, 61% female). The analytic strategy included: (1) ANOVA to evaluate between-group differences in IPSM, SPSI, and BSS, (2) MANOVA to examine possible group and sex differences in IPSM and SPSI, and (3) logistic regression to ascertain the effects of IPSM and SPSI scores on group status.

Results: ANOVA results suggest significant between-group differences in IPSM ($F(2,139) = 34.89$, $p < 0.001$) and SPSI ($F(2,139) = 42.5$, $p < 0.001$). Tukey post-hoc tests revealed that NSSI (100.25 ± 18.1, $p < 0.001$) and SA (84.50 ± 18.50, $p = 0.001$) groups had significantly higher interpersonal sensitivity than TDCs (72.0 ± 10.40). NSSI group scores were significantly higher than SA group scores ($p < 0.001$). For social problem-solving, the NSSI group (44.00 ± 14.90, $p < 0.001$) and SA group (50.60 ± 17.90, $p < 0.001$) had significantly lower scores than the TDC group (71.90 ± 11.00); however, there was no significant difference between the NSSI and SA groups ($p = 0.07$). Similarly, there was no significant difference in suicidal ideation (SI) between the NSSI and SA groups, $t(97) = 1.70$, $p = 0.093$. SI was not reported in any participants in the TDC group.

A 3X2 MANOVA indicated no significant interaction effect of group and biological sex differences on IPSM and SPSI total scores. The overall MANOVA model for group was significant [$F(4,270) = 21.04$, $p < 0.001$]. Univariate analyses showed significant group effects for both the IPSM [$F(2,136) = 25.23$, $p < 0.001$] and SPSI [$F(2,136) = 34.06$, $p < 0.001$] total scores. Specifically, the NSSI IPSM mean [$MNSSI = 99.95$] was significantly higher than the TDC mean [$MTDC = 71.72$, $p < 0.001$, Cohen's $d = 10.04$] and the SA mean [$MSA = 84.00$, $p < 0.001$, Cohen's $d = 5.79$]. The SA group IPSM mean total score was also significantly higher than the TDC group mean total score [$p = 0.002$, Cohen's $d = 4.90$]. The TDC SPSI mean [$MSPSI = 71.60$] was significantly higher than both the NSSI group mean [$MNSSI = 44.22$, $p < 0.001$, Cohen's $d = 10.60$] and the SA group mean [$MSA = 50.22$, $p < 0.001$, Cohen's $d = 9.28$]. There was no significant difference between the SA group and NSSI group SPSI mean total scores.

The logistic regression model was statistically significant, $\chi2(2) = 17.027$, $p < .001$. The model explained 21.1% of the variance (Nagelkerke R2) in group membership, and correctly classified 66.7% of cases, demonstrating that higher levels of interpersonal sensitivity were associated with an increased likelihood of NSSI-only adolescents. Higher levels of social problem solving were not significantly associated with either NSSI or SA group membership.

Conclusions: Our results suggest that higher interpersonal sensitivity is uniquely associated with adolescents' likelihood of engaging in NSSI, but not SAs. Such findings are in line with existing research theoretical frameworks that emphasize interpersonal factors such as rejection sensitivity in the development and maintenance of NSSI (e.g., the Four-function model of NSSI). This study can inform future research that uses multimodal and longitudinal assessment (e.g., behavioral tasks, imaging, genetic mediators, etc.) to probe and evaluate different facets of interpersonal functioning to better understand unique interpersonal risk factors for suicide vs. self-harm in adolescents. Ultimately, greater understanding of interpersonal dysfunction can translate to evaluation of if/how current frontline psychosocial treatments for suicidal adolescents directly target these constructs.

Keywords: Suicide Attempt, Non-Suicidal Self-injury (NSSI), Social Factors and Functioning, Suicide Behavior Severity, Self-Harm

Disclosure: Nothing to disclose.

P166. Multimodal Brain Imaging of Methylphenidate Treatment in Patients With ADHD

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Background: Attention deficit hyperactivity disorder (ADHD) impacts a significant number of adult patients, with a small percentage receiving an accurate diagnosis and/or proper treatment. The goal of this double-blinded, placebo-controlled, crossover study was to identify ADHD-related signatures in the brains of adult patients and examine how brain activity, metabolites, and cognitive performance are altered with the commonly prescribed stimulant medication methylphenidate.

Methods: The 7 participants were 20-36 years old, non-smoking, right-handed, not actively taking a stimulant medication, and had no other Axis 1 psychiatric disorders. We combined task-based 3T functional magnetic resonance imaging (fMRI) with high-resolution 7T magnetic resonance spectroscopy (MRS) to examine brain activity and brain chemistry, respectively. Two main fMRI tasks were utilized - the N-back working memory task and the flanker attention task to evaluate response inhibition. A standard cognitive battery including the NIH Toolbox Cognitive Battery was administered along with the Connors Adult ADHD Rating scale (CAARS), a self-report survey of ADHD symptoms.

Results: As expected, methylphenidate level correlated with frontal cortical activity during working memory ($p = 9.9e10\text{-}5$, slope$=0.08073$ $r2 = 0.932$). The ADHD index, a CAARS measure of symptom severity, was shown to be sensitive to a single dose of methylphenidate ($p = 0.021$), and drug level positively correlated with frontal cortical activity during working memory ($p = 0.0227$, slope$=6.165$, $r2 = 0.6067$). Increased glutamate levels in the anterior cingulate cortex and the dorsolateral prefrontal cortex (dlPFC) were associated with positive changes in composite cognitive function score ($p = 0.0047$, slope$=30.89$, $r2 = 0.95$) and fluid cognitive composite score ($p = 0.0066$, slope$=18.91$, $r2 = 0.94$), respectively. We also observed negative correlations between age and both processing speed ($p = 0.0171$, slope $= -2.266$, $r2 = 0.8852$, uncorrected scores) and dlPFC glutamate levels ($p = 0.0339$, slope $= -0.091$, $r2 = 0.82$).

Conclusions: The novelty of this study is in the combination of approaches used to probe ADHD neural pharmacology. By combining functional measurements with high-resolution spectra of key brain metabolites and cognitive data, we created a richer picture of how stimulant medications impact the brain of those with ADHD. The fact that we were able to capture significant and synchronous changes in cortical activity and glutamate levels suggests that multimodal brain imaging may be a viable, noninvasive tool for ADHD drug development efforts.

Keywords: ADHD, fMRI, 7T MRS, Methylphenidate

Disclosure: Nothing to disclose.

P167. Viloxazine Increases Interstitial Levels of Norepinephrine and Serotonin in a Dose Dependent Fashion in Rat Medial Prefrontal Cortex

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Background: Viloxazine ER (viloxazine extended-release (ER) capsules; Qelbree®), a non-stimulant drug, FDA-approved for attention-deficit/hyperactivity disorder (ADHD) in adults and children (≥6 yrs), is pharmacologically distinct from other approved treatments. Most FDA-approved ADHD treatments share a primary mechanism of increasing norepinephrine (NE) and/or dopamine (DA) levels. Viloxazine increases NE but is only a weak norepinephrine (NE) reuptake inhibitor (IC50 = 0.3 μM at human NE transporters) and was found to have moderate activity at serotonin (5-HT) receptors. Prior in vivo micro-dialysis experiments utilizing viloxazine have demonstrated strong increases in NE, DA, and 5-HT in the rat prefrontal cortex (PFC). As the PFC is associated with ADHD pathophysiology and attentional control, monoamine (5HT, DA, and NE) increases in this area may be crucial to the therapeutic effects of viloxazine. However, the viloxazine dose used in these prior microdialysis experiments (50 mg/kg, ip, rat) produced higher unbound viloxazine brain concentrations than the unbound concentration estimated in plasma of pediatric ADHD subjects receiving viloxazine ER 400 mg/day in clinical trials. Consequently, the current microdialysis study was undertaken to further evaluate dose-dependent effects of viloxazine on prefrontal monoamine concentrations at therapeutically relevant concentrations of Qelbree for ADHD.

Methods: Freely-moving Sprague-Dawley rats (male, 8 weeks old, 5-6/group) were implanted with I-shaped microdialysis probes into the PFC. Following recovery from surgery, the rats' probes were connected to a microperfusion pump, which perfused artificial cerebrospinal fluid containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl2, and 1.2 mM MgCl2. After a 2-hour baseline was established, viloxazine (1, 3, 10, or 30 mg/kg) was administered (ip). Dialysate samples were collected from the interstitial fluid (ISF) of the PFC and measured at 30-minute intervals for the 2-hour baseline and 4-hour post-viloxazine administration periods. The dialysate samples were evaluated by LC-MS/MS to determine the concentration of viloxazine and viloxazine-induced changes in NE, DA, 5-HT and their respective metabolites, 3,5-dihydroxyphenylglycine (DHPG) and 5-hydroxyindoleacetic acid (5-HIAA), and precursor, 3,4-dihydroxyphenylalanine (L-DOPA). Plasma samples were collected at 30- and 90-minutes post-dosing for determination of corresponding viloxazine plasma concentrations.

Animal research was performed at Charles River Laboratories (South San Francisco, CA, USA) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 2011). The experiments were conducted under approved protocol US19002 by the site's Animal Care and Use Committee.

Results: Pharmacokinetic evaluation showed a dose-dependent increase in viloxazine plasma and ISF concentrations. Plasma concentrations at 30-minutes post-dose were 2.4 ± 1.0 μM and 5.7 ± 3.6 μM for 10 and 30 mg/kg doses, respectively, yielding calculated free viloxazine plasma concentrations of ~0.9 and ~2.4 μM, respectively (based on prior murine studies showing viloxazine is 40% unbound to plasma proteins). The determination of viloxazine concentration in the dialysate collected from ISF in PFC showed that the viloxazine Tmax occurred at 60 minutes, with Cmax of 1.2 ± 0.3 μM and 3.5 ± 1.6 μM for viloxazine doses of 10 and 30 mg/kg, respectively.

Viloxazine administration resulted in significant dose-dependent effects on interstitial NE levels at all doses tested. The analysis of study results showed that viloxazine treated animals had significantly higher interstitial levels of NE compared to vehicle treated animals at t = 60 minutes, and the 30 mg/kg dose continued to show elevated NE over vehicle up to t = 180 min. The increase in NE interstitial level at 60 minutes reached 236%, 418%, and 558% over baseline for the 3, 10 and 30 mg/kg dose groups, respectively. Dose-dependent decreases in DHPG (NE metabolite) interstitial concentrations corresponded with NE interstitial increases, reflecting viloxazine's mechanistic activity as a NET inhibitor. Viloxazine treatment also resulted in a dose-dependent elevation of interstitial 5-HT concentrations in the PFC. Following the 30 mg/kg dose of viloxazine, 5-HT elevations reached 213% over baseline at a Tmax of 60 minutes and also were higher than the vehicle group; however, the lack of coincident changes in 5-HIAA (5-HT metabolite) interstitial concentrations reaffirmed viloxazine's lack of activity as a 5-HT reuptake inhibitor.

Conclusions: The viloxazine-mediated interstitial increases in NE and 5-HT were directly related to viloxazine interstitial concentrations, demonstrating a clear pharmacokinetic/pharmacodynamic relationship in the PFC, a critical target region for the therapeutic effect in ADHD. These results add to our understanding of viloxazine's mechanism of action and show that, at the unbound plasma concentrations produced by clinically effective doses of viloxazine ER, viloxazine is increasing interstitial monoamine levels in the PFC. Thus, induction of noradrenergic and serotonergic activity in the PFC may be implicated in viloxazine ER therapeutic effects in treating pediatric and adult patients with ADHD.

Keywords: ADHD, Serotonin, Microdialysis, Viloxazine, PFC

Disclosure: Supernus Pharmaceuticals Inc.: Employee (Self)

P168. Adolescents Engage Orbitofrontal Cortex and Dorsomedial Striatal Neurons Differently During Response Inhibition

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Background: Adolescence is a vulnerable developmental period. Neuronal underpinning of this vulnerability is poorly understood but has been attributed to an immature frontal cortex and its connections to striatal and other subcortical areas that modulate action selection. We have observed that action-guided reward processing by lateral orbitofrontal cortex (OFC) and dorsomedial striatum (DMS) neurons differ between adolescents and adults. Here, we test the specific hypothesis that action-outcome associations and response inhibition are processed differently in DMS-OFC circuits in adolescents compared to adults.

Methods: We used a Cued Response Inhibition Task (CRIT) to assess response inhibition in male and female adolescent or adult rats. To characterize the role of OFC to DMS projections in adults and adolescents in CRIT, we used DREADDs to inhibit DMS projecting OFC neurons. In addition, we simultaneously recorded single units and local field potentials (LFPs) in the OFC and DMS from adolescent and adult rats performing CRIT. The neural data was analyzed using multiple computational approaches to investigate the interaction and synchrony between two brain regions during specific behavioral events. We performed mixed design analysis of variance (ANOVA) for all dependent variables with group factors sex and age.

Results: Relative to adults, adolescents make more premature responses and fewer correct responses in CRIT. We find that inhibiting the OFC- > DMS projections decreases correct responses

and increases premature responses during CRIT, suggesting this pathway is necessary for successful execution of response inhibition. Adolescents exhibit a reduced response to inhibitory cue presentation in the OFC and an enhanced response to reward in the DMS. Adolescent spiking variability in the OFC was also increased during inhibitory cue presentation. Lastly, adolescents show reduced OFC and DMS theta power and reduced OFC-DMS theta synchrony during reward.

Conclusions: These results indicate that while the OFC-DMS circuit is critical for response inhibition in both adults and adolescent, adolescents engage this circuit differently by tuning stronger to reward and weaker to inhibitory cues and action. These data expand our knowledge of how the adolescent brain differently guides behavior relative to adults and highlights the importance of OFC-DMS circuits for mediating response inhibition in adolescents.

Keywords: In Vivo Electrophysiology, Corticostriatal Circuit, Neurodevelopmental Disorders, Local Field Potentials, Childhood-Onset Schizophrenia

Disclosure: Nothing to disclose.

P169. Maturation of Prefrontal Circuits Underlying Learned Threat Avoidance

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Background: The medial prefrontal cortex (mPFC) plays a key role in evaluating and responding to threats. In the prelimbic (PL) subregion of mPFC, coordinated activity of output circuits targeting basolateral amygdala (BLA) and nucleus accumbens (NAc) determines whether animals approach or avoid threatening stimuli. In development, as animals establish independence from caregivers, PL circuits undergo an extended maturation. Circuit maturation may promote age-appropriate levels of exploration, allowing mPFC to ultimately encode a nuanced repertoire of threat responses. Yet the causal relationships between mPFC circuit development and maturation of learned threat responding remain poorly understood.

Methods: We developed a modified version of platform-mediated avoidance (PMA) that is compatible with study of developing mice. In this assay, a conditioned tone prompts rodents to navigate to a safety platform. Sweet swelling odor pots that are just out of reach entice animals to explore in between tone presentations. We combined PMA with projection-specific optogenetic manipulations to test the hypothesis that maturation of PL-BLA and PL-NAc circuits determines age-specific avoidance behaviors.

Results: We found that learned threat avoidance behaviors are developmentally regulated. While adult threat memories are strong and long-lasting, juveniles exhibit weak threat memories, and adolescents show a temporary suppression of threat memories (Time on platform during retrieval, age main effect: $P = 0.02$; trial # main effect: $p = 0.23$; interaction: $P = 0.16$; Tukey-corrected post-hoc test: P35 vs. P60: $P = 0.017$). When we combined PMA with projection-specific optogenetic manipulations to test the hypothesis that maturation of PL-BLA and PL-NAc circuits determines age-specific avoidance behaviors, we discovered that PL behavioral contributions to PMA are age-specific. Activating PL-BLA reduced avoidance in juveniles by increasing latency to enter the safety platform. At P35, the same manipulation had no net effect on time on platform, but promoted entries onto the safety platform while reducing the length of platform stays. Finally, activating PL-BLA mildly enhanced PMA in adults (Time on platform during retrieval, age main effect: $P = 0.002$, opsin main effect: $P = 0.82$, interaction: $P = 0.002$; Turkey corrected post-hoc test: P23: $P = 0.01$, P35: $P = 0.99$, P60: $P = 0.04$). In contrast, activating PL-NAc had no effect at P23 or P35, but reduced avoidance in adults by enhancing the latency to enter the safety platform (Time on platform during retrieval, age main effect: $P = 0.07$; trial # main effect: $p = 0.13$; interaction: $P = 0.08$; Tukey-corrected post-hoc test: P60: $P = 0.01$). Silencing PL-BLA at P23 had no effect on PMA ($P = 0.2$, Student's t-test), while silencing PL-NAc at P35 enhanced avoidance ($P = 0.02$, Student's t-test) by decreasing the latency to enter the safety platform and increasing the duration of platform stays.

Conclusions: Together, these findings support in a model in which, instead of simply strengthening across development, pronounced rearrangements of frontolimbic circuits determine age-specific threat behaviors. In juvenile stages, PL circuits may not play a key role in avoidance; rather, BLA and NAc may control learned threat avoidance. In adolescence, changes in PL dynamics may favor activation of PL-NAc, which promotes exploration even during encounters with threats. In ongoing work, we are using fiber photometry to monitor neural dynamics across development. By performing circuit-specific activity manipulation and monitoring in development, our studies will be among the first to establish causal links between mPFC circuit dynamics and the maturation of threat-induced behaviors. By revealing the trajectory along which threat avoidance emerges, our research will establish important foundations for understanding how genetic and environmental insults alter developmental trajectories and shift the balance toward developmental disorders.

Keywords: mPFC, Developmental Trajectory, Active Avoidance, Threat Conditioning, Frontolimbic Network

Disclosure: Nothing to disclose.

P170. Sex Differences in the Transcriptional Networks Underlying Playfulness Suggest a Distinct Function for Play in Males Compared to Females

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Background: Social play is a dynamic, well-conserved behavior known to be sexually differentiated; in most species, males play more than females, a sex difference driven by the medial amygdala (MeA). To investigate whether the transcriptional signatures underlying play also differ by sex, we performed RNA-sequencing of MeA samples from high- and low-playing juvenile rats of both sexes. Using Weighted Gene Co-expression Network Analysis (WGCNA), we identified 22 co-expression modules, or networks of genes highly correlated in expression across samples. Of the 12 modules (for $p < 0.05$) significantly associated with play, almost all (11/12; ~92%) are sex-specific in expression, correlating with expression of play in one sex only. These data suggest there is a distinct transcriptomic profile associated with playfulness in the MeA of males compared to females, a noteworthy finding given the MeA regulates many sex-typical adult social behaviors. We propose that this is no coincidence: play-associated gene networks in the MeA are sex-specific because play modulates circuitry driving different adult behaviors depending on sex. To investigate this, we deprived male and female rats of play during the peak juvenile play window and assessed the effects on various behaviors in adulthood, predicting

that this would result in impairments in later-life behavior that would differ by sex.

Methods: For 2 weeks during the peak of the play period (postnatal day 26-42), juvenile rats experienced either control housing conditions or were deprived of play via two different methods: full social isolation, or by being placed in modified cages containing a perforated Plexiglass cage divider to prevent play while allowing for other social interaction ("play barriers"). All subjects ($n = 11$-13/group) were then returned to normal housing and later assayed in a battery of behavioral tests in adulthood. One- or two-way ANOVAs were conducted when appropriate followed by Tukey's post-hoc tests.

Results: Supporting our hypothesis, we found that preventing juvenile play impaired object memory ($p = 0.004$), decreased sexual behavior ($p = 0.01$), and enhanced social preference ($p < 0.001$) in adulthood in males only. There was no effect of play deprivation on anxiety-like behavior, sex preference, or social recognition in either sex, or on any assessed behavior, including maternal behavior, in adult females.

Conclusions: Consistent with the proposed hypothesis, play deprivation indeed resulted in sex-specific effects on later-life behavior: males prevented from playing as juveniles had impairments in object memory and in two tests of sociosexual behavior in adulthood. Surprisingly, there were no effects on females across any assessed behavior, suggesting female resilience to juvenile isolation. We are currently repeating these experiments with a longer play deprivation window (postnatal days 21-45) to determine if this may reveal additional deficits in females. These findings, together with our previous RNA-sequencing results, provide novel insight into the ultimate function of play and how and why this may differ by sex.

Keywords: Social Play Behavior, RNA Sequencing, Sex Differences, Medial Amygdala, Behavior

Disclosure: Nothing to disclose.

P171. Maternal Transmission of Preconception Stress and Impact on Offspring Hypothalamic Development

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Background: Cumulative stress –including adverse childhood experiences (ACEs), repeated trauma, or racial discrimination stress– experienced prior to pregnancy is a predictor of adverse perinatal and neurodevelopmental outcomes. Although adversity experience is not unique to women, pregnancy is a pervasive female-specific physiological challenge. However, little is known about the biological mechanisms involved in transmitting this lasting signal of stress history. We have developed a novel mouse model of maternal preconception stress (MPS) where female, but not male, offspring are hypersensitive to stress as adults. Our previous work has demonstrated a dramatic shift in circulating extracellular vesicle (EV) protein content towards proteins involved in metabolism in MPS dams, specifically in pregnancy. This shift is associated with impaired glucose tolerance in MPS dams at mid-gestation. We hypothesize that the increased metabolic demands of pregnancy unmasks programming, from preconception adverse experiences, that alter signaling at the maternal:fetal interface, leading to changes in offspring neurodevelopment in a sex-specific manner.

Methods: Adult female mice were exposed to chronic stress from 4-10 weeks of age. Mice were provided two weeks post-stress recovery to minimize confounding effects of acute stress prior to mating. To assess lasting effects of MPS on the maternal and fetal compartments, placentas, apposing uterine tissue, and fetal brains were collected at mid-gestation, E12.5, coinciding with full differentiation of the placenta and development of the hypothalamus. Utilizing Sim1-Cre x Ribotag mice, RNA transcripts specific to hypothalamic neurons, enriched in the paraventricular nucleus (PVN), were isolated and sequenced to assess the effect of MPS on offspring hypothalamic development and adult function (PN2, PN21, and PN70). Body weight and food intake was tracked in a separate cohort of offspring that were challenged with a high-fat low-fiber diet (HFD) from 4-10 weeks of age.

Results: At mid-gestation, reproductive tissues at the maternal:fetal interface in MPS dams showed broad changes in cellular and lipid metabolism in an offspring sex-specific. Female offspring showed a greater magnitude of differential hypothalamic gene expression in response to MPS and altered metabolic gene expression in PVN-enriched hypothalamic neurons across development, linking metabolic changes at the maternal:fetal interface to lasting offspring sex-specific effects on development of the hypothalamus. Unlike control female offspring, when challenged with a high-fat low-fiber diet, female MPS offspring were susceptible to elevated weight gain.

Conclusions: These data highlight the lasting effects of cumulative stress experienced preconception that surface during pregnancy to alter metabolic signaling at the maternal:fetal interface, ultimately altering offspring sex-specific hypothalamic development and susceptibility to a metabolic challenge in adulthood, effects that may also indicate risk for other neurodevelopmental disorders.

Keywords: Stress Models, Hypothalamic Development, RiboTag, RNA Sequencing

Disclosure: Nothing to disclose.

P172. A Prospectively Randomized Pharmacogenetic Trial of Pregabalin for the Treatment of Co-Occurring PTSD and AUD in Black/African American Adults

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Background: Post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD) commonly co-occur. This comorbidity is associated with worse symptom severity and treatment outcomes for each disorder. Black/African American (B/AA) individuals are more likely than other racial groups to be exposed to traumatic events, experience more severe symptoms of PTSD, and face worse consequences from alcohol consumption. Because they have been historically excluded from treatment trials, treatments for PTSD and AUD that are effective for B/AA individuals are sorely needed. The anticonvulsant pregabalin, via its affinity for the alpha-2-delta auxiliary site of voltage gated calcium channels, may help restore balance to the dysregulated glutamate and GABA systems that appear to similarly underlie both PTSD and AUD. Pregabalin has shown preliminary efficacy in treating AUD, generalized anxiety disorder, and PTSD. The goal of this randomized, controlled trial was to test whether pregabalin, vs. placebo, reduces alcohol consumption and improves PTSD symptoms in B/AA individuals with co-occurring PTSD and AUD. Additionally, a personalized medicine approach was employed to examine whether variation of SLC6A1, i.e. the gene encoding the GABA transporter GAT-1, affected response to pregabalin. There is evidence that GAT-1 is downregulated in AUD and upregulated by pregabalin administration. In B/AA, variants at the SLC6A1 gene promoter region insertion (i.e., non-insertion/insertion or insertion/insertion (NI/I or I/I) compared with those of Non-insertion/Non-insertion (NI/NI) type have significantly higher levels of GAT-1 promoter activity.

Methods: This study (NCT02884908) was a double-blind, placebo-controlled, and prospectively genotyped 12-week clinical trial to test the efficacy of pregabalin vs. placebo in reducing both alcohol consumption and PTSD symptoms. Participants were randomized to receive pregabalin 450 mg/day or placebo for 12 weeks in a 1:1 scheme with each medication group also stratified by SLC6A1 genotype (NI/I/II vs. NI/NI). Because of a low allelic frequency, individuals with the double copy insertion were combined into one group with those with the single copy (i.e., NI/I/II). At each of the 12 weekly visits, participants received standardized, guided interviews to enhance medication compliance and completed measures to assess alcohol consumption and PTSD symptoms. Primary outcomes were number of heavy drinking days (HDD) assessed by Timeline Followback Interview (TLFB) and severity of PTSD cluster B and E symptoms assessed by the PTSD Symptom Checklist for DSM-5 (PCL-5). Secondary outcomes were number of days abstinent and drinks per drinking day assessed by TLFB and PTSD total symptom severity as assessed by the PCL-5 and Clinician-Administered PTSD Scale for DSM-5 (CAPS-5). The study was actively recruiting from 07/2017 to 11/2021 with enrollment ceased for roughly one year (03/2020 – 02/2021) due to the COVID-19 pandemic. Power analyses indicated a need to enroll $n = 307$ and randomize $n = 203$ in order to detect a medium sized medication effect. In actuality, this study enrolled $n = 149$ and randomized $n = 54$ B/AA participants. A modified intent to treat analysis was performed which included $n = 44$ B/AA individuals who received at least one dose of medication ($n = 26$ pregabalin; $n = 18$ placebo). Repeated measures mixed effects models, adjusted for a pre-baseline assessment of the respective PTSD or drinking outcome, were used to compare change over the twelve weekly assessments between the two medication conditions. To test whether medication response differed by genotype, models were rerun with SLC6A1 genotype added as a main effect and interaction term.

Results: Participants reported a significant reduction in alcohol use (HDD, days abstinent, and drinks per drinking day) and PTSD symptoms (B and E clusters and total PTSD symptom severity) over the 12-week trial. However, there was no difference between medication condition nor was there a pharmacogenetic effect on any of the primary or secondary outcomes.

Conclusions: Pregabalin did not reduce alcohol use or improve PTSD symptoms in B/AA individuals with comorbid AUD and PTSD, and genetic variation in GAT-1 activity was not associated with treatment outcomes or medication efficacy. However, this study was severely underpowered, and these results, particularly the pharmacogenetic findings, should be viewed with caution given the small sample size. Further studies are needed to identify novel and targeted pharmacological treatments for B/AA with AUD and/or PTSD.

Keywords: Alcohol Use Disorder - Treatment, Post Traumatic Stress Disorder, Randomized-Controlled Trial, Pharmacogenetics, African American

Disclosure: Nothing to disclose.

P173. Open Trial of Transcranial Direct Current Stimulation for Warriors Experiencing Chronic Pain (tDCS for Warriors)

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Background: Chronic pain, or "pain that persists or recurs for more than 3 months"5 costs the United States hundreds of millions of dollars annually.2 Severe pain is about 1.5 times more prevalent in veterans than non-veterans.3 Among veterans, chronic pain is a robust risk factor predicting suicide across all service eras.6 Commonly prescribed medications to treat chronic pain such as opioids and NSAIDs pose increased risks to patients. This study explores the effectiveness of a safe, non-opioid, self-administered treatment - transcranial direct current stimulation (tDCS) - in the treatment of chronic pain in veterans completing an intensive outpatient treatment program for mental health concerns. tDCS is a non-invasive, painless brain stimulation treatment that uses direct electrical currents to stimulate specific parts of the brain and modulate neuronal activity. tDCS produces analgesic effects and improves pain system function through direct effects on the motor, somatosensory, and frontal cortices implicated in pain sensitivity.1,4 tDCS has yet to be examined for chronic pain in veterans. We investigate acceptability and effectiveness of tDCS for chronic pain in veterans completing the Emory Healthcare Veterans Program Intensive Outpatient Program (EHVP-IOP)7.

Methods: To date, 8 veterans with chronic pain in the EHVP-IOP underwent staff-monitored tDCS sessions. Starting on Day 1, tDCS was administered with a constant current intensity of 2 mA for 20 minutes per daily session for up to 10 sessions over the 2 weeks using a Soterix 1x1 tDCS mini-CT Stimulator (Soterix Medical Inc., NY). Staff reviewed a Medical Pain Assessment. To measure the degree of chronic pain, participants completed the Defense and Veterans Pain Rating Scale (DVPRS) daily both before and after each session and overall. On days 1 and 12 they completed Patient-Reported Outcomes Measurement Information System (PROMIS) 3a and 8a questionaries. To assess for co-morbid conditions such as PTSD and depression, on days 1,3,5, 8, 10, and 12 participants completed the Posttraumatic Stress Disorder Checklist (PCL-5) and the Patient Health Questionnaire (PHQ-9). Post-treatment follow-up occurred at 1, 3, 6, 9, and 12 months.

Results: We have currently enrolled 8 veterans in the EHVP-IOP plus tDCS program and will present all available veterans who enroll through mid-November (18 veterans estimated). Preliminary analyses show promise and address feasibility and acceptability. In brief, veterans find using the tDCS device with remote monitoring acceptable and can successfully self-administer under the tele video supervision of study staff with few reported issues. Worsening of headache occurred in one veteran who chose to discontinue. Significant and large reductions in PTSD are reported as expected with the EHVP-IOP program ($t(5) = -2.79$, $p = .03$, $d = -1.3$). Reductions in pain as measured with DVPRS show a small to medium effect size ($t(5) = 1.00$, $p = .18$, $d = .41$) that is not statistically significant possibly due to small sample size. Updated results will be presented in the poster including patient level pain data to illustrate veteran response over the two weeks of EHVP-IOP.

Conclusions: Based on this open trial, tDCS is a feasible and acceptable pain intervention for concurrent use during mental health intensive outpatient treatment. Impact on pain is promising with small to moderate reductions in pain across the daily tDCS program. Additional controlled trials with large sample are needed to establish efficacy. Reported pain in the sample is heterogeneous in focus (fibromyalgia, knee, migraine, etc.). Additional analysis within subgroups is needed.

Keywords: Pain therapeutics, veterans, Treatment, PTSD, Depression

Disclosure: Nothing to disclose.

P174. Chronic Stress Prior to Cocaine Exposure Produces Sex Differences in Cocaine Consumption and Memory Retrieval in Rats

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Background: A correlation has been recognized between post-traumatic stress disorder (PTSD) and substance use disorder (SUD), and stress has been associated with a higher likelihood of relapse to addiction. Moreover, stress is associated with a higher likelihood of relapse. Studies using different models of PTSD and a short-access cocaine self-administration paradigm in male rodents have produced varying results, depending on the specific experimental conditions. For example, a preclinical study revealed that rats exposed to modified single prolonged stress before cocaine short access self-administration (2 hours) showed a reduced cocaine self-administration, while rats exposed to single prolonged stress prior to cocaine showed reduced cue-induced primed reinstatement, without any effects on the acquisition or extinction of cocaine self-administration. On the other hand, early-life stress was shown to facilitate the acquisition of cocaine, but it did not alter the reinstatement of cocaine-seeking behavior. However, the effects of chronic stress and extended-access cocaine self-administration on cocaine-seeking in either male or female rats have not been reported. We hypothesize that chronic stress before cocaine self-administration will increase cocaine consumption in rats of both sexes. Additionally, we predict that forced abstinence will result in increased active lever presses in cue-reactivity after 30 days of abstinence, compared to 15 days, in both sexes, with male rats exposed to stress and cocaine showing higher incubation of cocaine craving, compared to female rats.

Methods: We employed an unescapable footshocks paradigm for 5 days at an intensity of 0.50mA (presented randomly), followed by 6-hour sessions of extended-access cocaine self-administration for 10 days, and subsequently, by either a 15 or a 30-day period of forced abstinence in male and female rats. Subsequently, we examined cue-primed and cocaine-primed memory retrieval.

Results: Our data show that chronic stress before cocaine acquisition decreases cocaine consumption in female rats, compared to males. In addition, cue-reactivity after 15-day withdrawal seems to decrease in both, male and female rats, without affecting cocaine-induced memory retrieval. Interestingly, after 30 days of withdrawal female rats show an increase in cue reactivity in the stressed group compared to non-stressed rats, while males show similar cue reactivity in both groups. Furthermore, stressed male rats show an increase in cocaine-primed memory retrieval compared with the non-stressed group after 30 days of withdrawal, while female rats did not show any difference.

Conclusions: Our findings suggest that the effects of chronic stress before cocaine exposure on incubation of cocaine-seeking behavior depend on the withdrawal period's length, indicating that there are different neurophysiological phases during forced abstinence. Moreover, chronic stress before extended-access cocaine self-administration produces sex differences in cocaine acquisition, consumption, cue-reactivity, and cocaine-primed memory retrieval. Our findings represent evidence that stress before cocaine exposure induces sex-dependent differences in cocaine-seeking behavior.

Keywords: Cocaine Sex Differences, Chronic Stress, Incubation of Cocaine Craving

Disclosure: Nothing to disclose.

P175. Inhibition of Hippocampal and Thalamic Inputs to the Nucleus Accumbens Restores Dopamine System Function in a Rodent Model Used to Study PTSD and Comorbid Psychosis

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Background: Post-traumatic stress disorder (PTSD) is a psychiatric illness that afflicts approximately 8% of the United States population. In addition to core symptoms of the disorder, patients with PTSD commonly present with a comorbid diagnosis, including psychosis. Symptoms of psychosis are thought to be driven by increased mesolimbic dopamine transmission, however, no clear histopathology has been identified within these cells. Rather, hyperactivity in upstream brain regions that regulate ventral tegmental area (VTA) dopamine neuron activity contributes to psychosis-like behavior. Two such regions are the paraventricular nucleus of the thalamus (PVT) and ventral hippocampus (vHipp), which synergistically regulate VTA dopamine neuron activity through a multisynaptic circuit that begins with convergent inputs to the nucleus accumbens (NAc). We have previously demonstrated that activation of PVT-NAc or vHipp-NAc projections significantly increases VTA dopamine neuron population activity, defined as the number of neurons firing spontaneously. These data suggest that hyperactivity in either the PVT or vHipp, following stress, may contribute to psychosis-like behavior in a rodent model used to study PTSD. Interestingly, hippocampal and thalamic regulation of VTA population activity requires simultaneous activity from both regions, suggesting that targeting either region may be a novel site of intervention for comorbid psychosis related to PTSD.

Methods: In this study, we induced stress-related pathophysiology in male Sprague Dawley rats using the two-day inescapable foot shock procedure. To selectively inhibit PVT-NAc or vHipp-NAc projections we used a chemogenetic approach. A Cre-dependent inhibitory DREADD was expressed in either the PVT or vHipp. Concurrently, a retrograde Cre virus was injected in the NAc. This allowed for selective inactivation of these projections following administration of Compound 21 (3 mg/kg; i.p.). To examine alterations in dopamine system function, we used in vivo electrophysiology.

Results: Two-day inescapable foot shock resulted in increased dopamine neuron population activity, consistent with what is observed in other rodent models used to study psychosis. Chemogenetic inhibition of either PVT-NAc projections or vHipp-NAc projections, following stress, restored dopamine system function.

Conclusions: These results demonstrate that aberrant dopamine neuron activity that is observed after stress can be reversed by inhibiting the activity of PVT-NAc or vHipp-NAc projections. Here, we provide evidence that the PVT or vHipp may be novel therapeutic targets for decreasing psychosis symptoms observed in PTSD.

Keywords: Psychosis, Chemogenetics, Electrophysiology

Disclosure: Nothing to disclose.

P176. Effects of Cannabis Legalization in Canada on Cannabis Use Patterns in Mental Health Disorders

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Background: Since the legalization of recreational cannabis use in Canada in 2018, one emerging public health concern is whether cannabis use will increase in people with mental health disorders. Relative to the general population, problematic cannabis use is elevated in individuals with mental health disorders, including those with psychotic-spectrum, mood, and anxiety disorders. Moreover, problematic cannabis use is associated with more severe psychopathology and poorer functional outcomes. Thus, there is a need to evaluate whether legalization has led to

changes in cannabis use patterns among psychiatric populations. We explored whether there are significant changes in cannabis use patterns pre- and post-legalization in a nationally representative sample of Canadian adults experiencing a mental health condition within the previous 12-months of survey administration.

Methods: Data came from Canadian respondents (N = 13,527) between Waves 1 (pre-legalization; Aug – Oct 2018) 2 (post-legalization; Sept – Oct 2019), and 3 (post-legalization; Sept – Oct 2020) from the International Cannabis Policy Study (ICPS). Binary logistic regressions and repeated measures ANOVAs examined changes in daily and 30-day cannabis use respectively, pre- and post-legalization among those who self-reported 12-month experiences of anxiety, depression, PTSD, schizophrenia/psychosis, or bipolar disorder.

Results: Respondents reporting schizophrenia symptoms were significantly more likely to use cannabis daily post-legalization (OR = 5.9, CI = 1.9-15.9; p < .01;). Daily cannabis use did not increase in other diagnostic groups or in nonpsychiatric populations post-legalization. Similarly, past 30-day cannabis use increased post-legalization only among respondents reporting schizophrenia symptoms (p < .05).

Conclusions: Our preliminary findings suggest that patients with schizophrenia have had selective increases in daily and 30-day cannabis use in the first two years of cannabis legalization in Canada.

Keywords: Cannabis Use, Schizophrenia (SCZ), Depression and Anxiety, PTSD, Cannabis Use Disorder

Disclosure: Nothing to disclose.

P177. Risk for Antipsychotic Induced Weight Gain in a Large Population Health Sample is Attributable to Weight Dependent and Weight Independent Genetic Factors

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Background: Obesity and its metabolic consequences contribute substantially to accelerated aging and reduce lifespan in psychiatrically ill individuals. The risk for antipsychotic induced weight gain (AIWG) is thought to be highly genetic, but our understanding of these contributors remains limited. Characterizing the genetic variation that influences AIWG is critical for the development of precision treatments and decision support tools in Psychiatry.

The present analysis represents the initial step in the NIH-funded multi-omic ORAcLE (Obesity Related to Antipsychotic Liability and Exposure) study, a multi-study consortium which aims to combine genetic and clinical data from large health systems, existing cohort studies, and randomized clinical trials both to validate previously reported genetic contributions to risk for AIWG and to identify new common variants that impact risk for AIWG.

The Geisinger MyCode Community Health Initiative, a population health genomics project with genetic and clinical data on ~185,000 individuals, was used to test the initial analytic approach for the overall ORAcLE consortium. Using the current interim genome wide association study (GWAS) of BMI from the Genetic Investigation of ANthropometric Traits (GIANT) consortium, which includes >1.1 million European participants, we derived polygenic risk scores (PRS) for cross-sectional body mass index (BMI), then tested those PRS for effect on AIWG, as measured by longitudinal change in BMI during treatment with an atypical antipsychotic.

Methods: Individuals in the MyCode data set with > 2 BMI measures > 30 days apart while prescribed an atypical antipsychotic medication were eligible for inclusion in the analyses. Non-physiologically plausible baseline and change values for weight, height or BMI were removed. Changes in height, weight or BMI between visits due to unit conversion error or injury, such as amputation were also removed. Weights collected during pregnancy and 6 months post-delivery, as well as post-bariatric surgery weights, were excluded from the analyses.

We employed a linear mixed model, where BMI was regressed with intercept and time (years) as both fixed and random effects, to derive the slope of AIWG, stratified by sex and ancestry. We conducted analyses of overall change in BMI in the population of all eligible individuals, as well as for "gainers" only (i.e., only individuals with a positive AIWG slope). PRS weights were estimated in SBayesR.

Association analyses between standardized PRS and AIWG were done using generalized linear models while accounting for families using general estimating equation (GEE). All models were adjusted for baseline age, age2, sex, baseline BMI, follow-up duration, and five genetically-informed principal components of ancestry [European (EUR), African Ancestry (AA), American Indian/Native American (AMR), East Asian (EAS), South Asian (SAS)]. Incremental R2 values were estimated in an unrelated subset of participants using linear regression. The association between PRS and baseline BMI was also assessed.

Results: There were 8,493 participants (97% European ancestry, 68% female, 61% who gained weight while prescribed antipsychotic medication) with usable data who met criteria for inclusion in the analysis. There was an average of 33 observations (SD = 58) per individual participant, with a mean of 3.7 (SD = 3.7) years of follow-up, and mean AIWG of 0.18 kg/m2 per year (0.93).

The final PRS included 1,080,060 variants and was significantly associated with baseline BMI (P = 1×10-305, β = 4.03, R2 = 14.4%). We observed significant relationships between the cross-sectional BMI PRS and AIWG among all individuals (R2 = 0.3%, P = 4×10-8, β = 0.069 BMI units/year) and among "gainers" (R2 = 0.3%, P = 6.5×10-6, β = 0.051 kg/m2/year), respectively.

Finally, a small but significant difference (0.125 kg/m2/yr, P = 2×10-4) in adjusted mean AIWG between the lowest and highest quintiles of the PRS was also observed.

Conclusions: These results demonstrate that genetic variants associated with cross-sectional measures of obesity, represented by a PRS derived from the GIANT Consortium's ongoing BMI GWAS are associated with AIWG. This result extends observations from smaller studies that there is a clear heritable component to AIWG that is detectable using a PRS originally designed to detect genetic risk for obesity.

The proportion of variance explained in AIWG by the cross-sectional BMI PRS was significant but small, suggesting that generalized risk for obesity, and its related pathways, might not be the primary mechanism for AIWG. This further motivates the primary goal of the consortium to bring together relevant genomic and clinical data to discover new genetic variants associated with AIWG. Our ongoing analyses in larger population samples and representing greater diversity will identify the modifiers specific to longitudinal AIWG and the affected molecular pathways.

Keywords: Antipsychotic Induced Weight Gain, Population Genetics, Molecular Genetics, Human Genetics

Disclosures: Alkermes, Novartis: Consultant (Self), LB Pharmaceuticals: Grant (Self)

P178. Dissecting Hypothalamic Dysfunctions in the Olanzapine Induced Metabolic Syndrome

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Background: Olanzapine is a second- generation antipsychotic used for the treatment of schizophrenia and mood disorders. The mechanism of action of this drug includes the modulation of several G-protein coupled receptors (GPCR). Olanzapine chronic administration is burdened by an increased risk of metabolic syndrome, a clinical condition characterized by the co-occurrence of obesity, dyslipidemia, diabetes, and hypertension. Mechanisms by which second generation antipsychotics trigger metabolic syndrome remain unclear. However either central or peripheral signaling functions have been put forward. The hypothalamus is a major regulator of energy metabolism, therefore an involvement of this brain structure in metabolic syndrome induced by antipsychotic medications could be suspected. Here we examine the potential contribution of hypothalamic GPCRs in the onset of the metabolic dysfunction induced by antipsychotics.

Methods: Animals: All animal procedures were performed in accordance with Canada Council of animal care guideline and were approved by the University of Toronto animal ethics Committee. The experiments were carried out on C57BL/6J wild-type female mice of approximately 7 weeks old. For the Olanzapine treatment pure Olanzapine was compounded into high fat diet. The Vehicle group received the high fat diet only. The mice were treated for 28 days.

Stereotaxic Injection: The mice were divided into three batches: H1R- Knockout, CB1R-Knockout, and Control group ($n = 24$/each). The knockout (KO) was induced with a double viral CRISPR approach using AAV-MeCP2Cas9. The viruses were administered through hypothalamic stereotaxic injection.

Phenotype characterization: For the phenotype characterization the following parameters were measured: food intake, weight gain, blood glucose, serum insulin, HDL, LDL, and Triglycerides. The Fatty liver disease was evaluated using the Oil-Red- O staining and Masson's Trichrome staining.

Statistical analysis

The data were presented as means ± SEM. For comparison between multiple groups one-way ANOVA was used followed by Bonferroni correction. The differential gene expression (DEG) analysis, the pathways analysis, and the gene co-expression network analysis were performed using mattest matlab package, Ingenuity pathway analysis (Qiagen IPA) and WGCNA R-studio package respectively.

Results: Analysis of hypothalamic gene expression in adult female mice allowed the identification of a transcriptomic signature of olanzapine induced metabolic syndrome. Then, using an unbiased approach we have identified two GPCRs: Histamine Receptor 1 (H1R) and Cannabinoid Receptor 1 (CB1R) that seem to play a crucial role. We thus implemented CRISPR/Cas9 mediated approaches to knockout each receptor in neurons of the hypothalamus.

The hypothalamic- neuronal KO of H1R explains weight gain but not glucose impairment neither the changes in lipid metabolism.

The mice with H1R-KO in hypothalamic neurons show a statistically significant increase in weight compared to controls ($p < 0.001$). The present weight gain appears fully comparable to the effect of the treatment with Olanzapine in control mice. Moreover, the treatment with Olanzapine in H1R-KO mice does not result in further increase in weight. Analysis of others metabolic parameters (glucose, insulin, triglycerides, LDL, HDL) indicate that H1R-KO in hypothalamic neurons seems not to lead by itself to glucose impairment or to a lipid dysfunction, with no statistical differences between H1R-KO mice and Control mice. The pathway analysis of the DEGs between H1R-KO and Control mice reveals that in H1R-KO mice the Neuropeptide Y (NPY) pathway is up-regulated. On the other hand, the comparison of the gene expression of H1R-KO mice treated with Olanzapine with H1R-KO mice treated with Vehicle highlights that Olanzapine not only increase the up-regulation of the NPY pathway but also induces a complete deregulation in the metabolic homeostasis inducing an up-regulation also of the Pro-opiomelanocortin (POMC) pathway.

The KO of CB1R in hypothalamic neurons is able to restore the full phenotype of Olanzapine-induced Metabolic Syndrome.

The CB1R KO mice do not show any difference in terms of weight gain compared with the controls. However the administration of Olanzapine to both groups reveals that CB1R-KO mice show less increase in weight compared with controls ($p < 0,01$). Moreover the Olanzapine treated CB1R-KO mice have a reduced glycemic dysfunction ($p < 0,001$), decreased levels of triglycerides ($p < 0,001$) and LDL ($p < 0,001$) and increased level of HDL ($p < 0,0001$) compared to the treated controls. The pathway analysis of the DEGs between CB1R-KO and Control mice both treated with Olanzapine reveals that in this case the NPY pathway is down- regulated in CB1R-KO mice. Moreover comparing the hypothalamic co-expression network of CB1-KO mice with the Control both treated with Olanzapine appears that the Knockout of CB1 receptor induces a dissociation of the expression of H1R and the main regulators of metabolic homeostasis (NPY, AGRP, POMC, NPY2R).

Conclusions: These results suggest that metabolic syndrome is a complex clinical phenotype where central and peripheral deregulations may act together resulting in a lost of the metabolic homeostasis. The possibility of reverting Metabolic Syndrome targeting the hypothalamic CB1R on the other hand opens promising perspective for therapeutic development

Keywords: Metabolic Side Effects, antipsychotic-Induced Metabolic Dysfunction, Metabolic Syndrome, Antipsychotic Induced Weight Gain, Cannabinoid Receptor

Disclosure: Nothing to disclose.

P179. Microglia are Implicated in Risk-Taking Behavior in a Mouse Cancer Model

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Background: Breast cancer is the most common cancer among females worldwide. Following diagnosis but before cancer treatment, up to 30% of these patients report mood disturbances (e.g., anxiety, depression), which reduce quality of life and inhibit curative treatment compliance. Remarkably, our mouse mammary tumor model recapitulates the negative affective-like behaviors observed in cancer populations, which coincide with increased proinflammatory mediators in the midbrain and hippocampus. Indeed, neuroinflammation is a proposed mechanism underlying the etiology of mood disorders and therefore may be contributing to cancer-associated mood disturbances by modulating neurotransmission (e.g., serotonin metabolism). However, the cellular mechanisms by which tumor-induced neurobehavioral changes occur remain unknown. We hypothesize that microglia are the primary cells driving tumor-induced neuroinflammation, which contributes to affective-like behavior and altered serotonin metabolism.

Methods: Young adult female Balb/c mice were induced with an orthotopic, syngeneic, non-metastatic 67NR mammary tumor; tumor-free controls underwent a sham surgery. Experiment 1 assessed brain region-specific microglia morphology (Iba1 immunohistochemistry) and serotonin-related gene expression (Tph2, Th, Slc6a4), as well as gene expression of Percoll-enriched brain microglial cells (Il1b, Il6, Tnfa, Tlr4). Experiment 2 tested the necessity of microglia in tumor-induced behavioral and neuroinflammatory/serotonin outcomes via microglia depletion using a colony stimulating factor 1 receptor inhibitor (PLX-5622) in chow.

Results: In Experiment 1, mammary tumors increased gene expression of midbrain serotonin transporter (Slc6a4) and a rate-

limiting serotonin enzyme (Tph2) relative to tumor-free controls (t-test: $p < 0.05$). Specifically, within brain microglial cells, tumors increased the toll-like receptor 4 gene relative to controls (t-test: $p < 0.05$); microglial immunohistochemistry analyses are underway. In the open field test of Experiment 2, tumor-bearing mice unexpectedly increased locomotion in the risky center of the field compared to tumor-free mice; these tumor-induced risky behaviors were attenuated by PLX-5622 chow (2-way ANOVA interaction: $p < 0.05$). Similarly, tumor-bearing mice were less hesitant to approach a novel object, a risk-taking behavior that was reversed when microglia were depleted (2-way ANOVA interaction: $p < 0.05$).

Conclusions: These results suggest that microglia may have region- and mediator-specific involvement in tumor-induced neuroinflammation and neurotransmission. This research will advance our understanding of the mechanisms underlying behavioral disorders associated with cancer in order to identify specific cellular and/or molecular targets to prevent or mitigate them.

Keywords: Microglia, Risky Behaviors, Neuroinflammation, Serotonin, Cancer

Disclosure: Nothing to disclose.

P180. TAAR1 Agonist Ulotaront Improves Glycemic Control, Reduces Body Weight and Modulates Neurocircuits Governing Energy Balance and Feeding in Rodents

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Background: Ulotaront (SEP-363856) is a trace amine-associated receptor 1 (TAAR1) and 5-HT1A agonist currently in Phase 3 clinical trials for the treatment of schizophrenia. Metabolic Syndrome (e.g. central obesity, dyslipidemia, hypertension, hyperglycemia, etc.), which can be induced or exacerbated by the current class of antipsychotic drugs (APDs), is highly prevalent in schizophrenia patients. The need for novel treatments that lack APD class-specific metabolic side effects is therefore apparent. Ulotaront has no significant activity at receptors commonly associated with APD-induced metabolic changes (i.e. D2, 5-HT2C, H1 and M3). Recent preclinical evidence has identified TAAR1 as a novel regulator of metabolic control and a promising target for obesity and type 2 diabetes. Here we evaluated the risk-benefit profile of ulotaront for the treatment of schizophrenia by assessing its effects on metabolic parameters in rodent models of diabetes, obesity, and iatrogenic weight gain.

Methods: Effects of 15-day and 35-day oral ulotaront treatment (0.3-10 mg/kg) on body weight, food intake and metabolic parameters were investigated in Sprague Dawley rats on high-fat diet (HFD) and in a mouse model of diet-induced obesity (DIO), respectively. In addition, body weight effects were determined in a rat (8-day treatment) and mouse (21-day treatment) model of olanzapine-, and corticosterone-induced body weight gain, respectively (1-10 mg/kg, po). Glucose tolerance was assessed in C57BL/6J and diabetic db/db mice following acute oral administration (0.3-10 mg/kg) as well as in DIO mice following 15 days of dosing (0.3-10 mg/kg, po). The acetaminophen absorption test and phenol red test were used to evaluate effects on gastric emptying in C57BL/6J mice (0.3 – 10 mg/kg, po). To obtain insights into the neurocircuits modulated by ulotaront, whole-brain 3D c-fos imaging was performed in C57BL/6J mice. Comparisons between groups were analyzed using One-Way ANOVA followed by appropriate post-hoc tests. Treatment effects over time were assessed with Repeated Measures ANOVA. Statistical comparisons of c-fos data were based on either ROIs of the Allen Brain atlas or evenly spaced voxels, using negative binomial regression.

Results: Acute ulotaront administration dose-dependently reduced plasma glucose excursion in C57BL/6J ($F(4, 47) = 15.07$, $p < 0.0001$, $n = 10$/group) and diabetic db/db ($F(4, 45) = 51.41$, $p < 0.0001$, $n = 10$/group) mice during an oral glucose tolerance test (oGTT). Delayed gastric emptying was seen in response to ulotaront treatment in mice, which is likely the main mechanism driving reductions in glucose excursion during the oGTT ($F(5, 56) = 29.90$, $p < 0.0001$, $n = 10$/group). Administration of ulotaront to rats on HFD resulted in significant and dose-dependent reductions in body weight ($F(4, 55) = 40.12$, $p < 0.0001$, $n = 12$/group), food intake ($F(4, 55) = 22.43$, $p < 0.0001$, $n = 12$/group) and liver triglyceride content ($F(4, 55) = 3.3$, $p < 0.02$, $n = 12$/group) compared to vehicle controls. A more rapid reversal of olanzapine-induced weight gain ($F(5, 67) = 17.04$, $p < 0.0001$, $n = 12$-13/group) and food intake ($F(5, 67) = 30.69$, $p < 0.0001$, $n = 12$-13/group) was observed in rats switched to ulotaront treatment compared to vehicle alone. Consistent with the body weight-lowering effects in rats, chronic treatment with ulotaront normalized corticosterone-induced body weight gain in mice ($F(4, 45) = 46.1$, $p < 0.0001$, $n = 8$-12/group) and reduced body weights in the DIO model in a dose-dependent manner ($F(4, 55) = 5.5$, $p = 0.0009$, $n = 12$/group). In addition, chronic ulotaront treatment improved glycemic control in DIO mice as indicated by reduced fasting plasma glucose ($F(4, 55) = 6.3$, $p = 0.003$, $n = 12$/group) and insulin levels ($F(4, 55) = 2.9$, $p = 0.03$, $n = 12$/group) and increased glucose tolerance ($F(4, 55) = 4.51$, $p = 0.003$, $n = 12$/group). Whole-brain imaging revealed neuronal activation (increased c-fos expression) of appetite-regulating pathways related to meal termination (initiated by signaling in the hindbrain nucleus of the solitary tract and relayed through the lateral parabrachial nucleus). Neuronal activation was also seen in hypothalamic subregions associated with the regulation of food intake and integration of peripheral metabolic signals including the arcuate and paraventricular nucleus. The c-fos signature of ulotaront was distinct from that of the typical APD haloperidol.

Conclusions: The current data indicate that ulotaront not only lacks APD-induced metabolic liabilities but can reduce body weight and improve glycemic control in rodent models. The underlying mechanisms likely include TAAR1-mediated peripheral effects on glucose homeostasis and gastric emptying, and/or direct modulation of homeostatic and hedonic neurocircuits regulating energy balance. The positive effects of ulotaront on metabolic parameters may suggest an improved risk-benefit profile compared to the established pharmacological class of APDs. In addition, the current preclinical results further support evaluation of TAAR1 agonists for the treatment of metabolic disorders.

Keywords: TAAR1, Schizophrenia- Novel Treatment, Antipsychotic-Induced Metabolic Dysfunction, Metabolic Side-Effects

Disclosure: Sunovion Pharmaceuticals: Employee (Self)

P181. Serum Biomarkers of Liver Fibrosis Identify Changes to Striatal Metabolites Consistent With Lipid Accumulation

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Background: The presence of hepatitis C virus (HCV) affects the levels of magnetic resonance spectroscopy (MRS)-detectable metabolites in the brain. In particular, the levels of striatal Cho (i.e., choline-containing compounds) were found to be higher in HCV seropositive relative to seronegative individuals.

Methods: To determine whether liver fibrosis independent of HCV status would affect brain metabolite levels, MRS data collected in 3 regions of interest [ROIs, striatum (10.6 cc), cerebellum (9.8 cc), and pons (5.9 cc)] were evaluated for their relations with 2 serum-derived, liver fibrosis markers. Cutoff scores for liver fibrosis were aspartate aminotransferase to platelet ratio index (APRI) > 0.7 and a fibrosis score (FIB4) > 1.5 calculated in a cohort of 170 participants: 62 healthy controls (47.8 ± 19.9yrs), 37 individuals with alcohol use disorder (AUD) (48.5 ± 10.6yrs), 33 individuals seropositive for HIV (50.0 ± 9.1yrs), and 38 individuals with comorbidity for AUD + HIV (51.9 ± 8.6yrs).

Results: Among all participants, 13 had APRI > 0.7 (1 control, 2 AUD, 4 HIV, 6 AUD + HIV) and 34 had FIB4 > 1.5 (4 controls, 5 AUD, 13 HIV, and 12 AUD + HIV). Sex did not differentiate the number of individuals with fibrosis (APRI $p = .26$; FIBR4 $p = .30$). Using nonparametric Wilcoxon-rank tests, an APRI > 0.7 was associated with higher striatal Cr (total creatine, $Z = 3.3$, $p = .001$), higher striatal Cho ($Z = 4.2$, $p < .0001$), and higher striatal mI (myoinositol, $Z = 2.7$, $p = .008$); metabolites in the other 2 ROIs did not differ by the APRI cutoff. FIB4 > 1.5 was associated higher striatal Cho ($Z = 3.1$, $p = .002$) and higher striatal ($Z = 2.6$, $p = .009$) and pontine ($Z = 2.8$, $p = .005$) mI. For striatal Cho, a multiple regression including HCV, APRI, and FIB4 explained 16.6% of the variance and was driven by the APRI score ($p = .0009$, alone contributing 14.7% to the variance). A similar model (i.e., including HCV, APRI, FIB4) resulted in the same pattern for striatal Cr (5.9% of the variance explained, driven by APRI $p = .02$); the model was not significant for striatal mI; pontine mI was more affected by HCV (11.1% of the variance explained, HCV $p = .01$). An APRI > 0.7 was associated with lower verbal fluency scores (F-A-S, $Z = -2.7$, $p = .006$; Freda's, $Z = -2.6$, $p = .01$). A FIB4 > 1.5 was also associated with lower verbal (Freda's, $Z = -3.1$, $p = .002$) and figural (RUFF, $Z = -2.6$, $p = .009$) fluency scores.

Conclusions: Together, these data suggest that the presence of liver fibrosis can selectively affect striatal metabolite concentrations. High levels of striatal Cho and mI suggest the accumulation of lipids, which may contribute to T1 signal hyperintensities observed in basal ganglia structures in liver cirrhosis. Correlations between higher fibrosis scores and compromised fluency suggest functional ramifications of these neurometabolite abnormalities measured in vivo.

Keywords: Liver Brain Axis, Alcoholic Liver Disease, HIV, Hepatitis C, Basal Ganglia

Disclosure: Nothing to disclose.

P182. Shape and Volume Alterations in Habit-Centered Basal Ganglia Structures Present in Atypical Anorexia Nervosa

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Background: Anorexia nervosa (AN) is a serious eating disorder characterized by maladaptive restrictive eating. Restrictive eating patterns often develop as goal-directed dieting behaviors and transition to habitual restrictive eating, unmotivated by initial rewarding goals. The transition to habit-governed behaviors may perseverate as a result of structural alterations in the basal ganglia that occur as a consequence of extreme weight loss. It is unknown whether morphometry of the basal ganglia is altered in individuals with atypical anorexia nervosa (atypical AN) who do not have low-weight (BMI < 18.5 kg/m2) but are otherwise clinically similar to AN. Based on prior literature, we hypothesized that participants with AN would show lower volume and greater shape deformations in regions associated with habitual responding, including the bilateral putamen and caudate, compared to participants with atypical AN and healthy controls.

Methods: The sample included 77 females with AN ($n = 30$, $\bar{x}$ age = 19.7, $\bar{x}$ duration of illness 5.0 years), atypical AN ($n = 21$, $\bar{x}$ age = 18.3, $\bar{x}$ duration of illness 3.6 years), and healthy controls ($n = 26$, $\bar{x}$ age = 18.0). T1-weight images were processed using a standard internal pipeline, which included: visual quality control, manual masking, and outlier detection. The recon-all function of Freesurfer v7.1 was used for segmentation of the basal ganglia to extract regional volumes. Between-group clinical characteristics were assessed using chi-square tests, analysis of covariance (ANCOVA), and Tukey post-hoc tests. Group volumetric comparisons were corrected for age and head size, Bonferroni corrected, and $p < .016$ was considered statistically significant. Shape of the basal ganglia was processed using the ENIGMA Shape Pipeline to extract the logarithm of the Jacobian determinant. For group comparisons of basal ganglia shape, we conducted vertex-wise regression analyses for each vertex on each region of interest, with age and estimated total intracranial volume (eTIV) as covariates, in a pairwise manner. Benjamini-Hochberg correction for multiple comparisons was utilized with a false discovery rate of 5% ($q = .05$).

Results: Contrary to our hypothesis, we found a significant main effect of group on the volume and shape of the bilateral putamen (Left: $F2,72 = 6.88$, $p = .0018$; Right: $F2,72 = 7.23$, $p = .0013$). Individuals with atypical AN had lower volume and greater shape deformations in these regions compared to both individuals with AN (R: $p = .0013$; L: $p = .0014$) and healthy controls (R: $p = .0016$; L: $p = .002$). We also found significant differences in the shape of the right caudate between atypical AN compared to AN, but not between atypical AN and HC.

Conclusions: Our findings suggest structural brain differences between individuals with both AN and atypical AN who share similar clinical presentations. However, the group differences in basal ganglia structures are even more pronounced in those with atypical AN relative to AN. Gray matter differences in AN are often attributed to the severe low-weight status present among individuals with AN. Our study suggests that basal ganglia structural differences persist regardless of the low-weight clinical delineation. Our findings also complement clinical data demonstrating that habitual restriction occurs in atypical AN. These results underscore the importance of weight restoration based on individual history, in order to target the habitual features seen in both AN and atypical AN.

Keywords: Eating Disorders, Basal Ganglia, Habit

Disclosure: Nothing to disclose.

P183. Using Causal Discovery to Identify Heterogeneity in the Causal Relationship Between Negative Affect and Binge-Eating and Compensatory Behavior in Bulimia Nervosa

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Background: Negative affect has been implicated as a precipitant and maintenance factor of binge eating and purging behavior in bulimia nervosa (BN). This relationship has been supported by laboratory data and group analyses of ecological momentary

assessment (EMA) data. However, treatment outcomes in BN intervention studies targeting negative emotions have been mixed, suggesting that there may be heterogeneity in these causal relationships at the individual level. Identifying individual patterns of negative emotion and BN behaviors can be used to more accurately target causal relationships that maintain eating disorder behaviors. We used causal discovery analysis of EMA data to identify the causal network structure that best fits the individual data for adults with BN.

Methods: To examine individual causal relationships, we analyzed data from 128 adult women with a minimum of 60 EMA data points collected over 14 days. EMA data included measures of negative/positive affect, anger/hostility, binge eating, vomiting, and exercise. We used the Greedy Fast Causal Inference (GFCI) algorithm to learn the partial ancestral graph and to compute a hypothetical causal model for each participant from their data. Structural equation modeling was used to estimate effect sizes for each model. Counts for the number of unconfounded causal edges between variables of interest were computed across all participants.

Results: We found a direct causal relationship with high negative affect preceding binge eating in 28/128 (22%) of the participants. Other causal relationships identified included low positive affect preceding binge eating: $n = 5$ (4%), anger/hostility preceding binge eating: $n = 5$ (4%), vomiting preceding binge eating: $n = 45$ (35%), and binge eating preceding vomiting: $n = 26$ (20%).

Conclusions: Prior studies examining negative affect and binge eating in BN have been limited by group-level analyses. By using causal discovery, we found that only 22% of participants had a causal relationship between negative affect and binge eating. Causal discovery also revealed heterogeneity in the sequence of binge-eating behaviors, with binge eating following self-induced vomiting (35%) as well as preceding it (20%). Identifying causal models at the individual level may optimize precision medicine approaches in the design of future trials for eating disorder treatments.

Keywords: Computational Psychiatry, Eating disorders, Ecological Momentary Assessment, Personalized Medicine

Disclosure: Nothing to disclose.

P184. Meal Challenge Revealed Differential Associations of Anorexia Nervosa Psychopathology With Soluble Epoxide Hydrolase-Derived Oxylipins

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Background: Oxylipins are bioactive lipid mediators derived from polyunsaturated fatty acids (PUFA) via cytochrome P450 (CYP), lipoxygenase (LOX), cyclooxygenase (COX), or non-enzymatic pathways. Soluble epoxide hydrolase (sEH) converts a class of CYP-derived oxylipins, epoxy fatty acids (epoxides), to corresponding diol fatty acids (diols). This process modulates important biological processes such as inflammation and metabolism. sEH-encoding gene was previously found to associate epidemiologically with anorexia nervosa (AN) risk. This study focused on clarifying the differential impact of sEH, PUFA, and oxylipins measured directly on AN risk and psychopathology.

Methods: Blood samples were analyzed from 70 women with AN (age: 32 ± 12) and 96 age-matched control women (age: 29 ± 8) at both fasting and 2h after consumption of a high-fat study meal. Fasting and postprandial levels of 59 oxylipins from CYP, LOX, COX, and non-enzymatic pathways, sEH protein expression and enzyme activity, and 13 diol/epoxide ratios (as sEH in vivo activity markers) were examined. AN clinical phenotypes were studied using EDI-3 and EDE-Q (eating disorder severity scales), anxiety, depression, and food aversion. Statistical analyses were conducted using t-tests and covariate-adjusted models.

Results: 17 fasting oxylipins (29% of 59 assayed), mostly derived from n-6 arachidonic acid (ARA, $n = 9$) and n-3 docosahexaenoic acid (DHA, $n = 7$), were significantly decreased in AN compared to controls (all $p < 0.01$). CYP-pathway oxylipins ($n = 9$ epoxides and 3 diols, 38% of total CYP oxylipins) were decreased in AN by 30% to 60%, while 5 LOX-pathway oxylipins (29% of total LOX oxylipins) were lowered by 42% to 82%. At the postprandial timepoint, levels of 6 CYP oxylipins (19% of total CYP oxylipins) and 3 LOX oxylipins (18% of total LOX oxylipins) were significantly different between groups. In AN, CYP epoxides were significantly elevated whereas CYP diols and 3 LOX oxylipins were lowered relative to controls (all $p < 0.05$). Significant between-group differences in the changes of oxylipin levels were found for 10 CYP epoxides (31% of total CYP oxylipins) (all $p < 0.03$), which all increased (range: 1 to 48 nmol/L) in AN but decreased (range: -2 to -306 nmol/L) in controls after eating. One LOX oxylipin increased in both groups but with a significantly greater magnitude in controls ($p = 0.02$).

In AN, of the 17 fasting oxylipins associated with AN risk, none of the CYP epoxides were significantly associated with psychopathology using EDI-3 and EDE-Q scores. However, CYP diol derived from n-6 ARA (5,6-DiHETrE) was linked to higher psychopathology in 5 of the EDI-3 scale scores, whereas n-3 DHA-derived diols (10,11- and 4,5-DiHDPE) were associated with less disorder severity, depression, and food aversion (all $p < 0.05$). LOX-pathway's 11- and 15-HETE were linked to less severe perfectionism score whereas 5-HETE was linked to higher interoceptive deficits score (all $p < 0.05$). None of the oxylipins were significantly associated with anxiety in either AN or controls.

sEH protein expression and enzyme activity were 16% ($p = 0.04$) and 22% ($p = 0.01$) higher in AN compared to controls at the fasting timepoint only. After eating, sEH expression and activity increased by 56% and 63% in AN ($p < 0.05$) and by 111% and 155% in controls ($p < 0.10$). None of the fasting oxylipin ratios (sEH in vivo activity markers) differed significantly between groups. At the postprandial timepoint, 7 oxylipin ratios (54%) were 44% to 75% lowered in AN (all $p < 0.05$) compared to controls. sEH expression, activity, and oxylipin ratios were not significantly associated with EDI-3 or EDE-Q scores.

Conclusions: sEH affects human health by converting PUFA-derived epoxides into their metabolic products, diol oxylipins. Association between sEH and AN was established previously at the genome wide level. Here, we present further evidence of sEH's link to AN risk by demonstrating differential associations between sEH substrates (epoxides) and products (diols) with AN risk and phenotypes that are PUFA class-dependent. Direct measurement of sEH expression and activity confirmed higher sEH level in AN. Significant between-group differences in meal-induced oxylipin changes were marked by directionally opposite changes in sEH substrates (epoxides), which increased in AN but decreased in controls. Surprisingly, although both epoxide and diol oxylipins were associated with AN risk, only diols were associated with AN phenotypes. Moreover, n-6 diols were associated with worsening AN phenotypes while n-3 diols were associated with better AN phenotypes. Our results confirmed the role sEH plays in modulating not only AN risk but also eating disorder severity. sEH action on n-3 PUFA oxylipins versus n-6 PUFA oxylipins led to differential impact on AN phenotypes, highlighting the importance of accounting for PUFA source in sEH studies and the potential for targeted dietary guidance in the future.

Keywords: Metabolites, Polyunsaturated Fatty Acids, Anorexia Nervosa

Disclosure: Nothing to disclose.

P185. Characterizing Midbrain Dopamine Function in Anorexia Nervosa Using Neuromelanin-Sensitive MRI

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Background: Anorexia nervosa (AN) is a devastating illness that most commonly emerges during adolescence and has a mortality rate among the highest of any psychiatric disorder. Several prior studies have identified altered dopamine function in AN, suggesting that abnormalities in the dopamine system may play a role in the pathophysiology of persistent maladaptive dietary restriction in AN. Although prior research utilizing positron emission tomography (PET) and lumbar puncture to assess metabolic byproducts of dopamine metabolism (e.g., homovanillic acid) broadly indicates dopamine dysfunction in AN, the nature and direction of this disturbance have not been clarified. This gap in knowledge may have arisen, at least in part, because AN often emerges and becomes entrenched during adolescence, when the use of in vivo assessments of dopamine function such as PET or lumbar puncture have limited appeal due to invasiveness, ethical considerations, cost, and methodology. Neuromelanin-sensitive magnetic resonance imaging (NM-MRI) is a high-resolution neuroimaging technique that provides a noninvasive proxy measure of dopamine function by measuring the concentration of neuromelanin, a byproduct of dopamine metabolism, in the substantia nigra (SN) and ventral tegmental area (VTA). This ongoing study examines dopamine in the SN and VTA in adolescents with AN as compared with healthy teens (HC).

Methods: NM-MRI data were collected in 14 female, post-menarchal adolescents with AN and 10 HC (ages 14-18 years); data collection is ongoing. Extracted neuromelanin (NM) signal (contrast-to-noise ratio) was averaged across the SN pars compacta (SNpc; SN region with greatest concentration of DA neurons) and the VTA. Independent samples t-tests were used to compare between-group differences and Hedge's g to measure effect size.

Results: Relative to HC participants, adolescents with AN had lower NM signal within the SNpc ($t_{1,22} = 2.07$, $p = 0.05$; $g = 0.86$). Preliminary analyses also suggest lower NM signal in the VTA ($t_{1,12.5} = 2.0$, $p = 0.067$; $g = 0.91$), though this result does not reach significance in this small sample.

Conclusions: Neuromelanin-sensitive MRI (NM-MRI) is a validated, non-invasive proxy measure of dopamine function. Our results indicate lower NM-MRI signal in the substantia nigra of adolescents with AN relative to healthy teens, suggesting reduced midbrain dopamine function in AN. NM-MRI may provide a noninvasive approach to measuring dopamine function which can be used safely and routinely in pediatric and non-pediatric populations with AN. Examining the relationship between NM-MRI signal in the midbrain and stage, severity, and trajectory of illness through longitudinal assessment in adolescents with AN will be a fruitful avenue for future investigation.

Keywords: Anorexia Nervosa, Dopamine, Human Neuroimaging

Disclosure: Nothing to disclose.

P186. Translocator Protein Distribution Volume, A Positron Emission Tomography Marker of Gliosis in Anorexia Nervosa

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Background: Anorexia Nervosa (AN) has a lifetime prevalence of ~0.8% and is arguably the most lethal psychiatric illness in the world, with a mortality rate 5.86% higher than in the general population. Unfortunately, there are no medications with indications for this disease, and a lack of therapeutic targets for AN in the brain.

Presently it is unknown whether gliosis occurs in AN, but there are several plausible reasons why it may occur. Peripheral inflammation may cause gliosis and there is evidence that greater peripheral inflammation may occur episodically in AN. Anorexia nervosa occurs at prevalence rates as high as 20% in children with Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections and Pediatric Acute-onset Neuropsychiatric Syndrome. The prevalence of autoimmune diseases, which may lead to gliosis, is ~2 fold greater in AN. Inducing peripheral inflammation in humans, such as with vaccination or lipopolysaccharide administration, the latter which also raises gliosis marker translocator protein (TSPO) in the brain, induces the symptom of anorexia, and direct cerebral inflammatory stimulation in rodents also induces anorexia. In addition, inverse relationships between body mass index (BMI) and TSPO VT, an index of translocator protein density and gliosis, have been frequently reported in neuropsychiatric disease.

TSPO VT can be measured using [18F]FEPPA, a radiopharmaceutical with excellent properties for use in positron emission tomography (PET) imaging. We hypothesized that TSPO VT will be elevated in circuitry implicated in AN, including the anterior cingulate cortex (ACC), insula, dorsal putamen, dorsal caudate, orbitofrontal cortex (OFC), ventral striatum, and thalamus.

Methods: Eleven AN (9 F, 2 M, average age 25.09) untreated with antidepressant, antipsychotic or anti-inflammatory medication and eleven healthy controls (8 F, 3 M, average age 25.82) underwent [18F]FEPPA PET scanning with concurrent arterial sampling. Exclusionary criteria included cigarette smoking and substance use disorders. A two-tissue compartment model was applied to measure TSPO VT. Regions of interest included the ACC, insula, dorsal putamen, dorsal caudate, OFC, ventral striatum, and thalamus. Groups were compared using a repeated measures ANOVA (rmANOVA), with group (healthy or AN) as the independent variable and region of interest as the repeated measure. ANOVAs to compare individual regions between groups were also done. The genotype of a single nucleotide polymorphism of the TSPO gene (rs6971) was applied as a factor in all analyses.

Results: There was an interaction between group and the a priori selected regions, indicating that some regions were more different between groups than others (rmANOVA, $p = 0.04$). ANOVAs comparing individual regions between groups found greater TSPO VT in ACC (32% difference, $F_{1,19} = 8.1$, $p = 0.01$) and thalamus (41% difference, $F_{1,19} = 7.2$, $p = 0.01$).

Conclusions: To our knowledge, this is the first investigation of gliosis in AN. Differences in TSPO VT are more prominent in the anterior cingulate cortex and thalamus, which implies that gliosis is more elevated in these regions in AN. This suggests that anti-inflammatory approaches should be considered for development as treatment in AN and these two regions should be a focus for future investigations of inflammatory processes in AN.

Keywords: Anorexia Nervosa, TSPO, Positron Emission Tomography Imaging, Gliosis, Eating Disorders

Disclosure: Nothing to disclose.

P187. Neural Correlates of Decreased Impulsivity During Delay Discounting Task in Obese Patients After Laparoscopic Sleeve Gastrectomy

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Background: Individuals with obesity (OB) prefer immediate rewards of food-intake over the delayed reward of healthy well being achieved through diet management and physical activity more often than normal-weight individuals (NW). This may reflect deficits in intertemporal decision-making function and heightened impulsivity. Overeating resulting from greater impulsivity is an important factor contributing to the development and maintenance of obesity. Laparoscopic sleeve gastrectomy (LSG) is one of the most effective treatments for morbid obesity and produces sustained weight-loss. In addition, LSG improved problematic and disordered eating behaviors, such as enhanced control of food-intake and decreased frequency of snacking on high-calorie foods. However, it remains unclear whether these improved eating behaviors were associated with decreased impulsivity in OB after LSG and how LSG regulates associated brain functions. Therefore, the current study employed functional magnetic resonance imaging (fMRI) with a monetary delay discounting (DD) task to examine LSG-induced changes in impulsivity and its neural correlates.

Methods: Twenty-nine OB underwent whole-brain fMRI scan with DD task before (PreLSG) and one-month after LSG (PostLSG). Thirty NW gender and age matched to the OB participants were recruited as controls and underwent the same fMRI scan. The discounting rate (K) based on the hyperbolic function and the area under curve (AUC), which is independent of theoretical assumptions regarding the discounting function were quantified as measures of impulsivity. Because the discounting rate did not follow a normal distribution, the log-transformed K (lg(K)) was calculated. Preprocessing of fMRI data was performed with Statistical Parametric Mapping 12. In the current study, two different statistical analyses were performed with the General Lineal Model (GLM). First, a GLM including hard- and easy-choice regressors was constructed for each participant with hard choices defined as those with similar subjective values for immediate and delayed reward options. Second, we constructed a GLM including SIR (choices for smaller immediate rewards) and LDR (choices for larger delayed rewards) condition regressors for each participant. Then, individual beta images responded to hard/easy-choices, SIR/LDR conditions and contrast images for "Hard - Easy" and "LDR-SIR" were calculated. To examine differences in activation between PreLSG and PostLSG, we performed second level analysis (paired t-tests) with those beta and contrast images. Clusters showing significant differences between PreLSG and PostLSG were selected as regions of interest (ROIs). In addition, whole-brain psychophysiological interaction (PPI) analysis was performed to investigate alterations in choice difficulty and subjective choice related functional connectivity with these ROIs as seeds. We also performed two-sample t-tests to compare the brain activations and functional connectivity between NW and LSG groups (PreLSG and PostLSG).

Results: LSG significantly reduced discounting rate in OB. During the hard trials in DD task, LSG significantly decreased activations in left dorsolateral prefrontal cortex (DLPFC) (Brodmann area (BA) 9), dorsomedial prefrontal cortex (DMPFC), which on average was equivalent to that in NW, and additionally increased activations in bilateral posterior insula (INS). Changes in activation in the left DLPFC were negatively correlated with changes in cognitive control measured with the Three Factor Eating Questionnaire (TFEQ, $r = -0.549$, $P = 0.004$) and AUC ($r = -0.484$, $P = 0.010$). Changes in activation in the left DMPFC were positively correlated with change in hunger (TFEQ) ($r = 0.513$, $P = 0.005$). Changes in activation in bilateral INS were negatively correlated with changes in body mass index (BMI) ($r = -0.551$, $P = 0.002$) and disinhibition (TFEQ, $r = -0.464$, $P = 0.011$). In addition, LSG significantly decreased activations in the left DLPFC (BA46) and right caudate during SIR condition, which on average was equivalent to that in NW. Changes in the left DLPFC activation was positively correlated with changes in BMI ($r = 0.555$, $P = 0.002$), lg(K) ($r = 0.467$, $P = 0.012$), and negatively correlated with changes in cognitive control (TFEQ, $r = -0.522$, $P = 0.004$), AUC ($r = -0.460$, $P = 0.012$). In PPI analysis, there was increased functional connectivity between the right caudate and left DMPFC (BA32) in responses to LDR versus SIR choices in PostLSG compared with PreLSG and NW. Changes in functional connectivity between the right caudate and left DMPFC were positively correlated with changes in cognitive control (TFEQ, $r = 0.588$, $P = 0.001$) and negatively correlated with changes in disinhibition (TFEQ, $r = -0.540$, $P = 0.004$).

Conclusions: These findings indicate that decreased impulsivity following LSG was associated with the normalization of activation in regions involved in executive control and reward evaluation, and compensatory effects through increasing interoception and prospection. This study may provide neurophysiological support for the development of nonoperative treatments such as brain stimulation for obese and overweight patients who do not qualify for LSG.

Keywords: Obesity, Task fMRI, Bariatric Surgery

Disclosure: Nothing to disclose.

P188. Expectation of Reward and Prediction Error Response and its Longitudinal Association With Treatment Response Among Young Adult Females With Anorexia Nervosa

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Background: Anorexia nervosa (AN) is a severe and often-chronic psychiatric illness with unknown etiology. Recent evidence from taste and monetary reward paradigms that investigated the reward prediction error suggests that dysregulation in reward processing may underpin symptoms and create a vicious cycle that contributes to chronicity and frequent relapse. However, whether conditioned reward expectation and receipt can predict long-term outcome has not been directly studied. Here we followed individuals who were treated for AN and tested whether brain imaging reward response could predict BMI. We anticipated that greater neural activity relative to expectation (higher arousal) and prediction error response (higher dopamine-related reward response) would predict lower BMI (poorer treatment response) at one-year follow-up.

Methods: The sample comprised a sub-set of a larger study of young-adult female patients with AN who received eating disorder treatment in a specialized partial hospital or residential program ($n = 35$, Mage [SD] = 23[7]). Functional brain imaging

was used to test brain response during a classical conditioning paradigm, where violations of learned associations between conditioned visual and unconditioned taste stimuli evoked the dopamine-related prediction error. Brain images were analyzed for expectation of the caloric stimulus as well as for prediction error response, which was comprised of the unexpected receipt or omission of the caloric taste stimulus sucrose. Regional brain response data were extracted for insula, orbitofrontal cortex and striatum. Study participants were contacted for long-term follow up assessment after discharge from the treatment program.

Results: Follow-up assessment occurred 1648 days (SD ± 1198) after brain imaging. Mean follow-up BMI = 18.9 (SD ± 3.3); mean BMI change between assessment time points = 2.5 (SD ± 3.2). Patients self-reported Eating Disorder Examination-Questionnaire (EDE-Q) Restraint = 2.2 (SD ± 1.9), Eating Concern = 2.3 (SD ± 1.3), Shape Concern = 3.2 (SD ± 1.5) and Weight Concern = 2.9 (SD ± 1.7); participants also rated mood (Beck Depression Inventory [BDI] = 20.2 [SD ± 12.9]), and anxiety (State/Trait Anxiety Index [STAI] = 49.3 [SD ± 12.3]). Long-term follow up BMI was positively correlated with discharge BMI ($r = .59$, $p < .001$) and days from scan ($r = .44$, $p = .009$). Adjusting for multiple comparisons, no significant associations were found for prediction error or expectation and follow-up BDI, STAI or EDE-Q scores. A pattern emerged whereby significant associations (at $p < .05$) in prediction error-related activity and follow-up BMI were positive, whereas associations in expectation-related activity and long-term follow-up BMI were negative. Adjusting for multiple comparisons ($p < .001$), a significant correlation remained only for expectation-related activity in the right superior orbitofrontal cortex with BMI, $r = -.58$, $p < .001$, 95%CI = [0.77, 0.31].

Conclusions: Results indicate that expectation, but not prediction error is related to weight outcomes. The study suggests that expectation response in the superior orbitofrontal cortex may be particularly important for long-term outcome. The superior orbitofrontal cortex is part of the superior frontal gyrus and is involved in executive function and inhibitory control, but also mood regulation and personality traits. The superior orbitofrontal cortex could be mechanistically involved in the persistent and cognitive emotional aspects of AN and could be a biomarker for long-term outcome.

Keywords: Anorexia Nervosa, Reward Expectancy, Superior Orbitofrontal Cortex

Disclosure: Nothing to disclose.

P189. Trait Anxiety is Associated With Amygdala Expectation and Caloric Taste Receipt Response Across Eating Disorders

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Background: Anxious traits are elevated in eating disorders (EDs), are considered risk factors for ED development, and trait anxiety has been linked to ED psychopathology. How trait anxiety relates to ED neurobiology is not well understood. Here we investigated a transdiagnostic study sample across the ED spectrum to test response to caloric stimulus (sucrose) expectation and expected receipt, both contrasted against non-caloric taste stimulus, and the effects of anxious traits. We hypothesized that elevated trait anxiety in individuals with EDs would be associated with brain response to caloric taste stimulus expectation and receipt. The amygdala is a brain region central to expectation, vigilance, anxiety and threat. We expected that the ED sample would show elevated amygdala response to expectation of a high caloric sucrose solution stimulus compared to the non-caloric stimulus and that expectation response would negatively bias and reduce response in brain reward regions during expected taste receipt, which could interfere with food intake.

Methods: In this study 179 individuals across the ED spectrum (anorexia nervosa n = 91; other specified EDs n = 34; bulimia nervosa n = 56; binge ED n = 16), and 120 healthy controls were assessed for anxious traits and learned to expect and receive caloric or neutral taste stimuli during fMRI of the brain. We developed first-level models to predict the response in each voxel as a function of the following conditions: (1) sucrose expectation: trials with CS predicting sucrose receipt contrasted against trials with CS predicting artificial saliva (non-caloric taste stimulus); (2) expected sucrose receipt: trials with expected US caloric sucrose receipt contrasted against trials with expected US non-caloric artificial saliva receipt. We extracted beta values from predefined regions of interest bilaterally. Group comparison studies were conducted with and without potential confounding covariates in the group-comparison (MANOVA, or MANCOVA for estimated marginal means). Regression analyses tested associations between behavior and brain activation. Moderator analysis (PROCESS, SPSS) was used to test the effects of anxiety on the relationship between sucrose amygdala expectation response (X) and reward circuitry taste receipt response (Y). Results were multiple comparisons controlled using false discovery rate (FDR).

Results: Amygdala sucrose expectation response differed across groups (Wilk's lambda=0.945, $p = 0.023$), and was higher on the left in anorexia nervosa compared to healthy controls ($p = 0.002$). Expected sucrose receipt response across taste reward regions was not different between groups. In the ED sample, trait anxiety negatively moderated the relationship between amygdala expectation and right dorsal ($p = 0.0062$) and ventral ($p = 0.0046$) anterior insula receipt response. A subgroup analysis showed similar results for anorexia nervosa, and partially in bulimia nervosa. Across EDs, appetitive motivation correlated positively with bilateral orbitofrontal cortex, caudate head, and ventral striatal sucrose receipt response ($r = 0.215$ to 0.179, $p = 0.002$ to 0.012). Across the study sample, trait anxiety showed an inverted-U-shaped relationship with right ($r = 0.147$, $p = 0.034$) and left ($r = 0.162$, $p = 0.016$) amygdala expectation response.

Conclusions: Elevated amygdala response in AN and the combined ED sample suggests elevated arousal to food stimuli. The relationship between amygdala expectation and right insular stimulus receipt response is moderated by trait anxiety in individuals with EDs. The influence of trait anxiety on brain taste response supports the notion that anxious traits may interfere with normal reward and interoception processing and thus have an important role in perpetuating ED pathophysiology and psychopathology. The study raises the question whether modifying the effects of trait anxiety and associated arousal via psychopharmacologic or psychotherapeutic interventions could have an important role in facilitating ED-specific treatment.

Keywords: Eating Disorders, Anorexia Nervosa, Reward Anticipation, Anxiety

Disclosure: Nothing to disclose.

P190. Attenuation of Anorexia-Related Phenotype in Recipients of Obese Adipose Tissue Transplant

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Background: Anorexia nervosa (AN) is observed predominantly in girls and women, and is characterized by hypophagia,

dangerously low body weight, and compulsive exercise. Recent large-scale human genome-wide studies have revealed that AN has a substantial metabolic component. Other recent studies point to adipose tissue as the location of 'metabolic memory' of earlier nutrition status. The activity-based anorexia (ABA) induces certain aspects of AN in rodents; mice are simultaneously exposed to running wheels and scheduled feeding, which induces hypophagia, weight loss, and hyperactivity. Here, we tested the hypothesis that transplanting adipose tissue from high fat diet (HFD)-fed obese mice into recipient mice would attenuate ABA-induced weight loss in recipients, possibly due to diffusible factors released by obese adipose tissue.

Methods: Nine-week-old mice received an intra-abdominal perigonadal adipose transplant harvested from either normal chow-fed mice, or high fat diet-fed obese mice (n = 21/group). All donors and recipients were female C57Bl/6 mice. Four weeks post-surgery, recipient mice were tested in the ABA paradigm. All mice underwent 2 days of acclimatization to single housing and constant running wheel access. Then, mice entered a baseline phase (4 days) in which food and running wheels were available continuously. Finally, mice entered a restriction phase (14 days) in which running wheels were continuously available, but food was only available 3 hours daily starting at 0900h. Body weight, food intake, and running distance were measured daily. During restriction, mice were removed from the study (termed dropout) after losing 25% of their baseline body weight. The number of days in restriction until dropout provided a measure of survival. Finally, this same experiment was performed using a new cohort, but within metabolic chambers (n = 7/group). Additional dependent measures were VO2 (volume of oxygen consumed, ml/kg/hr), VCO2 (volume of carbon dioxide produced, ml/kg/hr), respiratory quotient (RQ), food and water intake, body weight, as well as activity (x beams, y beams, wheel running counts).

Results: In the first study, no differences in body weight, food intake, or wheel running were observed between control adipose recipients and obese adipose recipients during baseline. However, during the restriction phase, obese adipose recipients remained in the ABA paradigm longer than control adipose recipients ($p < .01$). Despite the increase in survival time observed in obese adipose recipients, there were no differences in body weight, food intake, or wheel running between the groups. In the metabolic chamber study, no differences were found between groups for any measure.

Conclusions: Our results indicate that transplanting adipose tissue from high fat diet (HFD)-fed obese mice into recipient mice extends survival time in the ABA paradigm, defined as retaining > 75% of baseline body weight. These findings are consistent with the hypothesis that diffusible factors released by obese adipose tissue prevents weight loss. Studies are underway to examine the mechanisms underlying this effect.

Keywords: Food Intake, Wheel Running, Body Weight, Energy Metabolism, Transplantation

Disclosure: Nothing to disclose.

P191. A Hypothalamic to Infralimbic Melanocortin Circuit Regulates Food Intake and Food-Seeking Behavior

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Background: The melanocortin-4-receptor (MC4R) is implicated in metabolism and energy expenditure, and mutations of the MC4R are strongly linked to obesity in humans and mice. Its activity is regulated by peptides released from arcuate feeding and satiety neurons, agouti related protein (AgRP) and pro-opiomelanocortin (POMC). The MC4R is highly expressed in the hypothalamus, but deletion of MC4R from this region does not recapitulate the obesity induced by global brain knockout, suggesting MC4Rs in other regions are involved. The MC4R is expressed in the infralimbic cortex (IL), an area of the brain involved in decision making and habitual activity. Human imaging data implicates this region in obesity-related food cue responses. We hypothesized that MC4R activity in the IL (IL-MC4R) is regulated by peptides released from hypothalamic neurons, and also influences food intake and food-seeking behavior

Methods: We used a combination of genetically modified mice (male and female) with viral vectors to manipulate projections from molecularly defined melanocortinergic arcuate neurons (AgRP and POMC) at the terminal region in the infralimbic prefrontal cortex (IL), as well as the MC4R-expressing neurons in the IL directly. We used slice electrophysiology to test the pharmacologic function of the MC4R in the IL, and immunohistochemistry to further define the molecular identity of these ILMC4R neurons.

Results: We found that MC4R agonists applied in slice depolarized the membrane and increased excitability of IL-MC4R neurons. These neurons are also glutamatergic and project to areas associated with food-motivated behavior. Using optogenetic manipulation, we found that terminal stimulation of AgRP axons in the IL increases food intake acutely. Lastly, we used viral-cre manipulation in male MC4Rlox/lox mice to selectively delete IL-MC4R and observed an increase in food intake and body weight, as well as a delay in food-seeking and consuming behavior in an open field environment.

Conclusions: Our data highlights a novel population of MC4R-expressing neurons in the IL that receive functional input from hypothalamic feeding neurons, and influences food intake and other food-seeking behavior.

Keywords: Neuroendocrine, Eating Disorders, Melanocortin, Infralimbic Cortex, Food Intake

Disclosure: Nothing to disclose.

P192. Agrp Neurons Coordinate the Mitigation of Activity-Based Anorexia

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Background: Anorexia nervosa (AN) is a debilitating and deadly disease characterized by low body mass index due to diminished food intake, and oftentimes concurrent hyperactivity. A high percentage of AN behavioral and metabolic phenotypes can be replicated in rodents given access to a voluntary running wheel and subject to food restriction, termed activity-based anorexia (ABA). Despite the well-documented bodyweight loss observed in AN human patients and ABA rodents, much less is understood regarding the neurobiological underpinnings of these maladaptive behaviors. Hunger-promoting hypothalamic agouti-related peptide (AgRP) neurons have been well characterized in their ability to regulate appetite, yet much less is known regarding their activity and function in the mediation of food intake during ABA.

Methods: Female C57Bk6, AgRP-iCre, AgRP-iCre::hM3Dq, AgRP-iCre::hM4Di, or littermate controls, were used in these studies (n = 3-11). Mice were single housed and separated into one of three behavioral groups (Activity, FR, ABA) with access to ad libitum (Activity) or time restricted (FR, ABA) food and a voluntary running wheel (Activity, ABA only). Food access in FR and ABA cohorts was restricted to the first 3 hours of the dark cycle.

Feeding information was collected using FED3 devices, and detection or manipulation of neural circuits was performed using in vivo fiber photometry or chemogenetics, respectively.

Paired t-tests, unpaired t-tests, one-way ANOVAs followed by Bonferroni post-hoc tests (if applicable), two-way ANOVAs followed by Bonferroni post-hoc tests (if applicable), or two-way mixed ANOVAs followed by Bonferroni post-hoc tests (if applicable) were calculated as appropriate. Normality and homogeneity of variances were tested and, if necessary, accounted for using the Shapiro-Wilk and Levene's tests, respectively. Significance was determined for $p < 0.05$.

Results: We demonstrate that ABA mice decreased food intake due to increased interpellet interval retrieval and diminished meal number. Longitudinal activity recordings of AgRP neurons in ABA animals exhibited a maladaptive inhibitory response to food. We then demonstrated that ABA development or progression can be mitigated by chemogenetic AgRP activation through the reprioritization of food intake (increased meal number) over hyperactivity, but only during periods of food availability.

Conclusions: Taken together, we have ascertained the unique capability, necessity and circadian timeframe required for AgRP neurons to ameliorate ABA despite disrupted neural activity responses to food. These results elucidate a potential neural target for the amelioration of behavioral maladaptations present in AN patients.

Keywords: Feeding Behavior, Hypothalamus, Hyperactivity, Arcuate, Eating Disorders

Disclosure: Nothing to disclose.

P193. The Role of Insular Cortex NOS1 Neurons in Non-Homeostatic Feeding

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Background: Feeding is a complex motivated behavior that is modulated by both homeostatic and non-homeostatic factors. Changes in feeding behavior can result in increased consumption leading to obesity, as well as to decreased feeding in the case of anorexia nervosa. Both cases have profound clinical implications and have been largely intractable to pharmaceutical interventions. We have recently identified the insular cortex as a brain region implicated in controlling learned feeding behaviors that may contribute to obesity and eating disorders. Specifically, Nos1 neurons in the insular cortex were required for the expression of a learned overconsumption task. However, little is known about how insular cortex Nos1 neurons code for feeding-related behaviors and the mechanism by which they control overconsumption. Here we used a combination of chemogenetics and calcium recordings to gain a comprehensive understanding of how Nos1 neurons contribute to non-homeostatic changes in feeding.

Methods: Nos1-Cre mice ($n = 6$-10) were injected with a cre-dependent virus expressing the inhibitory DREADD, hM4Di (AAV-FLEX-hM4Di-mCherry) into the insular cortex. Mice were tested in a number of tasks including learned overconsumption, conditioned taste aversion, anxiety tasks (light-dark box, elevated zero maze, novelty suppressed feeding) and object recognition memory. Mice were also tested for homeostatic feeding of regular chow and palatable food (e.g. Ensure). Saline or CNO was injected 30 minutes prior to testing in both mCherry controls and hM4Di expressing mice. A separate cohort of Nos1-Cre mice ($n = 4$-6) were injected with a cre-dependent virus expressing the calcium indicator GCaMP in the insular cortex, and a fiber optic was implanted above the injection. Calcium recordings were done with the Neurophotometrics fiber photometry system during the previously mentioned tasks and data was analyzed with custom Matlab and Python scripts.

Results: Inhibition of insular cortex Nos1 neurons resulted in changes in behavior only in associative learning feeding tasks. While inhibition of Nos1 neurons prevented learned overconsumption, it surprisingly enhanced the conditioned taste aversion. We therefore tested whether Nos1 neurons lead to increased anxiety or altered memory, but we did not observe any changes in anxiety-related behaviors, object recognition memory, palatability, or homeostatic feeding. We therefore used fiber photometry recordings to correlate Nos1 activity to behavior. We found strong correlations between Nos1 activity and food consumption bouts, but not correlation to food approach or investigation. We also found increased activity in Nos1 neurons when mice were placed in a novel context and when exposed to novel, noxious odors.

Conclusions: We have found that Nos1 neurons are specifically involved in controlling the expression of learned feeding behaviors, but not anxiety, memory, or homeostatic feeding. Their calcium activity similarly correlates quite closely with food consumption bouts and olfactory and contextual cues that might be important for associating cues to food availability. Through Nos1 neurons, the insular cortex is therefore able to provide top-down control of complex feeding behaviors.

Keywords: Insular Cortex, Nos1, Feeding Behavior

Disclosure: Nothing to disclose.

P194. The Neuropeptide PACAP Enables Glutamate Signaling Between Nucleus Accumbens Astrocytes and Neurons to Regulate Behavioral Control

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Background: Prior work implicates system xc- (Sxc-) mediated glutamate signaling between astrocytes and neurons in drug seeking and other forms of maladaptive behavior. Here, we investigated which forms of cognition are dependent on Sxc- and how neurons regulate glutamate release from astrocytes to enable sophisticated cognitive control of behavior. Our hypothesis was that the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is released from cortico-striatal inputs to the nucleus accumbens where it regulates astrocyte to neuron signaling to enable cognitive control over behavior.

Methods: Rat nucleus accumbens afferent and efferents were labeled by injecting the retrograde neural tracer Cholera Toxin b into the nucleus accumbens or substantia nigra/ventral pallidum, respectively. Fluorescent-activated cell sorting was used to isolate nucleus accumbens astrocytes, efferents, and afferents. mRNA was measured using PCR or in situ hybridization. System xc-dependent glutamate release from nucleus accumbens astrocytes was measured using a cystine-evoked glutamate release assay coupled with reverse-phase HPLC. Neuronal activity in nucleus accumbens medium spiny neurons were measured using slice electrophysiology. Cocaine self-administration and primed reinstatement was performed using a long-access protocol (12 days). Working memory, impulsivity, decision-making, and cognitive flexibility were measured using Bussey-Saksida Touchscreens.

Results: Mutant rats lacking Sxc (MSxc rats) display significant deficits in decision making in the Rodent Gambling Task ($N = 10$/genotype; session x genotype x stimulus: $F_{42,1008} = 1.387$,

$p < 0.05$), cognitive flexibility in set shifting paradigm (N = 6-7/genotype; Trials to criterion: Extradimensional Shift: $t11 = 0.3.83$, $p < 0.05$ * relative to WT; Interdimensional Shift: $t11 = 2.96$, $p < 0.05$ * relative to WT; Perseverative Errors: $t11 = 3.39$, $p < 0.01$), increased impulsivity in 5-choice serial reaction time task (N = 6-7/genotype; $t11 = 2.217$, $p < 0.05$), increased levels of punished behaviors in Pavlovian conditioning (N = 9-10/genotype; session: $F4,68 = 7.633$, $p < 0.05$; genotype: $F1,68 = 6.187$, $p < 0.05$), and cocaine primed reinstatement (N = 13/genotype, $t11 = 3.26$, $p < 0.05$ * relative to WT). We also show that PACAP is expressed in nucleus accumbens inputs from the prefrontal cortex and that PACAP increases glutamate release from Sxc- in nucleus accumbens astrocytes (N = 6-12/genotype; genotype: $F1,40 = 275.645$, $p < 0.05$; treatment: $F3,40 = 4.492$, $p < 0.05$; genotype x treatment: $F3,40 = 4.003$, $p < 0.05$). To our surprise, we also found that PACAP acts at PAC1R expressed by MSNs to remove magnesium block from GluN2B-containing NMDA receptors, thereby enabling these receptors to be activated by Sxc- released glutamate. Lastly, intra-nucleus accumbens PACAP signaling blocks cocaine-primed reinstatement (treatment: $F1,18 = 13.03$, $p < 0.05$; test procedure: $F1,18 = 45.89$, $p < 0.05$; treatment x test procedure: $F1,18 = 13.82$, $p < 0.05$), and this effect requires intact Sxc- and GluN2B-containing NMDA receptor activity (treatment: $F3,52 = 5.646$, $p < 0.05$; genotype: $F1,52 = 3.666$, $p > 0.05$; treatment x genotype: $F3,52 = 10.148$, $p < 0.05$).

Conclusions: PACAP is expressed in cortico-striatal inputs to the nucleus accumbens and it enables a form of astrocyte to neuron signaling by increasing glutamate release from Sxc- and enabling activation of GluN2B-containing NMDA receptors. The consequence of PACAP-enabled astrocyte to neuron signaling is improved behavioral control resulting in reduced cocaine-primed drug seeking, only when Sxc- is intact. Next steps will be to determine whether PACAP release from cortico-striatal inputs is regulating behaviors dependent on Sxc- mediated signaling between astrocytes and neurons.

Keywords: Glutamate, Astrocyte, PACAP, Cognitive Control

Disclosure: Nothing to disclose.

P195. Dopamine D2 Receptors in Accumbens Cholinergic Interneurons Increase Delay-Based Impulsive Choice

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Background: Impulsive choice, often characterized by excessive preference for small, short-term rewards over larger, long-term rewards, is a prominent feature of substance use and other neuropsychiatric disorders. Growing evidence implicates nucleus accumbens (NAc) dopamine and its actions on D2 receptors (D2Rs) in various forms of impulse control. Because several NAc cell types and afferents express D2Rs, it has been difficult to determine the specific neural mechanisms linking NAc D2Rs to impulsive choice. Among those cell types, the cholinergic interneurons (CINs), which express D2Rs, are key regulators of striatal plasticity and local dopamine release, but their role in impulsive behavior is unknown. Our recent work showed that increased D2R expression specifically in NAc CINs impairs learning to suppress actions in a Go/No-Go task. While this is consistent with increased action impulsivity, here we set out to determine the contribution of CIN D2Rs to impulsive choice.

Methods: To determine whether increased D2R levels in NAc CINs contribute to impulsive choice, we first delivered Cre-dependent adeno-associated viruses (AAV) expressing D2R-EGFP or EGFP into the NAc of adult ChAT-Cre mice (8 mice/group, both sexes). Mice were trained on a delay discounting task that measures the choice between pressing a lever to obtain a small, immediate reward or pressing another lever to obtain a larger reward (3X) that was presented after increasing delays. After sessions in which both large and small rewards were delivered immediately following a lever press, delays to the large reward (2, 4, 6, 8, 10 s) were introduced across sessions in ascending order. We also used a probabilistic discounting task in a separate cohort of ChAT-Cre mice overexpressing either D2Rs or EGFP in NAc CINs (n = 8 mice/group). Mice had a choice between a small, certain reward and a large reward whose probability was progressively decreased (80, 60, 50, 40, 33, 20%). In both tasks, the mean percent of choices made on the "large" lever was analyzed using two-way repeated measures ANOVA. We further selectively inactivated the D2R gene in CINs using ChAT-IRES-Cre x Drd2flx/flx (CIN D2-KO) mice to measure the effect on both delay and probability discounting. Next, we determined whether CIN D2R upregulation altered the representation of time intervals using a peak interval task. After learning that lever presses were only reinforced after a 24-s fixed interval, mice received "peak" trials in which lever presses were not reinforced. Individual lever press rate data (peak location and width) were used to assess timing accuracy and precision. Furthermore, we used a temporal discrimination task to examine the ability to correctly categorize two auditory tones of different durations as short or long (2 vs. 8 s and 6 vs. 24 s).

Results: In the absence of any delays to the large or small reward, D2R-overexpressing and control mice similarly increased their preference for the large reward. As delays to the large reward increased, both groups showed discounting of the large reward, as shown by reduced choice of the large reward. This discounting, however, was significantly steeper in D2R-overexpressing mice compared to controls (virus x delay interaction: $F(5, 70) = 6.13$, $p < 0.05$). We then tested whether this manipulation would also alter probabilistic discounting, another dimension of impulsive choice in which rewards are discounted as they become more uncertain. Indeed, we found that greater uncertainty increased discounting (increased small, certain reward choices) in both groups, but there was no significant effect of D2R upregulation. To determine whether D2Rs in CINs are required for impulsive choice, we measured delay and probability discounting in CIN-D2KO and control Drd2flx/flx mice. Both groups discounted the large reward after greater delays, but CIN-D2KO switched less readily to the small, immediate option, indicating decreased delay discounting. In contrast, we found no effect of CIN D2R deletion on probabilistic discounting. Furthermore, D2R-overexpressing and control mice showed comparable response rates and distribution near the target of 24 s in the peak interval task. The temporal discrimination task also revealed no group differences in correctly categorizing long vs short tones, even when durations were proportionally increased.

Conclusions: Our findings show that increasing CIN D2R expression in the NAc leads to increased delay discounting without altering probabilistic discounting. This is supported by complementary data from CIN-D2KO mice showing that delay discounting, but not probabilistic discounting, is decreased compared to controls. These results suggest that CIN D2Rs promote impulsive choice involving delayed but not uncertain rewards. Because we observed no group differences in choices when both rewards were delivered immediately, the delay discounting effect is likely not due to different sensitivity to reward magnitude. Moreover, the lack of effect in peak interval and temporal discrimination tasks suggests that the ability to time intervals is not fundamentally altered by D2R upregulation. Together, these findings suggest that dopamine signaling via D2Rs expressed in CINs plays a key role in delay-based impulsive choice, providing new insight into the mechanisms by which NAc dopamine regulates CIN function and impulsive behavior.

Keywords: Cholinergic Interneuron, D2 Dopamine Receptor, Impulsive Behavior, Nucleus Accumbens, Delay Discounting

Disclosure: Nothing to disclose.

P196. Optogenetic Stimulation of ESR1-Expressing Neurons in PVT Reveals a Putative Neural Substrate for Outgroup, but Not Ingroup, Aggression

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Background: Navigating novel social environments is complex, multisensory, and cognitively demanding, where mammals have very limited initial information to guide their social behaviors. In some cases, this can lead to increased perceived threat and aggression towards an unfamiliar outgroup member, a phenomenon known as ingroup bias. Kinship and familiarity reduce aggression-related interactions in mice, however the neural circuits and mechanisms involved in ingroup versus outgroup social behavior biases remain to be determined. The Paraventricular Thalamus (PVT) is a critical node for integrating sensory features and emotional state information within novel and aversive contexts. However, the integration of social information in the PVT within these contexts and whether steroid hormones regulate PVT activity remains unresolved. Our study identifies a subset of steroid hormone receptor neurons in the posterior PVT involved in male outgroup aggression.

Methods: Using a multiplexed hybridization chain reaction assay, we characterized sex steroid hormone-related gene expression to identify distinct cell clusters in the PVT of male and female mice ($n = 3$ male, $n = 4$ female). With a transgenic Esr1-Cre + /- mouse line, we virally-targeted these steroid hormone receptor-expressing neurons in the PVT to investigate the role of steroid hormone genes in social behaviors. After testing a variety of behavior assays with optogenetic stimulation, (e.g., real time place preference, open field, elevated plus maze, and freely moving social interaction), we uncovered a male-specific aggression phenotype and focused the remainder of our study on male-male aggression in the resident intruder paradigm (group sizes of 8-10). In a resident-intruder paradigm, we tested male outgroup aggression bias in wildtype mice ($n = 8$) and recapitulated the behavior in the absence of optogenetic manipulation.

Results: Across the anterior-posterior axis of the PVT, we analyzed approximately 10,000 cells. Estrogen and androgen receptor-related genes, specifically Esr1, Esrra, and Rora, were highly expressed in the central and posterior areas of PVT, further corroborated by expression patterns of viral-labeled neurons in Esr1-Cre + /- mice. Additionally, Esr1, Esrra, and Rora were found to be highly co-expressed (>50%) within the same cell population, suggesting that sex steroid receptor-dependent activity in the PVT occurs in the same subset of cells, demonstrating the Esr1-cre + /- mouse a viable model for investigating sex steroid hormone modulation in the PVT during social behavior. During a resident intruder paradigm, optogenetic activation of Esr1+ PVT neurons in the resident mouse elicited an aggression phenotype specific to males, where male mice initiated aggressive attacks time-locked to light stimulation towards outgroup, but not ingroup, mice ($p < 0.05$). Additionally, we observed approximately 50% of wildtype C57BL/6 male mice with sexual experience exhibit outgroup, but not ingroup, aggression in the resident intruder paradigm in the absence of any optogenetic manipulation. Viral-labeling of Esr1+ PVT neurons in Esr1-Cre + /- mice also revealed dense projections to amygdala and extended amygdala regions, bed nucleus of the stria terminalis, ventromedial nucleus accumbens, insular cortex, and sensory cortices, providing us with downstream targets to dissect this behavior further.

Conclusions: From the data we have collected thus far, our results suggest that activation of the central and posterior PVT, specifically in subsets of neurons expressing estrogen and androgen receptor-related genes, gate outgroup aggression. Future directions of our study will aim to investigate 1) in vivo PVT neural activity in Esr1+ neurons during freely moving social behavior, 2) whether estrogen and/or androgen receptors in the PVT are necessary for outgroup attack behavior, and 3) if circulating hormones alter the activity in this neuron population.

Keywords: Paraventricular Nucleus of the Thalamus, Aggression, Ingroup Bias, Sex Steroid Hormone Receptors, Fluorescence in Situ Hybridization

Disclosure: Nothing to disclose.

P197. Distinct Behavioral and Neural Responses With Environmental Cues Associated With Addicted Behaviors

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Background: Behavioral addiction, such as addiction to gambling, Internet use, and gaming, has been recognized, but its concept and definition are still undetermined. Some impulse control disorders, such as kleptomania and paraphilia, have been suggested to meet the criteria of addiction, but are not legitimately categorized as addictive disorder, due to insufficient studies for comprehensively understanding these disorders as a behavioral addiction. In this study, we investigated how environmental cues associated with the disorder were processed in patients with kleptomania to gain further insights about it as behavioral addiction.

Methods: The images of the grocery store with (MKT + H) and without (MKT-H) a person, those of outside with (OUT + H) and without (OUT-H) a person, and those of foods (FOD) and stationary (STY) were presented to healthy adult subjects ($n = 27$; 11 males, 16 females) and patients ($n = 11$; 4 males, 7 females), who had been hospitalized for treatment of kleptomania with stealing foods in grocery stores. The areas of interest (AOIs) were set around the person within the MKT + H and OUT + H images. Their gazing patterns on the images were measured with eye-tracking, and oxy- and deoxy-hemoglobin changes while subjects gazed the images were simultaneously recorded at 10 regions within the prefrontal cortical (PFC) area with functional near infrared-spectroscopy.

Results: Bayesian ANOVA supported no overall difference in any of the number, duration, and dispersion fixations, the number of blinks, and changes (expressed as coefficient of variance) of pupil diameters over the course of gazing between the patients and healthy subjects as well as between images. Bayesian t-test or Mann-Whitney U test supported, although weakly, the alternate hypothesis over the null hypothesis in duration ($BF10 = 2.41$, error % = 0.007) and dispersion ($BF10 = 2.41$, error % = 0.009) of fixations made on the AOI of the MKT + H image, and those ($BF10 = 4.51$, error % = 0.001 in duration; $BF10 = 1.927$, error % =0.018 in dispersion) on the OUT-H image. The number of recording sites within the PFC where oxy- or deoxy-hemoglobin responses significantly increased or decreased from the baseline (quantified as area under the curve, with significance defined as $p < 0.05$ with t-test) over the courses of gazing the images was overall less in patients than that of healthy subjects. In addition, the principal component analysis with PFC oxy- and deoxy-hemoglobin changes unveiled that the correlations were strong

across all images (correlation coefficients in the range of 0.912 to 0.986) in the healthy subjects, whereas in the patients, the correlations were strong between images (correlation coefficients in the range of 0.865 to 0.987) except those between the MKT-H and any other images, where they were substantially lower in the range of 0.272 to 0.643.

Conclusions: These results suggest that patients with kleptomania exhibit distinct behavioral and neuronal responses than healthy subjects to the environmental stimuli associated with their uncontrolled behaviors.

Keywords: Addiction, Eye Tracking, fNIRS, Prefrontal Cortex

Disclosure: Nothing to disclose.

P198. Preliminary Evidence of Reduced Ventral Pallido-Striatal Synaptic Density in Opioid Use Disorder: A Pilot 11C-UCB-J PET Study

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Background: Decreased dendritic spine density has been demonstrated within the brain reward pathway (nucleus accumbens and prefrontal cortex) in rodents following abstinence from opiates [1, 2]. Whether similar micro-architectural alterations are present in humans with opioid use disorder (OUD) is unknown. Positron Emission Tomography (PET) imaging using the radioligand 11C-UCB-J, which selectively binds to the synaptic vesicle protein 2A (SV2A), is a novel method for visualization and quantification of synaptic density in the living human brain [3, 4]. Recently, PET imaging studies with 11C-UCB-J demonstrated relatively decreased synaptic density in other substance use disorders [5, 6].

Methods: Individuals with OUD, as diagnosed by DSM-5 criteria, ($N = 6$, $36.7 + /- 7.8$ years, BMI: $28.3 + /- 7.1$ kg/m2, all white male), underwent inpatient detoxification and monitored abstinence prior to PET imaging with 11C-UCB-J. Demographically matched healthy controls ($N = 6$, $38.5 + /- 8.7$ years, BMI: $25.0 + /- 3$ kg/m2, all white male) also participated in one 11C-UCB-J PET scan to assess SV2A density differences between the two populations. Female subjects also participated but unfortunately arterial line blood data was not able to be measured from these three individuals for a variety of reasons. The volume of distribution (VT) calculated using a one-tissue compartmental model with arterial input function with regions of interest analyzed and compared including the anterior cingulate (ACC), dorsomedial and ventromedial prefrontal cortex (PFC), lateral and medial orbitofrontal cortex (OFC), globus pallidus, and striatum. Groups were compared using independent two-sample, two-tailed t-tests.

Results: Initial PET results showed a significantly lower VT in regions of the pallido-striatal system, including globus pallidus (VT: $N = 6$; -18%; $p = 0.03$), putamen (VT: $N = 6$; -12 %; $p = 0.035$), and ventral striatum (VT: $N = 6$; -13%; $p = 0.07$) in OUD participants. Additionally, a significant negative correlation was found between smoking tobacco (cigarettes/day) and globus pallidus VT values ($N = 6$, slope = -0.1596, $P = 0.01$), other regions were tested but not found to be significant. No significant correlation was found between opiate dose used and VT values for this sample cohort.

Conclusions: These preliminary analyses 11C-UCB-J PET suggest decreased synaptic density within the striatum and globus pallidus among humans with OUD. To our knowledge, this represents the first in vivo clinical imaging demonstrating synaptic density differences in OUD as compared to HC participants. Though these preliminary findings align with literature preclinical work, there are several limitations such as small samples, no females, and potential confounding effects for nicotine. Limitations are being addressed with ongoing recruitment. Future analyses should employ partial volume corrections as well as in depth between groups' comparison for demographics. If these preliminary differences within OUD participants are confirmed, they would support future studies of agents with synaptotropic effects [7-13].

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Keywords: PET Imaging, Opioid Use Disorder, Synaptic Density, PET Tracer, [11C]UCB-J

Disclosure: Nothing to disclose.

P199. Correlates and Patterns of Cannabis Use in an ADHD Sample

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Background: Attention-deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized by inattention, hyperactivity, and impulsivity. According to the National Comorbidity Survey Replication the 12-month ADHD prevalence rate of 4.4%

for adults [1]. ADHD often leads to adverse outcomes across life domains, as well as an increase in comorbidity with other psychiatric conditions, such as anxiety and mood disorders and substance misuse [2,3]. Although effective the first line ADHD treatments are associated with side effects which often lead to poor treatment adherence [4]. Cannabis is one of the most commonly used psychoactive substances in the world; adult cannabis use in the last 12-months was globally estimated as 3.8%. It has been reported that cannabis has helped improve focus and hyperactivity in individuals with ADHD [5]. Among individuals with ADHD, the prevalence of cannabis use is higher than that in the general population, with estimates of 34-46% in treatment-seeking patients who use cannabis [6]. Although the relationship between ADHD and substance abuse is well-known, the literature examining the effects of cannabis on symptoms of ADHD is both scarce and equivocal. Few studies have examined the prevalence of cannabis use as a self-medication strategy in ADHD, and the effects of cannabis on specific symptoms. This cross-sectional, international survey study aimed to address this gap by asking individuals with ADHD about the frequency and benefits of cannabis use to treat ADHD symptoms.

Methods: Adults (all sexes) aged 18 and over with a self-reported lifetime diagnosis of ADHD, completed an online survey, posted between August 2020 and February 2022 in English. After signing an electronic informed consent, a questionnaire battery was completed, containing the Barkley Adult ADHD Rating Scale-IV (BAARS-IV) Quick-Screen, the Generalized Anxiety Disorder-7 (GAD-7), and the Patient Health Questionnaire (PHQ-9) to assess depression severity. Demographics and information regarding current and past substance use, was also collected. Validated cut scores on the GAD-7 (≥ 15) and PHQ-9 (≥ 15) were used to estimate rates of moderate-severe GAD and Major Depressive Disorder (MDD). Statistical methods included descriptive statistics, Chi square for normally distributed variables and Mann-Whitney analyses for non-normal distributions (only the BAARS-IV was non-parametric).

Results: The survey was completed by 838. Respondents were primarily female (71%), Caucasian (79%), and educated (41% with a graduate degree); with a mean age of 31.8 ± 9.9; primarily from Canada (56.2%), the U.S. (24.9%) and the U.K (4.4%). Most (76%) were receiving current pharmacotherapy for ADHD. The median BAARS-IV score was 16 (range 5.00 -20.00), indicating that most fell within the 98th to 99th percentiles. The mean scores on the GAD-7 (12.03 ± 5.3) and PHQ-9 (14.65 ± 5.8) were above the clinical threshold for diagnosis; 28.5% met threshold criteria for severe GAD, while 44.7% met criteria for moderately-severe MDD. Although lifetime rates of alcohol (89%) and tobacco (58.9%) use were similar to those in the general population, rates of cannabis use (77.3%), amphetamine/methamphetamine use (29%) and hallucinogens (33.6%) were nearly double the rates found in the general North American population[7,8]. Over half of the sample (55.6%) reported using cannabis in the past 6 months. Within this group of current users (n = 466), 51.1% reported daily or almost daily use of cannabis. Most (62.8%) of the total sample reported using substances to help manage their ADHD symptoms at some point in their life. Within this group (n = 526), 70% had used cannabis to manage their ADHD, reporting that they felt more calm (74.5%), got to sleep easier (70.1%) and felt less irritable or aggressive (59.3%). Although 50.6% reported that cannabis did not worsen their ADHD symptoms, respondents also said that cannabis worsened the ADHD symptoms of losing my train of thought (45.1%), having more difficulty staying focused (31.9%) and having more difficulty with organizing tasks and activities (27.8%). ADHD symptom severity was not associated with cannabis use or comorbid anxiety symptoms. However, those with moderate-severe MDD had significantly higher BAARS-IV scores ($p < .0001$). Individuals with moderate-severe GAD and MDD were significantly more likely to report current cannabis use ($p < .01$ – GAD; $p <= .05$ – MDD) as well as significantly higher frequency of current cannabis use ($p < .00001$ for both).

Conclusions: In this online sample of respondents with ADHD, the rates of lifetime substance use (excluding alcohol and tobacco) were much higher than North American population norms. This is especially surprising given that a high proportion of the sample was female. Substance use is typically higher in male populations. Furthermore, although most of the sample reported using cannabis to treat their ADHD symptoms, the benefits are unclear as the symptoms noted to have improved aren't core ADHD symptoms. It does appear that those with comorbid anxiety and depressive symptoms are using more cannabis than those without this comorbidity raising the possibility that cannabis maybe benefiting anxiety and depressive symptoms in this group.

Keywords: ADHD, Cannabis Use, Anxiety, Major Depression

Disclosures: Allergan, Almatica, Bausch Health, Brainsway, Elvium, Empowerpharm, Jazz, Lundbeck, Otsuka, Tilray, Vistagen: Advisory Board (Self), Biohaven, Otsuka: Contracted Research (Self), Canadian Institute for Health Research, Michael G. DeGroote Centre for Medicinal Cannabis Research, Elvium: Grant (Self), UptoDate: Honoraria (Self), Abbvie, Allergan, Elvium, Lundbeck, Otsuka, Pfizer, Sunovion, Takeda: Speakers Bureau (Self)

P200. CADM2 is Implicated in Impulsive Personality Traits by Genome- and Phenome-Wide Association Studies in Humans, With Further Support From Studies of CADM2 Mutant Mice

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Background: Impulsivity is a multidimensional heritable phenotype that broadly refers to the tendency to act prematurely and is associated with multiple forms of psychopathology, including substance use disorders.

Methods: We performed genome-wide association studies (GWAS) of eight impulsive personality traits from the Barratt Impulsiveness Scale and the short UPPS-P Impulsive Personality Scale (N = 123,509-133,517 23andMe research participants of European ancestry), and a measure of Drug Experimentation (N = 130,684). Because these GWAS implicated the gene CADM2, we next performed a phenome-wide study (PheWAS) of several of the implicated variants in CADM2 in a multi-ancestral 23andMe cohort (N = 3,229,317, European; N = 579,623, Latin American; N = 199,663, African American). Finally, we produced Cadm2 mutant mice and tested them using a battery of analogous behavioral tasks.

Results: In humans, impulsive personality traits showed modest chip-heritability (~6-11%), and moderate genetic correlations (rg = .20-.50) with other personality traits, and various psychiatric and medical traits. We replicated associations from earlier GWAS of these traits and found novel associations including DRD2, CRHR1, FOXP2, TCF4, PTPRF. PheWAS for CADM2 variants identified associations with 378 traits in European participants, and 47 traits in Latin American participants, replicating associations with risky behaviors, cognition and BMI, and revealing novel associations including allergies, anxiety, irritable bowel syndrome, and migraine. Cadm2 mutant mice recapitulated some of the associations found in humans, including impulsivity, cognition, and BMI.

Conclusions: Our results further delineate the role of CADM2 in impulsivity and numerous other psychiatric and somatic traits across ancestries, with further support from studies of Cadm2 mutant mice.

Keywords: GWAS, Impulsivity, Mouse Genetics, Polysubstance Abuse

Disclosures: Vivid Genomics: Advisory Board (Self), Own a patent: (Self)

P201. Investigating Fatty Acid Amide Hydrolase in Comorbid Borderline Personality Disorder and Major Depression: A [11C] CURB Positron Emission Tomography Study

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Background: Borderline personality disorder (BPD) is a debilitating psychiatric condition that is often comorbid with major depressive disorder (MDD). However, there is a lack of research investigating biomarkers that are common to both disorders. The endocannabinoid system (ECS) is a potent modulator of mood and stress circuits, and its dysregulation has been implicated in both animal and human models. Fatty acid amide hydrolase (FAAH), an enzyme of the ECS, plays a vital role in the metabolism of endogenous cannabinoids. Preclinical evidence suggests that FAAH levels are elevated within fronto-limbic brain regions in MDD, and a previous positron emission tomography (PET) study found elevated FAAH in the prefrontal cortex of humans with BPD. A FAAH gene variant (rs324420) also contributes to differential levels of FAAH expression. The ECS, however, has never been characterized in humans with comorbid BPD + MDD using in vivo imaging techniques. We hypothesized that brain FAAH would be elevated in the prefrontal cortex, hippocampus, and anterior cingulate cortex in humans with BPD + MDD compared to healthy controls using PET.

Methods: Ten BPD + MDD patients, aged 18 to 65 years, and 10 age-matched healthy controls were included in this pilot study. There were nine females in the comorbid group and eight females in the healthy group. Eligible psychiatric participants were unmedicated and met criteria for a current major depressive episode in addition to BPD. Control participants were excluded if they had any history of psychiatric disorder. All participants had negative drug urinalysis testing on the scanning days. FAAH binding was measured using PET with the radiotracer [11C]CURB. Participants also underwent genotyping to determine the FAAH gene polymorphism. Mood and personality questionnaires were administered for exploratory correlational analyses. A linear mixed effects model was employed to determine the differences in FAAH binding between the two groups, with genotype as a fixed factor.

Results: No significant differences in levels of brain FAAH were observed between BPD + MDD patients and controls in each examined brain region, including the prefrontal cortex, anterior cingulate cortex, and hippocampus. A whole brain analysis similarly did not find differences in the amygdala, temporal cortex, insula, thalamus, dorsal caudate, ventral striatum, and cerebellum between groups following Bonferroni correction. However, exploratory analyses revealed a significant positive correlation between FAAH binding in the ventrolateral prefrontal cortex (vlPFC) and impulsivity, as measured by the Barratt Impulsiveness Scale-11 (BIS-11), within the BPD + MDD group ($r = 0.635$, $p = 0.048$).

Conclusions: Considering the lack of response of current therapeutics by patients with BPD and MDD, research for novel biomarkers of these conditions is necessary. Our pilot study is the first to investigate FAAH in comorbid BPD + MDD using [11C] CURB PET. No significant group differences related to FAAH binding were observed. However, our sample at this point is likely underpowered to observe an effect. Our exploratory analyses suggest that prefrontal FAAH may be related to impulsive behavior in BPD + MDD, which is relevant because impulsivity is a core feature of BPD. Faulty vlPFC functioning has been implicated in impulsive decision-making, and it is possible that elevated FAAH in the vlPFC impacts neurotransmission to lead to impulsivity. Further research, however, is required to elucidate this potential mechanism.

Keywords: Borderline Personality Disorder, Major Depressive Disorder, Endocannabinoid System, Positron Emission Tomography (PET), Fatty Acid Amide Hydrolase, PET, Cannabis

Disclosure: Nothing to disclose.

P202. Sex-Dependent Effects in Dopaminergic Modulation of Risky Decision-Making in Rats

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Background: Dopamine (DA) modulates risky decision-making across species. Psychiatric disorders known to impact DA functioning (i.e., schizophrenia, bipolar disorder) exhibit worse performance in risky decision-making tasks, e.g., the Iowa Gambling Task, while DA agonist treatments, e.g., the DA-2 receptor (DA2R) agonist pramipexole (PPX), result in gambling problems. Using a risk-taking task, Zalocusky (2016) demonstrated the risk preference of male rats can be increased by systemic administration of PPX and decreased by optogenetic silencing of DA2R-expressing neurons in the nucleus accumbens (NAc). Sex-dependent differences were not examined however, and the role of sex or DA2R blockade in risky decision-making remains unclear. Here, we trained female and male rats in the same task to explore sex-differences in risk preference at baseline and in response to the pharmacological challenges of PPX, the DA2R antagonist sulpiride (SUL), and the dopamine transporter (DAT) inhibitor GBR-12909 (GBR).

Methods: In operant boxes, rats could choose from one of two nose-poke holes, one that delivered a 50 µl strawberry milkshake reward (safe-option), and the other a 10 µl reward with a 75% probability or a 170 µl reward with a 25% probability (risky-option). Once trained to a stable baseline of risk preference, rats were treated with PPX (0.15 or 0.3 mg/kg; Experiment 1) or SUL (30 mg/kg; Experiment 2) for 3 days, each separated by a saline washout. In experiment 3, animals were tested after an injection of GBR (5 or 16 mg/kg) or Vehicle. The primary outcome variable was percent risk choice (%RC).

Results: During baseline, females were less risk-adverse/more risk-prone than males. Experiment 1: collapsed across drug and saline tests, there was a main effect of drug on %RC change from baseline [$F(1,18)=10.5$, $p < 0.01$], with PPX increasing %RC. When analyzed across each testing day, a main effect of session [$F(6,108)=3.6$, $p < 0.005$] was observed, as was a session*sex*drug interaction [$F(6,108)=2.2$, $p < 0.05$]. Post hoc analyses revealed females differed from males in the timing of their response to PPX based on the dose administered. Experiment 2: collapsed across drug and saline tests, there was a main effect of drug on %RC change from baseline [$F(1,6)=20.0$, $p < 0.01$], with SUL decreasing %RC. There was also a trend toward a drug*sex interaction [$F(1,6)=3.6$, $p = 0.11$], with more pronounced attenuation of %RC by SUL in females. A similar pattern was observed when data was analyzed across all drug and saline tests. Experiment 3: there was a main effect of GBR on %RC change from baseline [$F(2,26)=4.0$,

$p < 0.05$], with the high dose of GBR (16 mg/kg) increasing %RC compared to VEH and low dose GBR (5 mg/kg; $p < 0.05$).

Conclusions: Together these data indicate a sex-specific modulation of baseline risk preferences, and that female rats may be more sensitive to DA manipulations on risky decisions, highlighting the necessity of tracking sex-based differences in such tasks. The data further support DAT inhibition (GBR) as recreating mania-like risk preference in rodents. Ongoing studies will determine whether DA2R NAc activity reveal a similar sex-specific change in DA during risk-preference. Understanding the neural basis of sex-based differences in risk, and DA modulation of risk, will enhance the management of psychiatric disorders that alter risky-decision making, particularly in those populations prescribed DA-based medications.

Keywords: Risky Decision-Making, D2 Dopamine Receptor, Sex-Differences

Disclosure: Nothing to disclose.

P203. Chemogenetic and Optogenetic Manipulation of Neuronal Projections From the Lateral Hypothalamus to the Paraventricular Nucleus of the Thalamus Impact Cue-Motivated Behaviors

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Background: The survival of an organism is dependent on their ability to properly respond to cues in the environment. Associative learning processes underlie an individual's response to such cues. For some, reward-paired cues are attributed with incentive motivational value (incentive salience), and can gain excessive control, leading to maladaptive behavior that is characteristic of psychopathology. To investigate the neural mechanisms that promote incentive learning, we utilize the goal-tracker (GT)/ sign-tracker (ST) animal model. With Pavlovian conditioned approach (PavCA) training, GTs attribute predictive value to reward-cues; whereas STs attribute predictive and incentive value to such cues. The attribution of incentive motivational value, or incentive salience, transforms the cue into an attractive and desirable stimulus. For STs, both food- and drug-associated cues gain excessive incentive value and elicit maladaptive behaviors. The GT/ST model, therefore, can be utilized to elucidate the neurobiological mechanisms that encode adaptive or maladaptive cue-driven behaviors. GTs and STs rely on distinct neurobiological mechanisms and the paraventricular nucleus of the thalamus (PVT) has emerged as a neural hub that mediates their characteristic differences in associative learning. Prior findings have led us to postulate that bottom-up projections to the PVT relay the incentive value of reward-associated cues, with the lateral hypothalamus (LH), which sends dense orexinergic (OXergic) projections to the PVT, acting as a critical neural node. Orexin (OX) is known to play a role in motivation, and administration of OX receptor antagonists into the PVT attenuates the incentive value of food-paired cues in STs. We hypothesize that OXergic transmission in the LH-PVT pathway encodes the incentive motivational value of reward cues and modulates the propensity to sign-track. Thus, we postulate that excitation of the pathway, via optogenetics, would elicit sign-tracking, and inhibition of the pathway following learning, via chemogenetics, would reduce sign-tracking behavior.

Methods: Here we investigate the role of the LH-PVT pathway in encoding the value of reward cues via (1) optogenetic excitation and (2) chemogenetic inhibition during PavCA behavior. In PavCA sessions, an illuminated cue (lever) extends for 8 seconds then retracts and a food pellet is dispensed into a food cup. In the optogenetics study, we used transgenic OX-Cre Long Evans male and female rats ($N = 11$), which express Cre-recombinase in OX neurons, and infused a retrograde Cre-dependent excitatory optogenetic virus (pAAVrg-Ef1α-DIO-ChR2-EYFP; channelrhodopsin, ChR2) or empty vector (pAAVrg-Ef1α-DIO-EYFP; control) into the anterior PVT. Animals received laser-induced excitation of LH-PVT neurons during cue (lever) presentation on sessions 1-5.

In the chemogenetic study, we utilized a dual-vector approach to selectively express an inhibitory (Gi) DREADD virus in the LH-PVT pathway in outbred Sprague-Dawley male rats ($N = 46$). A retrograde Cre virus (pAAVrg-hSyn-EGFP-Cre) was infused into the anterior and posterior PVT, while a Cre-dependent Gi virus (pAAV8-hSyn-DIO-hM4D-mCherry) was bilaterally infused into the LH. Following virus incubation, rats had 7 sessions of PavCA. After acquiring a conditioned response, rats received either vehicle or clozapine-N-oxide (CNO; 5 mg/kg) prior to sessions 5-7 of PavCA to activate the Gi-DREADDs.

Results: In the optogenetics study, preliminary results indicate that OXergic LH-PVT pathway stimulation during cue presentation results in increased goal-tracking behavior in ChR2 ($n = 6$) rats compared to controls ($n = 5$). This effect appears to be more pronounced in male ChR2 ($n = 3$) rats relative to male EYFP ($n = 2$), female ChR2 ($n = 3$), and female EYFP ($n = 3$); however, studies are ongoing to increase the sample size.

In the chemogenetics study, we assessed the impact of Gi DREADD activation on the expression of a learned conditioned response, session 5-7 of PavCA. A linear mixed-effects model analysis compared the effect of treatment (VEH vs. CNO) and phenotype (ST vs GT vs IR (intermediate responders)) for various behaviors in PavCA across sessions, and for all measures, there was a significant effect of phenotype ($P < 0.005$). When phenotypes were assessed independently using a repeated measures ANOVA, there was no effect of treatment on sessions 5-7 for either STs (VEH, $n = 3$; CNO, $n = 5$) or IRs (VEH, $n = 7$; CNO, $n = 7$). However, for GTs, there was a significant effect of treatment, such that CNO-treated GTs ($n = 8$) had less food-cup contacts ($p = 0.034$), a lower probability to approach the food-cup ($p = 0.002$), and a slower food-cup approach ($p = 0.01$) than VEH-treated GTs ($n = 16$).

Conclusions: These findings suggest that the LH-PVT pathway plays an important role in goal-directed behaviors. Preliminary findings indicate that optogenetic stimulation of OXergic neurons in the LH-PVT pathway of transgenic male rats increases goal-tracking behavior. Further, chemogenetic inhibition of the LH-PVT pathway in outbred male rats decreases the expression of goal-directed behaviors for GTs, without affecting the behavior of STs or IRs. Thus, the LH-PVT pathway appears to modulate predictive value encoding and the expression of goal-directed behaviors both early in training and after a conditioned response has been acquired. Ongoing studies are increasing the sample size in both studies to further investigate a potential role for the LH-PVT pathway in incentive learning and any sex differences that might arise.

Keywords: Individual Differences, Lateral Hypothalamus, Paraventricular Nucleus of the Thalamus, Incentive Salience, Pavlovian Conditioning

Disclosure: Nothing to disclose.

P204. Serotonin Modulation of Dorsal Striatal Medium Spiny Neurons During Reward Anticipation

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Background: Serotonin influences reward processing, threat detection, behavioral inhibition, and other components of motivation and behavioral control that are frequently disordered in a number of psychiatric disorders. In the dorsal striatum (DS),

pharmacological manipulation of serotonin signaling disrupts behavioral control and the prospective encoding of rewards. Additionally, many serotonin receptors, including the serotonin 1B receptor (5-HT1BR) have been implicated in regulating reward-motivated and impulsive behaviors. However, the nature of the behavioral encoding/control of reward-related behavior by serotonin is not well understood, nor have the circuit-level mechanisms through which serotonin modulates reward-related behaviors in the dorsal striatum been identified.

Methods: We used miniature microendoscopes for calcium imaging of dorsal striatal medium spiny neurons (MSNs) in adult mice during a simple appetitive operant task in the DIY-NAMIC system to analyze reward-related neural dynamics (n = 5; 3 male, 2 female). Additionally, mice lacking 5-HT1BR were also imaged to assess the effect of 5-HT1BR signaling on reward-related MSN activity. In parallel we used a G-protein coupled receptor activation based fluorescent sensor for serotonin (GRAB-5-HT) to measure serotonin release in the dorsal striatum of mice during reward trials delivered with the Davis lickometer (n = 10; 6 male, 4 female). These methods enable measurement of neural dynamics and serotonin signaling on a time scale compatible with single trial reward-related behaviors in Pavlovian and operant paradigms.

Results: We analyzed changes in calcium event rates in D1 and D2 MSNs during the different phases of reward seeking behavior and in response to an increased palatable reward. We find that the majority of cells decrease their rate to reward-predicting cues and reward delivery. Using supervised learning algorithms, we were able to accurately classify behaviorally correct, incorrect, and omitted trials based on the neural data during the trials. Calculated accuracy was >99%, and dropped to <40% when training labels were shifted. We also used recursive feature elimination to determine the number and pattern of cells that contributed the most to the classifier performance. Overall, our modeling work indicates that MSN activity in the dorsal striatum uses a relatively small number of cells for ensemble encoding of earned reward. Serotonin levels as measured by the GRAB-5-HT sensor in the DS increased prior to reward receipt. Interestingly, the increase in serotonin signal preceded reward consumption and was closely aligned with the initiation of approach to the reward following the cue signaling reward availability. The majority of licks for the reward (96%) occurred within 2 seconds after the onset of identified significant serotonin rises. Additional studies varying reward concentration with 2 and 6-bottle paradigms are ongoing, but preliminary data suggests there is some increase in the size and duration of serotonin rises with increases in reward value. Additional studies with GRAB-5-HT imaging are focused on parsing out the role of serotonin in encoding expected reward value, anticipation, and motivation.

Conclusions: Our results are important for understanding reward value coding in the DS and its modulation by serotonin. Current analysis of calcium imaging data is aimed at identifying the effect of 5-HT1B on MSN-reward related neural activity and determine how classifiers may change with the absence of this serotonin signaling. Characterizing real-time serotonin release and its influence on MSN neural activity and reward-related behaviors is important to understand how serotonin modulates reward processing and behavioral control.

Keywords: Serotonin, Reward, GRAB-5HT, Dorsal Striatum

Disclosure: Nothing to disclose.

P205. Transcriptional Effects of Neuropsychiatric Copy Number Variants in Postmortem Brain

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Background: Copy number variants (CNVs) are structural variants, where a large stretch of genomic DNA – typically spanning tens of kilobases – is either duplicated or deleted. Several CNVs have been associated with increased risk of neuropsychiatric disorders such as major depression, bipolar disorder, schizophrenia, and autism. These CNVs, while relatively rare, are highly penetrant and pleiotropic, but their impact on gene expression in brain has been little studied. Here we assessed gene expression changes in postmortem brain of carriers of neuropsychiatric CNVs compared to matched controls.

Methods: We identified 13 verified carriers of neuropsychiatric CNVs aged 10 or older from SNP genotyping array data across three postmortem brain repositories. Specimens included mirror CNVs on 22q11.2, 16p11.2, 1q21.1, 15q11.2 (BP1-BP2), and the 7q11.23 Williams-Beuren syndrome deletion. Whenever available, two brain regions – dorsolateral prefrontal cortex and anterior cingulate cortex – were profiled from a carrier and two matched controls, resulting in a total sample size of 62. RNA-sequencing data were quantified at the gene level and analyzed using linear mixed models to account for nested experimental design. Multivariate adaptive shrinkage was used to assess convergent effects across multiple CNVs.

Results: While few genes passed false-discovery rate of 5%, most genes within the respective CNV regions were under-expressed in deletion carriers and overexpressed in duplication carriers, as expected. Multivariate adaptive shrinkage showed moderate to low magnitude of differential gene expression effect sharing between CNVs, for the most part below 20%. The most prominent gene expression changes were observed in the 22q11.2 deletion and the least prominent ones in the 16p11.2 duplication, consistent with phenotypic severity.

Conclusions: We present the largest study of neuropsychiatric CNVs in postmortem human brain to date. Despite limited sample size of individual CNVs, initial assessment of differential gene expression showed prominent expression changes for genes within each CNV region that were consistent with copy number. Only moderate to low levels of convergence were found between different CNVs. Future analyses are directed toward functional enrichment and gene co-expression analyses of the shared and CNV-specific effects.

Keywords: Copy Number Variants, Human Postmortem Brain Tissue, RNA-Sequencing, Dorsolateral Prefrontal Cortex (DLPFC), Anterior Cingulate Cortex (ACC)

Disclosure: Nothing to disclose.

P206. Cell-Type-Specific Regulation of FosB Gene Expression in Nucleus Accumbens Moderates Effects of Chronic Stress on Sleep and Diurnal Rhythms

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Background: There is considerable evidence that Fosb in nucleus accumbens (NAc) medium spiny neurons (MSNs) regulates resilience to chronic social defeat stress (CSDS). Promoting Fosb function in dopamine type 1 receptor-expressing MSNs (D1-MSNs) confers resilience to CSDS as measured with social interaction and sucrose preference tests, whereas repressing Fosb in D1-MSNs

confers susceptibility. D1- and D2-MSNs also differentially regulate sleep architecture, such that DREADD-mediated inhibition of D1-MSNs increases duration of REM sleep. However, the ways in which changes in Fosb function in MSNs within the NAc affect CSDS-induced alterations in sleep architecture remains unknown. We hypothesized that enhancing Fosb function in D1-MSNs, through use of a construct that promotes histone acetylation at the Fosb promoter with a targeted zinc finger protein (ZFP) fused to p65, would prevent CSDS-induced alterations in sleep architecture, reflecting a pro-resilient phenotype.

Methods: Male Drd1-Cre mice were injected with a viral vector (AAV(DJ)-Fosb-ZFP-p65) that induces the endogenous Fosb gene in the NAc to physiologically relevant levels, or the corresponding control vector in which the ZFP lacks a functional domain (AAV(DJ)-Fosb-ZFP-ASSB). Two weeks later, mice were implanted with wireless transmitters to measure EEG, EMG, body temperature, and locomotor activity, followed by a week for recovery. Continuous telemetry was collected for 2 days of baseline, 10 days of CSDS, and 2 days of post-stress recovery. Active wake, REM sleep, and slow wave sleep (SWS) vigilance states, as well as activity and body temperature, were quantified as percent of baseline across all 14 days of the experiment. Data were analyzed using two-way repeated measures ANOVA and Sidak's post-hoc multiple comparisons tests.

Results: Elevated Fosb function (induced by Fosb-ZFP-p65) in NAc D1-MSNs prevented stress-induced alterations in REM duration observed in control mice (group: $F1,2 = 26.95$, $p = 0.0352$). In addition, Fosb-ZFP-p65 increased measures of slow-wave sleep during the light phase in the latter days of the CSDS regimen, an effect that persisted during recovery (group x time: $F13,26 = 2.594$, $p = 0.0187$). Body temperatures fell below baseline throughout the CSDS regimen, until recovery (time: $F1.905,3.80\ 9 = 2.594$, $p = 0.0243$).

Conclusions: Enhancement of Fosb function in D1-MSNs within the NAc mitigated the effects of CSDS on REM duration and SWS bouts, reflecting a potential pro-resilient effect. Across all groups, CSDS-induced changes in REM sleep and body temperature differed in direction and magnitude from those seen in naïve mice exposed to a similar regimen, raising the possibility that microinjections of these viral vectors into the NAc can produce non-specific changes in these endpoints. Future studies will characterize the ways in which repression of Fosb function in D1-MSNs, or corresponding changes within D2-MSNs, affect these same endpoints.

Keywords: Sleep, Social Defeat Stress, Telemetry, deltaFosB

Disclosure: Nothing to disclose.

P207. Contribution of the Integrated Stress Response to Behavioral Phenotypes Associated With Loss of TRNA Methylation in the Rodent Cortex

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Background: NSUN2, a mammalian tRNA methyltransferase, is expressed at high levels in brain and has been linked to neurodevelopmental defects in humans and mice due to its regulatory role in protein synthesis. We recently showed that there is a potent effect of Nsun2 depletion, via loss of tRNA methylation, on codon-specific tRNA expression and amino acid levels, producing proteomic shifts that impair synaptic transmission, cognition, and depressive-like behavior. Because dysregulation of amino acids and tRNA expression are known to produce the integrated stress response (ISR), we sought to explore whether this may be a mechanism by which loss of tRNA methylation alters behavioral outcomes.

Methods: We used Cre-driven conditional knockout of Nsun2 in the postnatal mouse cortex to deplete tRNA methylation levels and used western blot to measure hallmarks of the ISR, including Atf4 and phosphorylated Eif2a protein expression ($n = 5$ KO/5 WT males). Further, we injected Nsun2 KO mice with ISRIB ($n = 4$ KO males), an inhibitor of the ISR, or vehicle ($n = 3$ KO males) to alter the molecular phenotype. We also conducted behavioral testing (forced swim test) for depressive-like behavior on wild-type mice treated with tunicamycin ($n = 10$ males), a potent activator of the integrated stress response, or vehicle ($n = 10$ males). Data was analyzed using two-tailed t-tests.

Results: Nsun2 KO mice showed a marked increase in ATF4 expression ($p < 0.01$) and phosphorylated Eif2a expression ($p < 0.01$) compared to WT control mice. These molecular hallmarks were reversed after systemic treatment with ISRIB. Surprisingly, injecting wildtype mice with tunicamycin did not produce the same antidepressant phenotype as the Nsun2 KO but instead produced a pro-depressant phenotype in the forced swim test (time immobile: $p < 0.05$), suggesting another mechanism may be mediating changes in depressive-like behavior in the Nsun2 KO brain.

Conclusions: Data suggest that the ISR is activated following tRNA dysregulation but likely is not related to depression-related behavior in the Nsun2 KO mouse. Future studies will investigate alternate mechanisms including neuronal glycine content as potential molecular mechanisms for altered depressive-like behavior following loss of Nsun2-mediated tRNA methylation.

Keywords: Epigenetic, Noncoding RNA, Depressive-Like Behavior

Disclosure: Nothing to disclose.

P208. Enhancement of Cortical Plasticity on GABAergic and Glutamatergic Synapses by Targeting M1-Type Muscarinic Receptors in Somatostatin Interneurons Mediates Stress-Relevant Behavioral Outcomes

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Background: Major depressive disorder (MDD) and chronic stress impair both glutamate and GABA function in the mPFC, leading to altered connectivity and network dysfunction in cortical and limbic brain regions. Rapid-acting antidepressants, including ketamine and scopolamine, restore these deficits by targeting somatostatin (SST) interneurons in the mPFC, resulting in interdependent homeostatic enhancement of glutamate- and GABA-related plasticity, which restores the integrity of signal transfer to target regions. In particular, scopolamine, a non-selective muscarinic receptor antagonist, produces rapid antidepressant-like effects by targeting M1-type acetylcholine receptors (M1R) on SST interneurons, but these effects have largely been studied in male mice and have involved relatively short-term manipulations.

Methods: We generated mice with conditional M1R deletion in SST interneurons (M1f/fSstCre+) as an animal model to study long-term consequences of M1R deletion on GABAergic and glutamatergic synapses in the mPFC of male and female mice that lead to stress-relevant behavioral outcomes. We combined pharmacological, molecular, electrophysiological, chemogenetic and behavioral approaches to investigate whether the antidepressant-like effects of scopolamine can be mimicked

(baseline behaviors) or occluded (scopolamine challenge) in male and female M1f/fSstCre+ mice. Results were analyzed by Student's 2 tailed t-test and one- or two-way ANOVA, as appropriate, followed by post-hoc analysis, $p \leq 0.05$.

Results: Deletion of M1R in SST interneurons results in baseline decreases in stress-relevant behaviors in female mice accompanied by increased synaptic strength onto SST cells and enhancement of pre- and postsynaptic glutamate- and GABA-related signaling in the mPFC, but these baseline changes were not seen in male M1f/fSstCre+ mice. In contrast, both female and male M1f/fSstCre+ mice were resilient to stress following CUS exposure, with a more prominent effect in tests that evaluate coping strategies and motivation, which were positively correlated with levels of proteins relevant to glutamate and GABA signaling in the mPFC. In addition, SST M1R deletion in both male and female mice prevented the behavioral and molecular effects induced by scopolamine. Selective inhibition of SST interneurons in the mPFC of female M1f/fSstCre+ mice reversed the baseline behavioral changes, confirming that the mPFC somatostatin neurons are critical for stress-relevant effects of muscarinic receptor signaling.

Conclusions: Taken together, these results suggest that enhancement of both GABA and glutamate plasticity is necessary to restore signal integrity in the mPFC and other limbic regions, culminating in antidepressant-like states and stress resilience. These findings support the idea that restoring optimal excitation/inhibition function via M1R blockade in SST interneurons could represent a promising strategy for antidepressant development.

Keywords: Depression, Scopolamine, Antidepressant, Anxiety, Somatostatin

Disclosure: Nothing to disclose.

P209. Microglial BDNF Regulates Synaptic Plasticity and Behavior in Response to Stress and Ketamine Administration

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Background: Seminal studies demonstrate that brain-derived neurotrophic factor (BDNF) is a potent regulator of synaptic plasticity in the prefrontal cortex (PFC) and influences behavioral responses to stress and antidepressants. Prior studies indicate that neurons are the primary source of BDNF, but more recent work suggests that microglia produce BDNF as well. Still, it is unclear whether microglial BDNF is important for the effects of stress and antidepressant treatments.

Methods: In the first set of studies, mice with microglia-specific depletion of BDNF (Cx3cr1Cre/+:Bdnffl/fl) and genotype controls (Cx3cr1Cre/+:Bdnf + /+) were exposed to a shortened chronic unpredictable stress (CUS) paradigm (7 days). In separate studies, wild-type or transgenic mice ((Cx3cr1Cre/+:Bdnffl/fl; Cx3cr1Cre/+:Bdnf + /+) were administered a subanesthetic dose of ketamine (10 mg/kg, i.p.). In both experimental designs, molecular and histological analyses assessed markers of synaptic plasticity and microglia phenotype in the PFC as well as behavioral and cognitive outcomes.

Results: Flow cytometry and RT-PCR confirmed Bdnf depletion in sorted PFC microglia and revealed shifts in phenotypic markers, including reduced CSF1R and P2Y12 levels, due to CUS and BDNF depletion. Protein analyses of PFC synaptoneurosomes showed that Cx3cr1Cre/+:Bdnffl/fl mice had lower baseline expression of GluN2B in the PFC compared to genotype controls. Further, 7 days of CUS additionally reduced GluN2A expression in Cx3cr1Cre/+:Bdnffl/fl mice. Behavioral and cognitive testing showed that this coincided with exacerbated stress effects on temporal object recognition. Further studies showed that behavioral and synaptic changes caused by ketamine (10 mg/kg, i.p.) coincide with a 2-fold increase in Bdnf transcript in sorted PFC microglia. To test whether microglial BDNF signaling is important for these effects, we administered ketamine to Cx3cr1CreER/+:Bdnffl/fl mice and genotype controls. Protein analyses of PFC synaptoneurosomes again indicated that microglial BDNF depletion caused a reduction in GluN2B expression. Assessment in the forced swim test showed that ketamine reduced immobility in Cx3cr1CreER/+:Bdnf + /+ mice, but these effects were completely blocked in Cx3cr1CreER/+:Bdnffl/fl mice.

Conclusions: Collectively, our results show that microglial BDNF regulates expression of select glutamate receptor subunits in the PFC, which directs behavioral responses to stress and ketamine administration. These data indicate that microglia have an underappreciated role in promoting tolerance to stress and potential therapeutic utility in antidepressant treatment.

Keywords: Microglia, Brain-Derived Neurotrophic Factor, Stress, Ketamine, Synapses

Disclosure: Nothing to disclose.

P210. Increased Plasma Lactate and Lactate-to-Pyruvate Ratio in Patients With Treatment Resistant Depression

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Background: Major Depressive Disorder (MDD) affects roughly 264 million people worldwide, which translates to approximately 4.5% of the global population, being recognized by the World Health Organization (WHO) as one of the major causes of disability worldwide. Antidepressant medication or psychotherapy are common treatments for MDD; however, not all patients respond to treatment. Treatment-resistant depression (TRD) is a form of depression where standard medications tend to provide little to no relief. Mitochondria are organelles responsible, both directly and indirectly, for multiple cellular functions and signaling cascades, being considered a central platform in the execution of diverse cellular events. In this framework, it is particularly intriguing to think of the mitochondria as an active regulator of many of the biological phenomena involved in depression and the efficacy of or resistance to the most widely used pharmacological treatments. This study aims to evaluate biochemical markers associated with mitochondrial function in patients with MDD and TRD.

Methods: In this interim analysis of an ongoing pilot study, we included 17 healthy controls, 11 patients with MDD, and 14 patients with TRD. All subjects underwent a comprehensive clinical interview and diagnosis of MDD and TRD according to the DSM-IV-TR. Depressive symptoms and functional status were assessed with the Montgomery Asberg Depression Scale (MADRS) and Global Assessment of Functioning (GAF). Quantitative analysis of lactate and pyruvate plasma levels was performed using commercial kits.

Results: One-Way ANCOVA, after controlling for age and gender, showed that TRD patients had higher plasma levels of lactate and pyruvate when compared to healthy controls and MDD patients. Moreover, the lactate-to-pyruvate ratio was significantly different between TRD patients and healthy controls but not between TRD and MDD patients. Additionally, we evaluated the lactate-to-pyruvate ratio in a mitochondrial disease

cohort for comparison/validation purposes. Our results showed that the lactate-to-pyruvate ratio is higher in patients with mitochondrial diseases than in other groups. Another notable finding was that subjects with higher lactate-to-pyruvate ratio had higher MADRS scores and worse functional status.

Conclusions: In summary, our findings corroborate previous studies and support the notion that mitochondrial dysfunction is integral to the pathogenesis of MDD and may play a role in clinical and functional outcomes. Although preliminary, our results suggest that the lactate-to-pyruvate ratio might serve as one of the diagnostic bases of TRD. However, in this preliminary study, we cannot rule out the possibility of a type I error since it is a cross-sectional study with a small sample size. Therefore, our results should be seen as exploratory and require replication and validation.

Keywords: Major Depressive Disorder, Treatment Resistant Depression, Mitochondrial Dysfunction, Plasma Lactate and Pyruvate, Lactate-to-Pyruvate Ratio

Disclosure: Nothing to disclose.

P211. Cell-Type and Sex-Specific Molecular Signatures of Stress Resilience and Susceptibility in VTA

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Background: Mounting behavioral, circuit, and molecular-level evidence in humans and rodents shows that resilience to stress and depression is not an absence of susceptibility-associated changes, but an active process itself. The ventral tegmental area (VTA) has been strongly linked with depression and other psychiatric disorders. While a majority of studies have focused on changes within dopaminergic regions, there is mounting evidence for involvement of other cell types in stress response and resilience. We therefore sought to characterize the cell-type-specific molecular signatures associated with resilience and susceptibility to chronic stress within VTA.

Methods: Male and female mice were subject to chronic social defeat stress and tested on a battery of behavioral tests. VTA tissue was harvested 15-minutes after re-exposure to an aggressive encounter and processed for single-nucleus RNA-seq on a 10X chromium platform, to examine transcriptional signatures of cellular activity. Tissue from two mice of the same sex and group (control/susceptible/resilient) were randomly pooled for each sample, such that average social behavior was similar across samples within each group. Bioinformatic processing and analysis was performed with Cell Ranger (10X) and the Seurat package in R.

Results: We sequenced 185,908 nuclei from 16 samples, with an average of ~13,000 nuclei/sample, ~22,000 reads/nucleus, ~900 genes/nucleus, and ~72% of reads mapped to the genome. Clusters for expected VTA cell types were identified using UMAP. We show that resilience is associated with greater activation (immediate early gene expression) of dopamine, oligodendrocyte, endothelial, and GABAergic cells, while mixed glutamate/GABA cells were more active in susceptible mice. We present sex-specific characterization of excitatory/inhibitory balance, plasticity-related gene expression changes, neuroimmune, and neurovascular alterations associated with individual response to stress.

Conclusions: Distinct cell populations contribute to resilience or susceptibility to social stress. An imbalance in how each cell population adapts to stress may contribute to sex-specific vulnerability to psychiatric-disease-associated behavioral and physiological phenotypes.

Keywords: Ventral Tegmental Area (VTA), Social Defeat Stress, Resilience, Single-Nucleus RNA Sequencing

Disclosure: Nothing to disclose.

P212. Chronic Variable Mild Stress Alters Avoidance Behavior in Mice but has no Influence on the Transcriptome of the Anterodorsal Bed Nucleus of the Stria Terminalis and the M-Current of NPY Neurons

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Background: In humans, chronic stress leads to the development of mood disorders such as major depressive disorder and post-traumatic stress disorder. It has also been shown that women are more susceptible to the development of these disorders, suggesting a sex-related difference in how we process stress. The anterodorsal bed nucleus of the stria terminalis (abBNST) is a brain region that is essential for the central stress response. It is considered a part of the extended amygdala and serves as a relay between the amygdala and the prefrontal cortex. This region also has sexually dimorphic in expression of aromatase and estrogen receptors. Therefore, the adBNST may play a major role in the sex difference observed. We have previously demonstrated that chronic stress alters corticotropin-releasing hormone (CRH) expression and the M-current, a potassium current, in CRH neurons in the BNST from male mice. Other BNST neurons express Neuropeptide Y (NPY) which may plays a role in the stress response. We hypothesized that the CVMS paradigm would results in alterations in behavior of both male and female mice, sex-dependent differences in the transcriptome, and that adBNST NPY neurons would be affected by chronic stress, leading to a decreased M-current.

Methods: To study the effects of chronic stress on the adBNST, we used both wild-type and NPY-GFP male and female mice. Mice experienced six weeks of a chronic variable mild stress (CVMS) paradigm or no stress prior to behavior testing, adBNST tissue collection for RNA sequencing, and whole-cell patch clamp electrophysiology. The behavior tests conducted were the open field test (OFT), elevated plus maze (EPM), light dark box (LDB), and novelty suppressed feeding (NSF).

Results: Our results show that stress did cause sex-dependent differences in behavior depending on the parameters of the test. In the OFT, CVMS mice spent less time in the center (main effect of stress $P = .0019$) and more time in the corners (main effect of stress $P = .0209$). In the EPM, CVMS exposed mice spent more time in the closed arms than controls (main effect of stress $P = .0011$) and females had more open arm crossings than males. In the LDB, no effects were observed other than male mice had more stretch attend postures than females (main effect of stress $P = .0377$; interaction $P = .0002$). In the NSF, no effects were observed except that stressed mice weighed less than non-stressed mice (main effect of stress $P = .0136$). Serum corticosterone levels are currently being analyzed from blood collected before and after stress. RNA sequencing of the adBNST from stressed and unstressed mice found no effects of stress on any gene, potentially due to high variability between samples, but did find differences in expression between the sexes for ~30 genes. CVMS exposure did not affect neuronal excitability or the M-current in the adBNST NPY neurons, nor was there a sex-related difference. There was no effect on resting membrane potential yet there was an effect of stress on input resistance ($P = .0286$). The max peak of the M-current in control males was 30.4 pA, in stress males max peak was 34.0 pA (t-test: $P = 0.5175$), in females, control max peak was 23.4 pA, and stress max peak was 26.8 pA (t-test: $P = 0.6979$).

Conclusions: Our data suggests that CVMS is an effector of behavior in mice, but that adBNST NPY neurons may not be an important regulator of chronic stress or a mediator of the sex differences in the response to chronic stress.

Keywords: Chronic Unpredictable Mild Stress, BNST, Approach/Avoidance, Sex Differences, Neuropeptide Y

Disclosure: Nothing to disclose.

P213. The Involvement of SKA2, a Novel Glucocorticoid Receptor Interaction Partner, in Stress-Related Psychiatric Disorders

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Background: Mood and anxiety disorders represent a major disease and social burden worldwide, but the underlying molecular mechanisms are still poorly understood. In recent years, evidence has emerged for the crucial role of genes involved in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis, especially in the context of stress-related psychopathologies such as anxiety and depression. The glucocorticoid receptor (GR) is the main mediator of the HPA axis negative feedback loop in response to stress. The SKA2 gene, encoding the spindle and kinetochore associated complex subunit 2, has previously been identified as a GR interaction partner in peripheral cells in vitro. Interestingly, single nucleotide polymorphisms and DNA methylation within the SKA2 gene, as well as gene expression alterations, have been associated with posttraumatic stress disorder and suicide risk in human patients. Yet, little is known about the underlying molecular mechanisms and the role of SKA2 in the brain. Here, we set out to further investigate the function of SKA2 in the CNS of humans and mice in the context of stress-associated psychiatric disorders and behaviors.

Methods: We conducted Immunohistochemistry (IHC) to study the expression pattern of SKA2 in human postmortem hippocampus and amygdala samples. Additional co-immunoprecipitation (co-IP) assays addressed whether SKA2 physically interacts with the GR in the mouse brain and in human postmortem brain samples. Moreover we performed GR reporter gene activity (MMTV-Luc) assays and qPCR following Ska2 perturbation to investigate the receptors activity and downstream effects in neuronal cell culture. Western blot (WB) analysis were performed to examine SKA2 protein expression in basolateral amygdala (BLA) and hippocampus samples of individuals with bipolar disorder and matched controls. Stress-induced changes in SKA2 expression in mice were investigated via WB following fear conditioning.

Results: IHC analyses in human postmortem hippocampus and amygdala samples revealed a prominent expression of SKA2 in Camk2a-positive neurons. Moreover, co-IPs revealed a physical interaction between SKA2 and GR in the mouse brain and human postmortem samples. In addition, Ska2 knockdown in hippocampal neuroblastoma (HT-22) cells led to significantly reduced GR reporter gene assay activity, while overexpression of Ska2 resulted in the opposite effect (2-Way ANOVA, interaction: $F(14,42) = 16.38$, $p < 0.0001$). Along these lines, Ska2 knockdown led to significantly altered mRNA expression of GR target genes Fkbp5 (t-test, $T(21) = 2.683$, $p < 0.05$) and Id3 (t-test, $T(22) = 5.762$, $p < 0.001$). Interestingly, we detected significantly increased SKA2 protein levels in the BLA of individuals with bipolar disorder compared to matched controls (ANCOVA, $F = 5.83$, $p < 0.025$, $n = 14$ per group). Moreover, hippocampal SKA2 expression is increased in bipolar disorder, when 'presence of psychoactive drugs in the blood at death' is included as co-variate (ANCOVA, $p < 0.01$, $n = 20$ per group). Detailed mapping and co-labeling studies in mice also revealed a distinct pattern of SKA2 expression in neurons of the BLA, as well as in the hippocampus, medio-dorsal thalamus, the paraventricular nucleus of the hypothalamus and throughout the cortex. Most of the SKA2-positive neurons also expressed the GR. It has previously been reported that the synthetic glucocorticoid dexamethasone decreases SKA2 protein levels in human lung epithelial cells. Consequently, we assessed whether acute stress is able to modulate SKA2 expression in the mouse brain. Using WB, we found dynamic changes of SKA2 expression 8 and 12, but not 24 hours after stress (fear conditioning, 5 tone/foot shock pairings). Stressed mice showed significantly decreased SKA2 protein levels in the hippocampus (8 hrs: $T(8) = 2.626$, $p < 0.05$, $T(8) = 3.358$, $p < 0.01$, $n = 4$ (ctrl), $n = 4$ (stress)), while no expression changes were observed in the prefrontal cortex.

Conclusions: Our findings reveal SKA2 as a novel GR interaction partner in brain regions associated with emotion processing and cognition. Further experiments showed that Ska2 gene expression is dynamically regulated by stress exposure and that SKA2 is able to positively modulate GR signaling and its downstream targets, providing a mechanistic link to its association with stress-related psychopathologies. Collectively, our data point to an important, and thus far unappreciated, role of SKA2 in stress-related psychiatric disorders, which is relevant to our understanding of the molecular mechanisms underlying such diseases.

Keywords: PTSD, Bipolar Disorder, Suicide Risk Factors, HPA Axis, Glucocorticoid Receptor

Disclosure: Nothing to disclose.

P214. Sex and Region Differences in Microglia and Dendritic Spines in the Human Brain

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Background: Microglia are resident macrophages of the brain, performing roles related to brain homeostasis, including trophic support, phagocytosis of apoptotic cells and debris, as well as brain protection and repair. In healthy adults, microglia exhibit a ramified morphology, sampling the local environment by extending and retracting processes. Various stimuli, including infection, trauma, or altered neuronal activity, induce changes in microglia shape, gene expression, and function. Evidence from rodent studies reveal that microglia density, morphology, gene expression, and proliferation vary based on brain region and sex. In addition to being the resident macrophages of the brain, microglia participate in elimination and formation of synapses on pyramidal cells. The primary source of excitatory synaptic input onto pyramidal cells are dendritic spines, which are morphological protrusions on PC dendrites. Similar to microglia, evidence suggests brain region and sex differences in dendritic spine density. Notably, most studies describing brain region and sex differences in microglia and dendritic spines were performed in rodents. Whether similar differences exist in the human brain has not been fully studied, especially with consideration of whether there are correlations between microglia reactivity and dendritic spine density within the same subjects. Here, we simultaneously assess dendritic spine density and microglia reactivity state in the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) of human subjects unaffected by psychiatric or neurological disorders.

Methods: For DLPFC and ACC separately ($N = 5$/sex; same subjects used for DLPFC and ACC), 2 equally spaced coronal sections from the immersion-fixed left hemisphere were

processed for immunofluorescence. Dendritic spines are identified by labeling for Shank3, a spine scaffolding protein, and f-actin, which is concentrated in dendritic spines, using the phallotoxin phalloidin. Using the colocalization of two dendritic spine markers provides a robust approach for identifying spines. In the same sections, microglia are labeled using antibodies against the microglia specific marker TMEM119 to assess microglia morphology and CD68 to assess microglia phagocytic activity. NeuroLucida 360 was used to mask fluorescent signal in 3 dimensions. Microglia complexity was assessed using Sholl analysis; an average of 55 microglia were analyzed per subject per brain region. Two-way repeated measures ANOVA was used to assess the number of microglia process intersections at 10 micron radii surrounding each microglia soma; Tukey's multiple comparisons test was used for posthoc analyses. Unpaired t-tests were used to assess sex differences in colocalized shank + /phalloidin+ puncta (putative dendritic spines) and one-way ANOVA was used to assess layer differences in colocalized shank + /phalloidin+ puncta, with Tukey's multiple comparisons test used for posthoc analyses.

Results: We observed striking differences in microglia between the DLPFC and ACC. Microglia in the ACC exhibited a more activated morphology (more amoeboid; fewer, less complex processes) compared to microglia in the DLPFC. We note that assay methods, imaging, and processing were identical between the ACC and DLPFC. Thus, technical differences are not driving the robust morphological differences between microglia in the ACC and DLPFC. Within the ACC, we observed sex differences in microglia reactivity and in dendritic spine density. Specifically, Sholl analysis indicated that female microglia exhibit less complexity (main effect of sex: $p < 0.01$; sex-by-distance from soma: $p < 0.0001$), and female microglia had shorter process length ($p < 0.05$); both findings suggest that female microglia are more reactive. Interestingly, females also had lower density of shank + /phalloidin+ puncta (putative dendritic spines) within 12μm proximity of microglia ($p < 0.01$). When males and females were combined, we also observed cortical layer differences in microglia complexity (layer-by-distance from soma: $p < 0.01$) as well as in microglia process length, with layer 2 microglia exhibiting a more reactive profile compared to other layers. Additionally, layer 3 had more dendritic spines than other layers ($p < 0.05$ for comparisons to other layers).

Conclusions: Our findings suggest a high level of microglia heterogeneity in the human cortex in subjects without psychiatric or neurodegenerative disorders. In terms of microglia reactivity, we found noted differences based on brain region (ACC > DLPFC), sex (female > male), and cortical layer (layer 2 > other cortical layers). Assessment of dendritic spine density in the proximity of microglia within the ACC revealed striking sex differences (male > female), which is consistent with females having more reactive microglia. Ongoing studies are expanding this analysis to psychiatric disease cohorts.

Keywords: Sex Difference, Human Microglia, Dendritic Spines, Human Postmortem Brain

Disclosure: Nothing to disclose.

P215. GM-1020: A Novel, Orally Bioavailable NMDA Receptor Antagonist With Rapid and Robust Antidepressant Effects and Reduced Ataxia in Rodents

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Background: The discovery of the rapid antidepressant effects of the NMDA receptor (NMDAR) antagonist (R,S)-ketamine has ushered in a new wave of interest in identify molecules that can achieve the rapid and robust efficacy of (R,S)-ketamine but improve on some of its shortcomings. At the commonly administered antidepressant dose of (R,S)-ketamine (0.5 mg/kg, iv infusion), patients experience dissociative side effects and ataxia, which restrict safe administration to a supervised, in-clinic setting and limits the clinical uptake of this treatment. In addition, (R,S)-ketamine has poor oral bioavailability that necessitates parenteral dosing. Spravato®, an intranasal formulation of the more potent S-isomer of ketamine, has been approved for patients with treatment-resistant depression, but still requires supervised administration due to its dissociative effects. Early clinical data with intravenous R-ketamine indicate that this less potent enantiomer retains antidepressant efficacy at non-dissociative doses, suggesting an improved therapeutic index is possible for this mechanistic class. However, there remains an unmet need for a non-dissociative, orally bioavailable NMDAR antagonist with rapid and robust antidepressant efficacy. GM-1020 is an orally bioavailable, NMDAR antagonist with robust, durable antidepressant effects and attenuated side effects in rodents.

Methods: The binding affinity of GM-1020 at NMDAR was determined using displacement of [3H]MK-801 from rat cortical tissue homogenates. Functional antagonism was demonstrated using automated patch clamp in oocytes expressing human GRIN1/GRIN2A- or GRIN1/GRIN2B-containing NMDAR.

The metabolism of GM-1020 was evaluated in rat, mouse, minipig, dog, cynomolgus monkey, and human liver microsomes and hepatocytes. Oral bioavailability of GM-1020 was determined by comparing PK after IV and PO administration in rat, mouse, mini-pig, and cynomolgus monkey.

GM-1020 was assessed in a paradigm of Chronic Mild Stress (CMS), wherein male Wistar Han rats ($n = 8$/group) were subjected to stressors (e.g., wet bedding, tilted cage) for a period of 10 weeks. After two weeks of CMS, animals were dosed once per week with GM-1020 (0.75 - 9 mg/kg, ip), (R,S)-ketamine (10 mg/kg, ip), or vehicle for 5 weeks. Rats continued to be exposed to stress for an additional 3 weeks after the final dose to determine the durability of the antidepressant effect. Each week, anhedonia was assessed by measuring sucrose intake (24 h after dosing). Anxiety levels in the elevated plus maze (EPM) and memory impairment in the novel object recognition (NOR) test were assessed 48 and 72 h after the first dose, respectively.

Acute effects of GM-1020 (1- 32 mg/kg, sc) on locomotor activity were assessed in male C57BL/6 mice in an open field test. The effects of GM-1020 (1- 32 mg/kg, sc) and (R,S)-ketamine (3.2-32 mg/kg, sc) on motor coordination were assessed using an accelerating rotarod in mice ($n = 12$/group).

Results: GM-1020 bound to NMDAR in rat cortical tissue with low-micromolar affinity (Ki 3.25 μM). GM-1020 exhibited channel blocking properties of glutamate/glycine-induced NMDAR currents in the absence of Mg2+ at GRIN2A- (IC50 6.45 μM) or GRIN2B-containing (IC50 4.16 μM) NMDAR.

Oral bioavailability (%F) was determined to be 27-43% in non-clinical species. Microsomal clearance in vitro was found to be a good predictor of %F (32-52%); human microsomal clearance predicted human %F of >60%.

Rats exposed to CMS showed a robust anhedonic phenotype evidenced by a reduction in sucrose intake throughout the study in vehicle treated rats ($P = 0.0003$). GM-1020 reversed the effects of CMS on sucrose intake at doses ≥1.5 mg/kg ($P < 0.001$ vs stress vehicle group). CMS also resulted in increased anxiety in the EPM and memory impairment in the NOR test. Both effects were reversed by administration of GM-1020 (≥1.5 mg/kg; $P < 0.001$ vs stress vehicle group). Similarly, (R,S)-ketamine (10 mg/kg) reversed the anhedonia, anxiety and memory impairment caused by CMS. Efficacy of GM-1020 was maintained for 14 days, and ketamine for 7 days, after the final dose.

Evaluation of higher doses of GM-1020 (1-32 mg/kg) for effects on motor activity showed no change in distance moved in the open field (0-60 min post dose), while GM-1020 (≥10 mg/kg) produced a decrease in latency to fall off the rotarod 5 minutes after dosing ($P < 0.05$ vs vehicle). The dose of GM-1020 resulting in a 50% reduction in latency to fall was ~20 mg/kg, whereas for (R,S)-ketamine, it was ~6 mg/kg.

Conclusions: GM-1020 has a good oral bioavailability in non-clinical species and predicted bioavailability of >60% in humans. In the CMS model of depression, the minimum antidepressant-like dose of GM-1020 was 1.5 mg/kg, with robust efficacy observed after the first dose and maintained for 14 days after the final dose. The ED50 for ataxia was ~13-fold higher, while no effect on locomotion was observed even at >20-fold higher doses than the minimum efficacious dose in CMS. These data indicate that pre-clinically, GM-1020 shows large separation between antidepressant doses and those causing ataxia. Literature reports with (R,S)-ketamine indicate that in rodents, ataxia is observed at doses only 3-fold higher than antidepressant doses, whereas in humans, the antidepressant dose of (R,S)-ketamine is associated with ataxia and dissociation. This supports further development of GM-1020 as a putative orally bioavailable, rapid-acting, robust antidepressant with an improved therapeutic index with respect to dissociation and ataxia.

Keywords: NMDA Antagonists, Depression and Anxiety, Rapid-Acting Antidepressant

Disclosure: Gilgamesh Pharmaceuticals: Employee (Self)

P216. Baseline Differences and Treatment-Responsiveness to Reelin and (2R,6R)-HNK of IPSC-Derived Neurons From Participants With Treatment-Resistant Depression

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Background: Treatment-resistant depression (TRD) is of great clinical concern, with patients often not responding to first-line antidepressant treatments. Subanesthetic doses of ketamine, a non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist, has been found to have rapid and robust antidepressant effects in patients with TRD. However, certain psychoactive effects necessitate administration under medical supervision. Recent research suggests that the fast-acting antidepressant effects of ketamine are mediated through transient activation of the mammalian target of rapamycin (mTOR), which promotes an upregulation of proteins which strengthen synaptic signaling, such as surface insertion of GluA1. (2R,6R)-hydroxynorketamine (HNK) is a major metabolite of ketamine which, at therapeutic levels, does not antagonize NMDARs. Preliminary findings demonstrate that (2R,6R)-HNK may have similar effects to ketamine without the associated side effects. Reelin, a glycoprotein essential for proper cortical development, is an important mediator of synaptic plasticity and also parallels some of the fast-acting antidepressant-like behavioural and biological effects of ketamine, warranting further investigation.

Methods: Inducible pluripotent stem cells (iPSCs) were programmed from peripheral mononuclear blood cells collected from subjects with treatment-resistant depression ($n = 5$) and healthy controls (HCs) ($n = 2$) from both males and females. The STEMdiffTM embryoid body protocol was followed for 19 days to develop single-cell neural progenitor cells. Cells were supplemented and cultured for 10 weeks, then divided into 5 different conditions (vehicle + DMSO; 5nM, 10nM, and 50nM reelin; 1μM (2R,6R)-HNK) at 2 timepoints (1 hour; 24 hours) to assess short and long-term effects of reelin and (2R,6R)-HNK. Western blotting analyses were used to quantify mTOR, phosphorylated-mTOR (p-mTOR), post-synaptic density 95 (PSD95), GluA1, Synapsin 1 (Syn-I), Disabled-1 (Dab1), tyrosine kinase receptor B (TrkB), extracellular signal-regulated kinase (ERK), and p-ERK.

Results: Baseline differences of mTOR ($p < 0.05$), GluA1 ($p < 0.05$), TrkB ($p < 0.05$), p-ERK ($p < 0.01$), and ERK ($p < 0.001$) were observed between MDD cell lines and healthy controls. At 1 hr in cell lines from subjects with TRD, (2R,6R)-HNK and reelin significantly increased levels of PSD-95 (R50nM, $p < 0.05$; HNK1μM, $p < 0.01$), Syn-I (R10nM, $p < 0.05$; R50nM, $p < 0.01$; HNK1μM, $p < 0.01$), Dab1 (R10nM, $p < 0.05$; R50nM, $p < 0.01$; HNK1μM, $p < 0.01$), and p-ERK (R50nM, $p < 0.05$; HNK1μM, $p < 0.05$). With 24 hr application, these effects were reversed, demonstrating a significant down-regulation of expression of PSD-95 (R10nM, $p < 0.05$; R50nM, $p < 0.01$) and Syn-I (R10nM, $p < 0.05$; R50nM, $p < 0.01$; HNK1μM, $p < 0.01$). However, in HC, no significant differences were found in any treatment or time group.

Conclusions: High rates of TRD have necessitated the search for novel antidepressants that have lower long-term side effects, higher efficacy rates, and rapid therapeutic effects. This exploratory study shows both reelin and (2R,6R)-HNK hold promise in activating cellular pathways essential for fast-acting antidepressant responses. The differences between TRD and HC cell lines and their responsiveness towards treatment warrants further investigation. In addition, a limitation on the translatability of novel antidepressants has been due to the lack of in vitro or in vivo models that can encompass the heterogeneity of depression. While still preliminary, (iPSCs) could provide individualized options for evaluating treatment responsiveness. This study is a step forward in developing innovative antidepressant therapeutics and improving translation in pharmacological research using novel methodologies.

Keywords: Treatment Resistant Depression, (2R,6R)-hydroxynorketamine, Reelin, Induced Pluripotent Stem Cells (iPSCs)

Disclosure: Nothing to disclose.

P217. Cell-Type-Specific Dynamics of Histone 3.3 Lysine 27 Methylation Confers Susceptibility to Stress Across the Lifespan

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Background: A life history of stress is the strongest risk factor for several psychiatric disorders. Indeed, stress is known to "scar" the brain, leading to persistent transcriptional alterations in regions involved in reward and mood regulation, such as the nucleus accumbens (NAc). Here, we characterized the enduring changes in histone modifications in the NAc of mice exposed to stress, either in early life or in adulthood, and assessed their role in mediating life-long behavioral and transcriptional dysregulation.

Methods: We exposed female and male mice to early life stress (ELS), a paradigm known to heighten vulnerability to stress in adulthood, by combining maternal separation and reduced bedding during postnatal days (PND) 10 to PND 17. In parallel, an independent cohort of adult mice was subjected to chronic social defeat stress (CSDS), a validated model for the study of depression-like behaviors that separate mouse populations into susceptible (SUS) and resilient (RES) based on a social interaction test (SIT) which is predictive of numerous other behavioral

abnormalities. NAc tissue at PND21 and PND60 from mice exposed to ELS or from SUS and RES mice exposed to CSDS was processed for histone profiling via mass spectrometry. Finally, we mapped the genome-wide enrichment of the most changed histone modifications in both stress models using Cleavage Under Targets and Release Using Nuclease (CUT and RUN) followed by sequencing.

Results: Our results show that stress alters the methylation (me) dynamics of lysine (K) 27 of the histone variant H3.3—the predominant form of H3 present in adult brain neurons. We found a consistent increase in the abundance of H3.3K27me1, and decrease in the abundance of H3.3K27me2, in the NAc immediately after ELS exposure (PND21), effects that lasted into adulthood (PND60). Strikingly, we also observed that SUS mice to CSDS exhibit the same opposite pattern of H3.3K27me1/me2 abundance in the NAc. Genomic distribution shows that H3.3K27me1 is primarily enriched in gene bodies and proximal promoters, and correlates positively with gene expression, whereas H3.3K27me2 negatively correlates with gene expression and is weakly deposited across intergenic regions. Finally, we observed that changes in H3.3K27me1 and H3.3K27me2 are associated with elevated expression of SUZ-12, an important regulator of H3K27 methyltransferase activity. Indeed, cell-specific overexpression of SUZ-12 increases H3.3K27me1 expression in D1 medium spiny neurons and leads to stress-induced persistent social, behavioral, and cognitive alterations.

Conclusions: Together, these results suggest that H3.3K27me1 and H3.3K27me2 are important epigenetic "scars" that mediate stress susceptibility across the lifespan, and point at SUZ-12 as a novel epigenetic target for therapeutic interventions.

Keywords: Histone Variants, Chronic Stress, Psychiatric Disorders, Cognitive Function

Disclosure: Nothing to disclose.

P218. Psychedelics Show an Antidepressant Biosignature but a Sustained Response

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Background: Previous data suggest that the heterotrimeric G protein, Gsalpha (Gsα) is ensconced predominantly in lipid rafts in subjects with major depressive disorder (MDD), resulting in impaired stimulation of adenylyl cyclase. Both diminished Gsα-adenylyl cyclase coupling and an increase in the proportion of Gsα in lipid rafts have been observed in MDD. Antidepressants accumulate slowly in lipid rafts and evoke translocation of Gsα out of lipid rafts toward a more productive association with adenylyl cyclase, resulting in sustained cAMP elevation and sequelae such as increased brain-derived neurotrophic factor. This is also observed in cellular model systems and rodents. We hypothesize that heightened accumulation of Gsα in lipid rafts is a biomarker for depression, and that the translocation of Gsα from those rafts is a biomarker for clinical response to antidepressants. "Rapid-acting" antidepressant compounds, such as ketamine, have the same effect, but on an accelerated timescale. Some clinical data suggest that psychedelics, at hallucinogenic doses, evoke a long-lasting antidepressant response after a single dose

Methods: This study sought to compare the effects of monoamine-centric antidepressants, ketamine and psychedelics on cellular features of antidepressant response using cultured cells (C6 glioma, SK N SH neuroblastoma or astrocytes induced from fibroblasts derived from MDD or control subjects) exposed to fluoxetine, desipramine or ketamine (all 10 μM) or LSD (10 nM) or psilocin (psilocybin metabolite-50nM). cAMP was determined by Alpha Screen or fluorescent reporters.

Results: While the antidepressant biomarker activity (Gs translocation and augmented Gs activation of adenylyl cyclase) was seen with all compounds to which antidepressant activity has been attributed, there were distinct differences in the response profile for the compounds. Fluoxetine and desipramine required three-day exposure to translocate Gsα from rafts and increase Gsα-activated adenylyl cyclase. The effects of ketamine, while the most rapid in onset (15 minute exposure), declined over a 24-hour period, returning to pre-drug values after that time. However, both LSD and psilocin (1 hr exposure followed by washout) gave a prolonged antidepressant biosignature. All experiments were repeated 4-6 times in triplicate.

Conclusions: Antidepressants, psychedelics and ketamine all show an antidepressant biosignature, revealing a mobilization of Gsα from lipid rafts and an increased signaling of GPCRs working through Gsα. Previous studies in postmortem brain and in platelets showed that this biomarker was enriched in lipid rafts from depressed subjects and reverted toward control values after effective treatment (as indicated by HAM-D). The similarities and differences in the actions of these drugs at the cellular level may lead to a better molecular understanding of the distinct kinetic features of their actions.

Keywords: GPCR, cAMP Signalling, Ketamine, Depression, Psychedelics

Disclosure: Pax Neuroscience: Founder (Self)

P219. Sex Hormone-Driven X Chromosome Dynamics in the Adult Female Brain

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Background: Sex differences in brain physiology and disease result from the interplay of sex hormones and sex-chromosome-linked genes. From puberty to menopause, cyclical sex-hormone fluctuations represent a female-unique experience, which is associated with substantial brain plasticity and increased female risk for brain disorders such as anxiety and depression. One of the X chromosomes in females is typically inactivated by the process involving chromatin condensation, although there are genes that escape this process known as "X escapee genes". However, whether ovarian hormones can dynamically change 3D chromatin organization and gene expression of the X chromosome in the adult female brain is unknown and this knowledge is needed to understand female-specific brain dynamics and disease risk.

Methods: We examined 3D compartmental organization of the X chromosome in adult ventral hippocampal (vHIP) neurons across the estrous cycle and by sex using an unbiased chromatin conformation capture (Hi-C) method combined with candidate-loci DNA fluorescence in situ hybridization (FISH). Hi-C was performed in triplicates ($N = 6$ animals/group) on vHIP neurons isolated from 11 week-old male and female mice by fluorescence-activated nuclei sorting. We included females in two estrous cycle stages: proestrus (high estrogen-low progesterone) and diestrus (low estrogen-high progesterone), mimicking the human follicular and luteal phase, respectively. To address the effect of estrogen on the X chromosome 3D organization, we treated ovariectomized (OVX) mice with estradiol (5 μg, 4 hours) or vehicle and performed Hi-C assay on vHIP neurons (5 biological replicates or $N = 10$ animals/group). Compartmental analysis was performed by calculating the eigenvector on the Pearson correlation matrix in

25 kb bins. Motif analysis of differential compartmental regions was performed using meme-chip. The role of 3D genome organization in gene regulation was assessed by integrating Hi-C data with chromatin accessibility (ATAC-seq) and gene expression (RNA-seq) data (N = 6 animals/group).

Results: We show that ~16% of the X chromosome changes compartmental organization in vHIP neurons across the estrous cycle. With naturally rising estrogen levels in female mice, the X chromosome acquires an interaction profile more similar to males, and is likely to undergo a decondensation event and increase in volume during proestrus. We confirmed X chromosome compartmental dynamics across the cycle using FISH, as a complementary approach. We also found associated changes in the expression of neuronal function-relevant X-linked genes, describing for the first time estrous cycle-dependent X escapee genes. The estrous cycle-dependent compartments are enriched for estrogen response elements, implying the role of estrogen and its receptors in X chromosome dynamics across the cycle. This is further confirmed by estrogen replacement in OVX mice, which reproduces around 25% of the cycle-dependent compartmental changes in the X chromosome.

Conclusions: Overall, our data show unexpectedly high dynamics of the X chromosome in response to cycling sex hormones, with an indication that inactive X chromosome may undergo a decondensation event during the high-estrogenic phase of the ovarian cycle. Rhythmic "breathing" of the inactive X-chromosome could induce global changes in cellular environment affecting neuronal gene regulation and function, with critical implications for female physiology and disease risk. Our findings also open an intriguing possibility of hormonally-mediated X chromosome re-activation that may be of interest for the treatments of X-linked developmental disorders such as Rett syndrome.

Keywords: Sex Hormones, X chromosome, 3D Genome, Brain Plasticity, Epigenetic

Disclosure: Nothing to disclose.

P220. Interleukin-6 Decreases Dopamine Availability and Gene Pathways Related to Vesicular Transport and Release in Human Pluripotent Stem Cell Derived Dopaminergic Neurons

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Background: Chronic inflammation may impair dopamine (DA) neurotransmission in ventral striatum leading to motivational deficits including anhedonia, a core symptom of depression. For example, inflammatory cytokines such as interleukin (IL)-6 have been shown to decrease DA release in ventral striatum in laboratory animals and humans, and in the context of stress and inflammation, IL-6 has direct access to striatal DA'ergic neurons through disruption of the blood-brain-barrier.

Methods: To study the direct effects of IL-6 on DA neurons, in vitro studies were conducted on

DA neurons derived from human induced pluripotent stem cells (hiPSCs) of healthy female volunteers. Using standard protocols, hiPSCs were differentiated into neurons, ~80% of which expressed tyrosine hydroxylase or PITX3, indicative of DA neuron differentiation. Experiments (n = 3 or more per condition) were conducted in midbrain organoids or 2D cultures, and results between and among conditions were compared using Student's t-tests or one-way analysis of variance, respectively. Routine procedures were used for protein identification using immunohistochemistry as well as for RNA extraction and sequencing.

Results: iPSC-derived DA neurons exhibited positive staining for IL-6 receptors on the cell body and axonal projections. IL-6 treatment (5ng/ml) for 24 hours reduced DA availability in culture supernatants as measured by high performance liquid chromatography in the absence of neurotoxicity (as determined by caspase staining). Transcriptomic analyses of hiPSC-derived DA neurons treated with IL-6 (100 pg/ml) for 24 hours revealed significantly down-regulated gene pathways involving "vesicle-mediated transport", "synaptic vesicle exocytosis", "signal release from synapse", and "neurotransmitter secretion" (all -log(10)p > 5). These IL-6-induced molecular signatures were reversed by 24 hour co-treatment of cells with IL-6 (100 pg/ml) and baricitinib (200nM), an inhibitor of Janus Kinase (JAK) 1 and 2, which are pivotal signaling molecules in the response to IL-6. Finally, IL-6 treatment (100pg/ml) for 24 hours increased the relative expression of the short (versus long) form of the DA receptor 2 ($p < 0.001$), which is preferentially expressed on presynaptic DA neurons and inhibits DA vesicular release.

Conclusions: These data indicate that IL-6 has direct effects on DA'ergic neurons that can reduce DA vesicular transport and release as well as increase DAergic feedback inhibition. These effects were reversible by the JAK inhibitor baricitinib. Taken together, these findings support the use of iPSCs to reveal mechanistic insights into effects of inflammation on DA neurotransmission, while providing a platform for drug discovery.

Keywords: Dopamine, Neurons, Inflammation, IL-6, Induced Pluripotent Stem Cells (iPSCs)

Disclosure: Cerevel Therapeutics: Consultant (Self)

P221. Characterizing Disease-Associated Missense Mutations in the GABA Transporter, GAT1

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Background: Dysregulation of neural circuit excitatory/inhibitory balance may underlie multiple neuropsychiatric disorders. Genes involved in GABAergic neurotransmission, the main regulator of inhibition in the central nervous system, have been increasingly implicated in disease. SLC6A1 encodes the GABA transporter GAT1, one of two GABA transporters expressed in the brain. GAT1 primarily localizes to presynaptic terminals of GABAergic neurons, where it regulates signaling through the re-uptake of GABA from the synaptic cleft. Knockout mouse studies have demonstrated impairment of GAT1 leads to abnormal GABA receptor signaling, alterations to hippocampal oscillations, abnormal social behavior, impairments in learning and memory, as well as deficits in prepulse inhibition. To date, over 85 rare mutations in the coding sequence of GAT1 have been identified in patients and associated with a wide variety of disorders including epilepsy, neurodevelopmental delay (NDD), autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), schizophrenia (SCZ), and Tourette Syndrome (TS). Previous studies have investigated a select cohort of variants primarily associated with epilepsy, ASD, and/or NDD and found them to all be loss-of-functions mutations. Here, we set out to characterize an extensive list of missense mutations across the complete spectrum of disorders associated with GAT1.

Methods: The effects of 42 missense mutations in GAT1 on GABA transport were evaluated using a radiometric GABA uptake assay in transiently transfected HEK293T cells. From this characterization, three mutations (T46M, R211C, and G297R) were chosen for further evaluation. Subcellular localization and protein expression was measured using confocal imaging in transfected

MG-63 cells and western blot in transfected HEK293T cells. Transporter kinetics and affinity to two selective GAT1 antagonists (Tiagabine and NNC-711) were assessed by radiometric GABA uptake. CRISPR/Cas9 gene-editing was then used to introduce these mutations into human induced pluripotent stem cells (hiPSCs). Gene-edited hiPSCs were differentiated into cortical, GABAergic neurons and GABA transport was measured by the radiometric GABA uptake assay. Data were analyzed using a one-way ANOVA with a Dunnett correction for multiple comparisons and graphed as the mean + /- SEM. The significance level was set at $p < 0.05$.

Results: Radiouptake analysis in transiently transfected HEK293Ts demonstrated that 41/42 (97.6%) mutations significantly impacted GABA uptake ($n = 9$, 3 independent transfections, 3 replicates per transfection). Most variants (39/42, 92.9%) were characterized as loss-of-function, but two mutations (4.8%) were gain-of-function. To gain insight on how GAT1 function correlates with clinical phenotypes, we compared the level of GABA uptake for all mutations associated with a variety of disorders. Mutations associated with epilepsy, NDD, ASD, and ADHD showed on average severe loss-of-function (<25% of WT levels), whereas those associated with SCZ were only mildly impacted (71.5%). The unique mutation associated with TS showed a robust increase in function (293.7%).

Protein expression, membrane localization, and ER retention were not significantly different in the selected mutations (T46M, R211C, and G297R) as measured by confocal imaging ($n = 12$, 4 independent transfections, 3 replicates per transfection) and western blot ($n = 6$).

Mutation T46M showed increased GABA transport velocity, decreased GABA affinity, but no difference in affinity for GAT1 antagonists. R211C demonstrated decreased transport velocity, increased GABA affinity, and increased affinity for GAT1 antagonists. G297R had no active transport ($n = 9$, 3 independent experiments).

Preliminary experiments in hiPSC-derived GABAergic neurons show the neurons broadly recapitulate alterations to GABA transport found in transfected HEK293T cells ($n = 12$, 2 independent differentiations, 6 replicates per differentiation).

Conclusions: Using a radiometric GABA uptake assay, we functionally assessed 42 missense mutations in the GABA transporter, GAT1. Consistent with previous studies, most of the mutations were loss-of-function, yet two variants demonstrated enhanced GABA uptake. Our findings indicate bi-directional modulation of GABA uptake is relevant for neuropsychiatric disease. These changes to GABA transport could not be explained by protein expression or localization, but by transporter kinetics and affinity for GABA. Furthermore, we established hiPSC-derived GABAergic neurons can be used to model alterations to GABA transport in human neurons. Future experiments will focus on the role these mutations play on neuronal signaling using gene-edited, hiPSC-derived GABAergic neurons.

Keywords: SLC6A1/GAT1, GABA, hIPSCs, Disease Modeling

Disclosure: Johnson and Johnson: Employee (Self)

P222. A KRAB-Zinc Finger Transcription Factor Promotes Social Behaviors by Coordinating the Anti-Pathogen Immune Response in Prefrontal Cortex

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Background: Social behaviors are central to the health of society and the individual and are disrupted in a number of psychiatric illnesses. However, the neurobiological origins of complex social behaviors are incompletely understood. Earlier work employing co-expression analyses of RNA-sequencing (RNAseq) datasets of limbic brain areas from mice subjected to chronic social stress identified one member of the Kruppel-associated box (KRAB) zinc finger protein (KZFP) transcription factor (TF) gene family, Zfp189, as a key protective factor against stress-induced social deficits. Here, to more fully interrogate how ZFP189 may function in the PFC to govern social behaviors, we designed and synthesized novel synthetic ZFP189 TF variants each capable of exerting distinct forms of transcriptional control at in vivo ZFP189 target genes. These ZFP189 variants were virally delivered to mouse PFC and subsequent effect on pyramidal neuron dendritic spine morphology, social behaviors, and transcriptional response were characterized.

Methods: Three ZFP189 variants were synthesized: ZFP189-WT which is identical to the endogenously expressed ZFP189 and contains a transcriptionally repressive N-terminal KRAB domain and a C-terminal Cys2-His2 DNA-binding domain; ZFP189-VPR in which the endogenous KRAB domain is replaced with the robust transcriptional activator VP64-p65-Rta (VPR) yet retains the endogenous ZFP189 DNA-binding domain; and ZFP189-DN in which any transcriptional regulatory domain is entirely removed. Each variant was sub-cloned into identical (p1005) expression vectors and gene-targeted regulation was characterized in combination with a ZFP189 response element (RE) luciferase assay in Neuro2a cells ($n = 3$ in technical triplicate). Expression vectors were then packaged in herpes simplex virus (HSV) to enable brain-region targeted expression. Both male and female C57Bl/6J mice, aged 8-10 weeks, were utilized in these studies. All mice were used in protocols approved by the IACUC. HSV-ZFP189-WT, -ZFP189-VPR, or -ZFP189-DN were delivered by stereotaxic surgery bi-laterally to PFC (15°; +1.8 AP; ± 0.75 ML; -2.7 DV, 1uL). Three days after surgery dendritic spine morphology, social interaction, or RNAseq was performed. For dendritic spine morphology, mice were transcardially perfused with 4% paraformaldehyde, brains were sectioned coronally at 40um, and sections were imaged on a Zeiss 880 Airyscan confocal microscope. For social behaviors, a three-chamber social interaction test was performed involving a choice between an empty cage and novel mouse followed by a choice between a novel vs familiar mouse. Sample sizes are 10-15 mice per group and statistical analyses were performed with one-way ANOVAs, including a post-test comparing the test groups (HSV-ZFP189WT, -ZFP189VPR) versus the control group (HSV-ZFP189DN). Finally, we extracted RNA from virally infected PFC tissues, performed ribosomal RNA depletion, prepared libraries, performed RNAseq in the following configuration: 2x150 paired-end reads on an Illumina sequencing platform (HiSeq 2500) at a depth of >30M reads per sample ($n = 8$ mice for each condition). Differentially expressed genes (DEGs) were generated using DESeq2 in comparing either test group (HSV-ZFP189-WT, -ZFP189-VPR) to the control group (HSV-ZFP189-DN) with 5% FDR adjusted p-value < 0.05 and absolute log2 fold change > 1 DEGs called as significant.

Results: By co-transfecting the ZFP189 RE luciferase plasmid and iterations of our ZFP189 variants, we are able to observe, relative to GFP control: ZFP189-VPR induces robust targeted gene activation ($P < 0.0001$), ZFP189-WT induces gene-targeted repression ($P < 0.05$), and ZFP189-DN exerts no regulatory control in this assay ($P > 0.5$). In virally delivering each of these ZFP189 variants to mouse PFC, we observe that both HSV-ZFP189-VPR and HSV-ZFP189-WT significantly potentiate mature mushroom spine morphology on transduced pyramidal neurons ($P < 0.0001$). In three chamber social interaction testing, in contrast to all other groups, mice expressing ZFP189-VPR intra-PFC show no preference for social interaction with a novel mouse over investigating an empty cage, nor do they show a preference for social

interaction with the novel mouse over a familiar mouse ($P < 0.05$). By excising virally manipulated PFC and performing RNAseq, we identify significant DEGs regulated by either ZFP189-WT or ZFP189-VPR. Interestingly, in performing gene ontology analyses on these DEGs, we observe an opposing effect on the genes that comprise the anti-pathogen immune response, with particular opposite impact to the gene products involved in interferon gamma signaling.

Conclusions: By designing synthetic ZFP189 TFs capable of exerting opposing forms of transcriptional control in brain, we are able to identify that ZFP189-mediated transcriptional regulation in PFC governs social behaviors in rodents. By inverting the natural, repressive gene-regulation of ZFP189-WT to gene-activation with ZFP189-VPR, we observe that mice demonstrate pronounced social deficits with no other detectable behavioral abnormalities. In combining our transcriptional manipulations with RNAseq transcriptome profiling, we detect that ZFP189-VPR consequently down-regulates, and ZFP189-WT up-regulates, immune genes that comprise the anti-pathogen response, suggesting that the PFC function of ZFP189 has evolved to regulate cortical neuroplasticity as a means of connecting brain immune function to social behaviors.

Keywords: Adaptive Immunity, Social Behavior, Brain Transcription, Artificial Transcription Factors

Disclosure: Nothing to disclose.

P223. Patient-Derived Medium Spiny Neurons as a Model of Circadian Rhythms in Bipolar Disorder

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Background: Bipolar disorder (BD) is a serious mental illness characterized by recurrent mood episodes of depression and mania and disrupted circadian rhythms. BD patients show alterations in reward processing in the striatum, a brain structure in which the largest neuronal population is composed of GABA-producing medium spiny neurons (MSNs) that express D1 and D2 dopamine receptors. In animal models and humans, MSNs are critically important for directing motivated behavior and are the targets of antipsychotic drugs commonly used to treat BD. Moreover, genetic mutations to clock genes in animals alter dopamine signaling to MSNs. Existing human induced pluripotent stem cell (iPSC) models of BD have focused primarily on glutamatergic neurons.

Methods: We generated GABAergic MSNs from BD patient and healthy control-derived stem cells using a dual SMAD inhibition protocol. PCR was used to confirm the presence of MSN distinct markers. Expression of the clock genes BMAL1, CRY and PER was evaluated over a 24 h time course as a measure of cellular circadian rhythms. The cell population was characterized further using immunohistochemistry to quantify the percentage of neurons that express GABA versus glutamate.

Results: Our protocol yields cell populations highly enriched for GABAergic neurons that express a variety of MSN-specific markers, dopamine receptors, and clock genes.

Conclusions: BD patient-derived medium spiny neurons will provide a novel model to study BD and advance previous work examining circadian rhythm abnormalities in other cellular models.

Keywords: Bipolar Disorder, iPSC, GABA, Dopamine Receptor

Disclosure: Alkermes: Consultant (Self)

P224. Cytomegalovirus Antibodies are Associated With Increased Risk of Neuroinflammation, White Matter Damage, and Mental Illness: Evidence From Postmortem Samples

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Background: Herpesviruses such as cytomegalovirus (CMV) have attracted the psychiatric field's attention not only because they are neurotrophic and may cause neurological disease but because they can be reactivated by stress and inflammation. Importantly, lytic viral replication can in turn worsen inflammation, raising the possibility that poorly controlled CMV infections may contribute to the inflammation underlying the development of some cases of mental illness. We recently published some of the first work linking CMV infections to neuroimaging abnormalities in the context of major depressive disorder (MDD). Whether these findings result from a direct effect of viral damage to the brain or are from indirect inflammatory processes is unclear. Here, we investigated whether CMV infection increases the propensity of having mental illness, neuroinflammation, and brain cellular alterations using postmortem samples.

Methods: Postmortem brain data ($n = 364$) was obtained from the Stanley Medical Research Institute's brain collection ($n = 114$ with schizophrenia; $n = 78$ with bipolar; $n = 87$ with depression; $n = 85$ controls). IgG antibodies to CMV were measured using ELISAs. Neuroinflammation was determined on a recursive two-step cluster analysis using expression data for 8 inflammation-related genes in the dorsolateral prefrontal gyrus (DLPFC). Eighty-two cases ($n = 30$ with schizophrenia; $n = 23$ with bipolar disorder; $n = 29$ controls) were divided into "high" ($n = 30$) or "low" inflammation groups ($n = 52$). The density of astrocytes, oligodendrocytes, microglia, and neurons were quantified in white matter underlying DLPFC for 51 samples ($n = 19$ with schizophrenia; $n = 18$ with bipolar disorder; $n = 14$ controls).

Results: Relative to CMV negative (CMV-) samples, CMV positive (CMV+) samples were 2.12 times more likely to have mental illness (Odds ratio [OR] = 2.12, 95%CI = 1.20~3.80, $p = 0.01$) with controlling for age, sex, race, postmortem interval, and brain PH. Post-hoc analysis revealed that CMV infection did not significantly increase the odds of having schizophrenia. However, CMV seropositivity significantly increased the odds of having mood disorders (bipolar: OR = 2.49, 95%CI = 1.32~ 4.76, $p = 0.004$; depression: OR = 2.62, 95%CI: 1.41~ 4.95, $p = 0.002$). Furthermore, every 1 standard deviation increase of CMV antibody level was associated with 79% increase in odds of having neuroinflammation (OR = 1.79, 95%CI = 1.01~3.32, $p = 0.05$). Although not statistically significant, CMV + cases also had lower neuron density (Cohen's d = -0.51) and lower astrocyte density (Cohen's d = -0.42) in white matter.

Conclusions: Anti-viral IgG antibody levels are a surrogate marker of viral reactivation, with higher antibody levels generally indicative of an active infection. Together these results are therefore consistent with the possibility that the reactivation of CMV is a contributing factor to increased neuroinflammation, cellular deficits, and major affective disorders. Nevertheless, it is also possible that the arrow of causality is reversed and that reactivation of CMV could be due to cellular damage or increased cytokines. Experimental designs, potentially with anti-herpesvirus medications, are required to evaluate the potential pathophysiological effects of CMV.

Keywords: Postmortem Human Brain, Mental Illness, CMV, Neurons, Astrocytes, Neuroinflammation

Disclosure: Nothing to disclose.

P225. Using Computational Approaches and Exosomes to Integrate Biological Networks, Environmental Factors and Clinical Outcomes: Multidimensional Predictors of Antidepressant Response

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Background: There is a dearth of new mechanistic models for the development of objective tools to diagnose depression and monitor antidepressant responses. In rodent models, boosting mitochondrial metabolism of acetylcarnitine (LAC) leads to a rapid and persistent antidepressant response by epigenetic regulation of glutamatergic function in key brain circuits; we also reported decreased LAC levels in phenotypes of treatment resistant depression associated with childhood trauma.

Methods: Here, we used computational approaches and efficiently leveraged biological samples from previous randomized, placebo-controlled trials to determine the emergence of the pivotal mitochondrial metabolite LAC in relation to previously described individual predictors of antidepressant responses.

Results: Our newest data showed the lowest central levels of LAC as assessed by using exosomes enriched for L1CAM, a protein highly expressed in the brain, in subjects characterized by the highest severity of depressive symptoms. We also found the lowest peripheral LAC levels in those subjects with depression characterized by elevated BMI, high reported rates of emotional abuse and decreased leucocytes telomere length ($n = 47$). Furthermore, we used prediction profilers to show that decreased baseline LAC levels, elevated BMI and high reported rates of emotional abuse predict lack of antidepressant response to pioglitazone ($p = 0.02$, $r = 0.86$) with a stronger ability than each individual measure alone.

Conclusions: The current findings suggest that integrated factors spanning central and peripheral mitochondrial metabolism, cellular aging, metabolic function and childhood trauma contribute to define potential new mitochondrial phenotypes of depression. The further novelty of combining the exosome technology with computational approaches to study in-vivo molecular mechanisms of mitochondrial metabolism otherwise inaccessible in the human brain will aid in building individualized biological models of depression.

Keywords: Mitochondria, Epigenetics, Machine Learning

Disclosure: Nothing to disclose.

P226. Astrocyte- And Neuron-Specific CD44 Exon Variant Expression and Hyaluronan Levels in Postmortem Brains of Mood Disorders

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Background: Cell adhesion molecule CD44 and its ligand hyaluronan play critical roles in the central nervous system, including the regulation of glutamate neurotransmission and neuroplasticity. We previously reported that CD44 gene expression was associated in silico with SNP rs3829280, which was associated with anterior cingulate glutamate levels of major depressive disorder (MDD) and bipolar disorder (BD) patients. Since the relationship between CD44 and mood disorders has not been explored in human brain tissues, we conducted this study to validate the association between rs3829280 and CD44 gene expression and examine the associations of CD44 and hyaluronan in brain tissues of mood disorder patients.

Methods: All procedures were conducted according to the Declaration of Helsinki and approved by the Institutional Review Board of the University of Mississippi Medical Center, Jackson, MS, and the University Hospital Cleveland Medical Center, Cleveland, OH. Subjects' psychopathology was determined by a board-certified clinical psychologist and a board-certified psychiatrist. We selected gray matter tissues of the dorsolateral prefrontal cortex (DLPFC; BA9) and anterior temporal pole (TP; BA38) of subjects meeting DSM-IV criteria for MDD ($n = 10$) or BD ($n = 20$) and controls without a psychiatric diagnosis ($n = 10$) matched by sex and age. rs3829280 genotypes were determined by TaqMan genotyping assay, CD44 exon variant expression levels (astrocyte-specific "short" variant; neuron-specific v4, v5, and v10 variants) were ascertained by RT-qPCR, and hyaluronan was measured by immunoassay. We have previously performed genome-wide DNA methylomics analysis on the same brain tissue samples using the Illumina 850K Human MethylEPIC array. All statistical analyses were performed using SPSS v.25 and in a brain-region-specific manner. Exon variant expression levels and hyaluronan levels were Log10 transformed for analysis. To examine the association between SLC1A2 rs3829280 and CD44 exon variant expression, we compared CD44 exon variant expression levels between SLC1A2 rs3829280 genotypes by ANCOVA with age, sex, and subject group as covariates. To test the associations of CD44 exon variant expression and hyaluronan levels with mood disorders, we compared CD44 exon variant gene expression levels and hyaluronan levels between subject groups by ANCOVA with age and sex as covariates. The correlations between CD44 protein and hyaluronan levels were tested by partial correlation, controlling for age and sex, for each subject group. Comparisons that attained nominal statistical significance at unadjusted $p < 0.05$ were highlighted, but statistical significance was set at Bonferroni-corrected $p < 0.05$ to account for multiple comparisons. The Log10 values of the methylomic levels (β value) of 60 CpG site probes mapped to CD44 were compared between groups by ANCOVA with age and sex as covariates. Comparisons that attained nominal statistical significance at unadjusted $p < 0.05$ were highlighted, but statistical significance was set at Bonferroni-corrected $p < 0.05$ to account for multiple comparisons. CD44 CpG sites that showed between-group differences were entered into a linear regression model to test for their relationship with the gene expression levels of CD44 exon variants, controlled for age and sex.

Results: CD44 exon variant expressions in both DLPFC and TF were not different between rs3829280 A-allele carriers and non-carriers. In DLPFC, nominal statistical significance was observed in between-group comparisons of CD44s ($F2,40 = 4.185$; $p = 0.023$), CD44v4 ($F2,40 = 3.861$; $p = 0.031$), CD44v5 ($F2,40 = 3.937$, $p = 0.029$), and CD44v10 ($F2,40 = 4.923$; $p = 0.013$) expression levels, which was mainly due to lower levels in the BD patients compared to the controls (posthoc $p < 0.05$). However, comparisons did not survive multiple comparison correction (Bonferroni-corrected $p > 0.05$). In DLPFC, hyaluronan levels were marginally different between groups but without reaching the statistically significant difference ($F2,40 = 3.157$; $p = 0.055$) due to relatively higher levels in the BD group. In the DLPFC of the BD group, nominal negative correlations were found between hyaluronan levels and CD44 exon variant expressions: CD44s ($r = -.575$; $p = 0.013$), CD44v4 ($r = -.504$; $p = 0.033$), and CD44v5 ($r = -.535$; $p = 0.022$). Out of 60 CpG sites mapped to CD44, three CpG sites showed between-group differential methylation levels in the DLPFC at nominal level cg01766065 ($F2,40 = 3.378$; $p = 0.046$), cg23186333 ($F2,40 = 3.605$; $p = 0.038$), cg11478974 ($F2,40 = 3.493$; $p = 0.041$), and cg22768101 ($F2,40 = 5.100$; $p = 0.011$). Nevertheless, none of these findings remained significant after multiple testing correction.

Conclusions: Although we found no association between rs3829280 and CD44 exon variant expressions in human DLPFC and TP, our other findings suggest that astrocytic- and neuronal-related CD44 exon variants and hyaluronan in DLPFC may play a role in the neuropathophysiology of BD, but a larger sample size would be needed. Future studies may investigate how neuronal- and astrocytic-specific CD44 deficiencies and the balance between hyaluronan synthesis and degradation are related to BD symptoms and the mechanisms underlying treatment efficacy.

Keywords: CD44, Bipolar Disorder, Major Depressive Disorder, Postmortem Human Brain

Disclosure: Nothing to disclose.

P227. Lipidomic Signatures Informs Intravenous Racemic Ketamine Associated Remission in Treatment-Resistant Depression: A Pilot Study

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Background: Ketamine, a novel rapid-acting antidepressant, is increasingly being used for treatment-resistant depression (TRD), while its mechanism of action (MOA) is actively investigated. In a preliminary study leveraging an established pharmacometabolomic approach targeting acylcarnitines (ACs), we demonstrated rapid utilization of short-chain ACs within 40 minutes of ketamine treatment followed by restoration within 24 hours; this change in short-chain ACs with ketamine is consistent with our previous work identifying similar change but at 8-weeks with slower-acting SSRI treatment.

In this abstract, we report novel findings of change in triglycerides using a comprehensive metabolomic platform. We investigated an association between the change in baseline peripheral/blood metabolites after a 40-minute intravenous (IV) racemic ketamine infusion followed by recovery (change in metabolites in 24 hrs) with ketamine treatment response, in nine adults with treatment-refractory major depression.

Methods: This is an ongoing open-label, non-randomized, feasibility trial where 14 adults (18-65 years of age) with treatment-resistant major depression and moderately severe/severe symptoms of depression (PHQ-9 score ≥ 15), who provided written informed consent are enrolled. This study is approved by our Institutional Review Board. TRD is defined by the failure of at least two previous antidepressant treatments of at least 8 weeks duration within the index depressive episode. All patients received a single IV ketamine infusion (at the rate of 0.5 mg/kg body weight over 40 minutes) while in the MRI scanner, and the functional MRS (fMRS) sequence was completed. Patients were monitored for an additional 60 minutes after completing the fMRS scan and ketamine infusion. We are reporting data for nine subjects (2 subjects – data not yet analyzed, 3 subjects – data could not be used due to errors in the consent form). The fMRS data was available for five subjects.

Peripheral metabolites were measured at baseline, at the end of a 40-minute infusion, 60 min after the completion of infusion, and 24 hours post-infusion. A comprehensive non-targeted metabolomics platform, the Biocrates MxP® Quant 500 kit provided measurements of up to 630 metabolites from 26 biochemical classes including lipids (e.g., glycerophospholipids, sphingolipids, triglycerides [TGs]), and small molecules such as amino acids, biogenic amines, carboxylic acids, indoles, and bile acids.

Montgomery-Asberg Depression Rating Scale (MADRS) was used to measure depression symptoms. Early changes in the metabolites (baseline to end of infusion) and late changes (100 minutes to 24 hours post-infusion) were measured. Spearman correlation was conducted to test for a relationship between percent change in peripheral metabolites, quantified as a log2 change, with change in MADRS (baseline to 24 hours).

Results: Nine middle-aged (mean age 43 ± 16 years) subjects (3 males/6 females), had moderate depression (MADRS score 23 ± 5). Several metabolites including ceramides, TGs, sphingolipids, and cortisol showed significant changes within 40 min. The comprehensive metabolomic platform showed a significant decrease in 93 Triacylglycerols (TAGs), CE(16:0), CE(17:0), lysoPC a C28:0, PC aa C30:0, PC aa C32:3, PC aa C36:1, PC ae C34:3, PC ae C38:1, PC ae C40:1 within 40 min of ketamine infusion. A significant increase in sphingolipids/glycerophospholipids, TAGs, ceramides/ glycosylceramides, and cholesterol esters was observed within 24 hours (recovery phase). Several TGs, TG(14:0_36:4), TG(18:2_32:1), TG(20:2_34:3), TG(16:0_36:5), and ceramides correlated with change in MADRS. Early changes in blood TGs and other lipids were noted and TG change correlated with central GABA changes, thus suggesting a role of TGs and lipid metabolism in ketamine's rapid MOA.

Conclusions: This new data suggests rapid utilization of triglycerides with ketamine treatment followed by recovery within 24 hours, highlighting a possible role of lipid metabolism in ketamine's MOA. Lipid peroxidation plays a vital role in CNS homeostasis and a lipid-based pathway is possibly involved in depression pathology, activated by stress and inflammation. The rapid reduction of TAG levels in plasma likely is a consequence of energy deficiency after ketamine shock since fatty acid synthesis and lipoprotein assembly is an energy-consuming process, leading to the reduction of fatty acid biosynthesis and lipoprotein assembly/release. This can be tested by measurement of apolipoprotein and cholesterol/cholesterol-ester levels. TAG accumulation in the recovery phase represents the compensatory effects of energy mobilization and storage. Changes in metabolites that correlate with response to ketamine at 24 hours provide novel insights into the mechanism of ketamine's action. These changes when linked to brain imaging data inform how effects peripherally, such as in liver and many other organs, impact brain metabolic state and affect mood and other functions. These preliminary findings need to be investigated further to elicit the role of triglycerides and ketamine response.

Keywords: IV- Ketamine, Metabolomics Signature, Treatment Resistant Depression, Open Label Trials

Disclosure: Nothing to disclose.

P228. Efficacy of Lumateperone in Pooled Short-Term Late-Phase Clinical Trials for the Treatment of Major Depressive Episodes Associated With Bipolar II Disorder

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Background: Bipolar II disorder is associated with significant functional impairment, mood instability, and long periods of potentially disabling symptoms of depression. In December 2021, lumateperone was the second antipsychotic FDA-approved as monotherapy and first approved as adjunctive therapy to treat depressive episodes associated with bipolar II disorder.

Lumateperone is also FDA-approved to treat schizophrenia and depressive episodes associated with bipolar I disorder as monotherapy and as adjunctive therapy with lithium or valproate. Lumateperone is mechanistically novel, simultaneously modulating serotonin, dopamine, and glutamate neurotransmission. A

robust late phase clinical trial program established the safety and efficacy of lumateperone 42 mg in patients with a major depressive episode (MDE) associated with bipolar I or bipolar II disorder. This analysis evaluated the efficacy of lumateperone 42 mg across pooled short-term studies in patients with depressive episodes associated with bipolar II disorder.

Methods: All trials enrolled men and women (18-75 years) with a clinical diagnosis of bipolar I or II disorder who were experiencing an MDE (Montgomery-Åsberg Depression Rating Scale [MADRS] Total score ≥20 and Clinical Global Impression Scale-Bipolar Version-Severity [CGI-BP-S] score ≥4 at screening and baseline). Patients in each trial were stratified by their diagnosis of bipolar I or bipolar II disorder. Lumateperone 42 mg was administered once daily in the evening.

This post hoc analysis pooled efficacy data for the intent-to-treat (ITT) population and subgroup of patients with bipolar II disorder from 3 short-term, 6-week, placebo-controlled studies. Study 401 (NCT02600494) and Study 404 (NCT03249376) investigated lumateperone 42-mg monotherapy and Study 402 (NCT02600507) assessed lumateperone 42 mg adjunctive to lithium or valproate.

Assessments included change from baseline in MADRS Total score, CGI-BP-S Total score, CGI-BP-S subscores (Depression, Overall Bipolar Illness, and Mania), and Quality of Life Enjoyment and Satisfaction Questionnaire-Short Form (Q-LES-Q-SF) percent score.

Results: The pooled ITT population comprised 1,067 patients with bipolar I or bipolar II disorder (placebo, 539; lumateperone 42 mg, 528). Lumateperone 42 mg significantly improved symptoms of depression in the ITT population compared with placebo, as measured by the change from baseline to Day 43 in MADRS Total score (least squares mean difference vs placebo [LSMD], − 2.5; 95% confidence interval [CI] − 3.8, −1.2; P < .001). In the 174 patients (16.3%) with bipolar II disorder (placebo, 87; lumateperone 42 mg, 87) lumateperone also significantly improved MADRS Total score from baseline to Day 43 compared with placebo (LSMD, − 4.0; 95% CI − 7.2, −0.7; P < .05), with significant improvements beginning at Day 22 (P < .05). In patients with bipolar II disorder lumateperone significantly improved illness severity at Day 43 compared with placebo as measured by CGI-BP-S Total score (LSMD, − 1.0; 95% CI − 1.8, −0.2; P < .05), CGI-BP-S Depression subscore (LSMD, − 0.5; 95% CI − 0.9, −0.1; P < .05), and CGI-BP-S Overall Bipolar Illness subscore (LSMD, − 0.5; 95% CI − 0.9, −0.1; P < .05); change from baseline to Day 43 in CGI-BP-S Mania subscore was minimal with lumateperone treatment compared with placebo (LSMD, − 0.0; 95% CI − 0.2, 0.1; P = .61). Lumateperone 42 mg also significantly improved quality of life in the bipolar II disorder subgroup, as measured by Q-LES-Q-SF percent score compared with placebo at Day 43 (LSMD, 6.3; 95% CI 1.1, 11.4; P < .05).

Conclusions: In patients with bipolar II disorder experiencing an MDE, short-term treatment with lumateperone 42 mg as monotherapy or as adjunctive therapy significantly improved symptoms of depression, disease severity, and quality of life.

Keywords: Lumateperone, Bipolar II Disorder, Bipolar Depression

Disclosures: Intra-Cellular Therapies, Inc.: Employee, Stock/Equity (Self)

P229. ANC-501, A Novel V1B Receptor Antagonist: Association of MDD Response With MADRS Subscales

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Background: Depression is ranked as a leading cause of disability worldwide by the World Health Organization. Major depressive disorder (MDD) is a psychiatric disorder that is characterized by the occurrence of one or more major depressive episodes, along with an absence of any history of manic, mixed, or hypomanic episodes. In addition to the high mortality rate due to suicide, depressed patients are more likely to develop coronary artery disease and type 2 diabetes among many other related health conditions.

Stress mediated by the hypothalamus-pituitary-adrenal (HPA) axis has been hypothesized to be a pivotal factor in the pathophysiology of depression. Specifically, both corticotropin-releasing factor (CRF) and arginine vasopressin (AVP), both of which are produced in the paraventricular nucleus of the hypothalamus are considered primary factors in the regulation of HPA axis activity. Receptor subtypes for these neuropeptides, which may be involved in the regulation of HPA axis activity, have attracted much attention as potential targets for the treatment of depression and anxiety. ANC-501 (formerly TS-121) is an investigational new drug with antagonistic activity of the vasopressin receptor 1b (V1b receptor), which plays a role in the modulation of stress and mood. Based on nonclinical and early clinical studies, ANC-501 appears to be a promising candidate for clinical development with a novel mode of action that may benefit MDD patients.

Methods: The initial phase 2 study of ANC-501 was a randomized, double-blind, placebo-controlled phase 2 study, enrolled 51 MDD patients (43 of whom completed the study) randomly assigned to either ANC-501 10 mg, 50 mg or placebo for 6 weeks treatment period with a 2-week follow-up period. The primary endpoint was change from baseline on the Montgomery-Åsberg Depression Rating Scale (MADRS) score at week 6. A key secondary was the change in baseline at the end of the follow-up period. Repeated Measures analysis (MMRM)/non-parametric methods were used to compare the difference between the three treatment groups on the primary endpoint (change from baseline in MADRS score). To further characterize these patients, we performed an exploratory post-hoc analysis of individual baseline MADRS item scores in the high cortisol patients treated with ANC-501 to determine which symptom(s) may be associated with subsequent response at the end of the treatment or follow-up period. Association reported as Pearson's r uncorrected for multiplicity.

Results: The changes from baseline in MADRS score at week 6 (Least Square Mean [95% Confidence interval] were: ANC 501 10 mg (-9.0 [-13.9, -4.1]), ANC-501 50 mg (-9.0 [-13.4, -4.5]), and placebo (-6.4 [-10.7, -2.2]). ANC-501 groups showed greater numerical reductions in MADRS score change from baseline compared to placebo, though these reductions did not achieve statistical significance. However, higher baseline urinary and hair cortisol levels were associated with a greater separation between TS-121 groups and the placebo group in the primary endpoint.

In MDD patients with elevated urinary cortisol, baseline MADRS items 4-reduced sleep (r (9) = .61, p = .045) and 9-pessimistic thoughts (r (9) =.60, p = .049) were each correlated with the change from baseline in total MADRS score at the end of 6 weeks of treatment. Interestingly, items 1-apparent sadness (r(9)=.71, p = .013), 3-inner tension (r(9)=.73, p = .011), 5-reduced appetite (r(9)=.72, p = .014), 7-lassitude (r(9)=.71, p = .014), 8-inability to feel (r(9)=0.66, p = .03), and item 9-pessimistic thoughts (r(9)=.76, p = .005) were each strongly correlated with the subsequently change from baseline in total MADRS score at the end of 8 weeks (6 weeks of treatment and 2 weeks off-treatment follow-up).

Conclusions: In this trial, post-hoc analysis of the MDD patients with elevated cortisol and who responded to V1b antagonism were characterized by a specific sub-group of baseline symptoms broadly consistent with Melancholic depression, a subtype of depression previously shown to have HPA axis disruption. While this exploratory analysis is limited by a small sample size, these data, if replicated in subsequent studies, may characterize a group

of MDD patients whose symptoms are uniquely responsive to V1b antagonism, supporting a personalized, biomarker-driven approach to the treatment of MDD. A phase 2 study of ANC-501 in MDD patients with elevated cortisol is ongoing.

Keywords: Depression, Vasopressin 1b Antagonist, Arginine Vasopressin, Adjunctive Treatment, Precision Psychiatry

Disclosure: Ancora Bio: Employee (Self)

P230. Tractography Analysis of a Clinical Trial of Subcallosal Cingulate Deep Brain Stimulation for Depression

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Background: Open-label studies of subcallosal cingulate deep brain stimulation (SCC DBS) for treatment-resistant depression have demonstrated sustained response and remission rates that are clinically significant. Validation of a patient-specific tractography-based targeting approach has further streamlined lead implantation surgery with improved clinical outcomes. This is in contrast to the halted multi-site, double-blind, randomized sham-controlled clinical trial (BROADEN), where long-term response rates matched open-label results (50% responders at 2 years) but where the predefined 6-month endpoint fell short of projections. Heterogeneity in patient selection, as well as variability in targeting, accuracy of lead placement, and programming are potential contributors. As subjects in BROADEN were implanted using anatomical rather than tractographic methods, we hypothesize that response variability may be explained by differences in white matter activation pathways (WMAP) impacted by trial-and-error adjustments during the open-label follow-up period. We test this hypothesis here using a retrospective normative tractography analysis.

Methods: Fifty-five patients (of 90 enrolled) completed 2 years of open-label SCC DBS. WMAP was calculated for 47 patients (with adequate imaging data) using Lead DBS (https://www.lead-dbs.org). All 47 patients were assigned to one of four outcome groups: remitter (HDRS-17 ≤ 7, $n = 16$), responder (HDRS-17 improvement $\geq$40%, $n = 8$), low-responders (HDRS-17 improvement $\leq$40%, $n = 13$), and non-responders (no HDRS-17 improvement at any time over 2 years, $n = 10$). Standard imaging processing pipelines were used for generating common WM activation pathways. The anatomical location of active contacts was first compared among four groups. Then, common activation pathways were calculated using the HCP dMRI dataset, producing whole-brain common group activation pathways by Lead Group Analysis.

Results: Group tracts explained differences in outcomes. Remitters show complete WMAP of three bundles: forceps minor (FM), uncinate fasciculus (UF), and cingulum bundle (CB). Responders show similar CB and UF to remitters, but incomplete FM activation. Critically, non-responders showed no activation and low-responders showed incomplete activation of the left CB. There were no anatomical location differences in active stimulation contacts among the groups.

Conclusions: These findings provide evidence that poor outcomes in BROADEN were due to suboptimal lead implantation that could not be appreciated by anatomical location alone. As with independent experimental studies, patient-specific tractography-based targeting may be necessary to standardize and optimize SCC DBS.

Keywords: DBS, Diffusion Tensor Imaging (DTI), Treatment-Resistant Depression

Disclosure: Janssen Pharmaceuticals: Advisory Board (Self)

P231. Effects of Acute Estradiol or Progesterone Administration on Perimenstrual Exacerbation of Suicidal Ideation, Depression, and Perceived Stress: Preliminary Analysis of a Three-Period Crossover Randomized Controlled Trial

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Background: Suicide appears routinely among the top five causes of death worldwide among females of reproductive age. Perimenstrual (around menses) withdrawal from the ovarian steroids estradiol (E2) and progesterone (P4) correlates with suicidal ideation (SI) and behavior and therefore may represent a time-varying trigger for acute suicide risk. Our prior crossover placebo controlled RCT provided the first experimental evidence that administration of transdermal E2 (.1mg/day) and oral micronized P4 (200mg/day) in the perimenstrual weeks (days 7-21 following LH surge) reduces exacerbation of depressed mood, perceived stress, and suicidal ideation in female outpatients with SI. These beneficial effects were strongest around the onset of menses, suggesting a pathophysiologic role of perimenstrual E2 or P4 withdrawal in cyclical symptom exacerbation. The next step is to clarify whether E2 or P4 alone may be responsible for these effects.

Methods: The present three-period, double-blind, crossover randomized controlled trial (R00MH109667; NCT03498313) extended this work by testing the unique effects of perimenstrual E2 or P4 administration using the same doses/routes in the same timeframe. Inclusion criteria included endorsement of past-month suicidal ideation; seeing a mental health professional at least every 3 months; aged 18-45; no history of serious or chronic nonpsychiatric illness; no family or personal history of recurrent blood clots or known thrombophilia; regular menstrual cycles (25-35 days); not pregnant or breastfeeding and at least one year postpartum; not using hormonal medications or devices; BMI 18-34.99 kg/m2; not regularly using nicotine; no history of hospitalization for manic episode or psychosis; and a current substance use disorder that would interfere with the study. Medication use was allowed. After a baseline month, participants completed three subsequent experimental intervals, separated by two washout cycles, with each interval spanning the two weeks surrounding expected menses onset (with medication taken from days +7 to +21 following a positive 40mIU/ml urine luteinizing hormone test). Daily surveys evaluated depressed mood, perceived stress, and suicidal ideation (1=Minimal or Absent, 6=Extreme), and calls evaluated imminent suicide risk; no suicide attempts occurred. We hypothesized that experimental E2 and P4 would both reduce perimenstrual exacerbation of symptoms, such that the symptom trajectory in the PBO condition would be characterized by increases in symptoms from the early luteal phase (when EP levels are rising/moderate) to the perimenstrual phase, when dual EP withdrawal is most pronounced, and that these changes would be prevented in both the E2 and P4 conditions.

Results: Forty-five participants were randomized; 25 completed all three conditions, 34 completed two conditions, and 42 completed one condition. 34 participants with two conditions were included in the per-protocol sample for the present analyses. Power analyses indicated that the smallest detectible effect size (Given: person $N = 34$, daily $N = 2{,}234$; effective/corrected $N = 67$; $ICC = .50$, $\alpha = .05$, $1\text{-}\beta = .80$) was $f2 = .09$. Even though a clear perimenstrual exacerbation pattern was observed for all outcomes (including SI) in the pre-experimental baseline cycles, there was a mean perimenstrual decrease in SI across all experimental conditions, suggesting a marked placebo effect on SI that was

not present in the prior trial. Relative to placebo, E2 administration reduced perimenstrual increases in depression (Est = -.41, SE = .17, p = .01; Effect size note: response scale range=5) and perceived stress (Est = -.33, SE = .15 p = .03), although a corresponding greater reduction in suicidal ideation severity did not reach statistical significance (Est = -.15, SE = .10, p = .17). In contrast, relative to placebo, P4 administration did not significantly alter perimenstrual changes in depression or perceived stress (p's > .30), and showed less robust perimenstrual reductions (i.e., less robust placebo effect) in suicidal ideation severity (Est = .26, SE = .12, p = .03).

Conclusions: These preliminary analyses suggest that the beneficial effects of dual E2 and P4 administration observed in the first trial may have been due to a benefit of E2 specifically. These may represent critical mediators of acute perimenstrual suicide risk. Though preliminary, results combine to suggest a potential pathophysiologic role of E2 withdrawal in perimenstrual exacerbation of depression, perceived stress, and suicidal ideation. Forthcoming intent-to-treat analyses will provide information about the potential usefulness of perimenstrual E2 as a clinical intervention. Further, additional per-protocol analyses of these trial data are warranted to determine the robustness of our findings to the inclusion of covariates such as daily medication changes, the COVID-19 pandemic, and physical pain. Larger clinical trials with pre-selection of patients for perimenstrual exacerbation, as well as appropriate attention to placebo mitigation methods, are warranted to determine the underlying causes of steroid withdrawal sensitivity—and particularly E2 withdrawal sensitivity—across the menstrual cycle.

Keywords: Estradiol, Progesterone, Reproductive Affective Disorder, Suicide, Depression

Disclosure: Nothing to disclose.

P232. Metabolic Profile of Lumateperone in Late-Phase Clinical Trials for the Treatment of Major Depressive Episodes Associated With Bipolar I or Bipolar II Disorder

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Background: Atypical antipsychotics are correlated with a range of metabolic adverse effects including weight gain and increased risk of diabetes, hyperglycemia, and hyperlipidemia. Identifying treatment options with a favorable metabolic profile is important to improve patient outcomes and to reduce patient morbidity and mortality.

Lumateperone is an FDA-approved antipsychotic to treat schizophrenia and depressive episodes associated with bipolar I or bipolar II disorder as monotherapy and as adjunctive therapy with lithium or valproate. Lumateperone is mechanistically novel, simultaneously modulating serotonin, dopamine, and glutamate neurotransmission. A robust late phase clinical trial program established the safety and efficacy of lumateperone 42 mg in patients with a major depressive episode (MDE) associated with bipolar I or bipolar II disorder. This analysis evaluated the metabolic impact of lumateperone 42 mg across short- and long-term studies in patients with bipolar depression.

Methods: All trials enrolled men and women (18-75 years) with a clinical diagnosis of bipolar I or II disorder who were experiencing an MDE (Montgomery-Åsberg Depression Rating Scale [MADRS] Total score ≥20 and Clinical Global Impression Scale-Bipolar Version-Severity [CGI-BP-S] score ≥4 at screening and baseline). Lumateperone 42 mg was administered once daily in the evening.

Safety data were reported for 3 trial groups: (1) data pooled from 2 short-term, 6-week, placebo-controlled studies of lumateperone 42-mg monotherapy (Study 401 [NCT02600494]; Study 404 [NCT03249376]); (2) a 6-month open-label extension period (OLE) of Study 401 that evaluated long-term effects of lumateperone 42-mg monotherapy; (3) a Phase 3 placebo-controlled study (Study 402, NCT02600507) that investigated lumateperone 42-mg therapy adjunctive with lithium or valproate in patients with bipolar depression.

Metabolic assessments included changes in weight, body mass index (BMI), waist circumference, and cardiometabolic laboratory parameters. Potentially clinically significant (PCS) weight change defined as ≥7% change from baseline was assessed. Subgroup analyses were conducted in patients who were classified by baseline BMI as underweight (<18.5 kg/m2), normal weight (≥18.5 to <25), overweight (≥25 to <30), or obese (≥30).

Results: The short-term safety population comprised 746 patients in pooled monotherapy trials (placebo, 374; lumateperone 42 mg, 372) and 352 patients in the adjunctive study (adjunctive placebo, 175; adjunctive lumateperone 42 mg, 177). In the short-term studies, mean changes from baseline to end of treatment with lumateperone were similar to placebo for weight (kg; placebo, 0.19; lumateperone, 0.06; adjunctive placebo, 0.23; adjunctive lumateperone, 0.00), BMI (kg/m2; placebo, 0.07; lumateperone, 0.02; adjunctive placebo, 0.09; adjunctive lumateperone, 0.00), and waist circumference (cm; placebo, 0.03; lumateperone, 0.18; adjunctive placebo, 0.11; adjunctive lumateperone, 0.06). No patients treated with lumateperone had PCS weight gain during treatment. PCS weight loss was rare in all groups (<1%); PCS weight loss with lumateperone monotherapy or adjunctive therapy was only observed in those who were overweight (2 patients) or obese (2 patients) at baseline. Mean change from baseline to the end of treatment with lumateperone was similar to placebo for total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, glucose, or insulin in the monotherapy and adjunctive therapy trials.

The long-term OLE safety population comprised 127 patients. There was no mean increase from baseline to the end of treatment in weight (−0.01 kg), BMI (− 0.01 kg/m2), or waist circumference (−1.46 cm) with lumateperone 42 mg. At any time during the 175-day treatment period, 3.4% of patients (2 normal BMI, 2 obese patients at baseline) experienced PCS weight increase and 6.0% had PCS weight loss (1 normal weight, 3 overweight, 3 obese patients at baseline). There were no clinically significant changes from baseline to end of treatment in total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, or insulin.

Conclusions: In patients with bipolar I or bipolar II disorder experiencing an MDE, lumateperone 42-mg monotherapy or adjunctive therapy had a favorable metabolic profile for both acute and long-term treatment.

Keywords: Lumateperone, Bipolar Disorder, Metabolic Profile

Disclosure: Intra-Cellular Therapies, Inc.: Employee, Stock/Equity (Self)

P233. Personalized Connectivity-Based Accelerated TMS Therapy for Bipolar Depression: Preliminary Outcomes

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Background: Bipolar disorder (BD) is prevalent, severe and under studied, and is often excluded from clinical trials. The depressive

phase of Bipolar disorder I or II accounts for the majority of time a patient is ill –72% in BD-I and 81% in BD-II. Many patients fail to respond adequately or cannot tolerate pharmacotherapy due to adverse side effects. Thus, accessibility to alternative treatment options is important to help achieve and sustain remission. One treatment option, transcranial magnetic stimulation, (TMS) has demonstrated efficacy in treatment resistant major depression, however there have been relatively few studies in Bipolar Disorder, and none to our knowledge utilizing intensive high dose spaced theta-burst rTMS (HDS-TBS). Thus, the objective of this clinical trial is to determine the clinical and functional connectivity effects of intensive TMS in depressed patients with Bipolar Disorder I/II.

Methods: To date, a total of 10 patients have been screened using the Structured interview for DSM-5, the Montgomery-Asberg Depression Rating Scale (MADRS), the Young Mania Rating Scale (YMRS), and screened for dementia (MMSE > 24). Following informed consent, participants were randomized to active or sham treatment using HDS-TBS. Inclusion criteria: Bipolar depression, DSM 5 criteria; Age 18-70; Right or left-handed; All genders; Treatment resistant depression: 2+ failed antidepressant trials (< 50% reduction in symptoms) using ATHF criteria; Not in a mixed bipolar episode (YMRS ≤12) ; Not rapid cycling (> 4 episodes in the past year); Stable medication regimen, including mood stabilizer; MADRS ≥ 20.

Treatment sessions: iTBS was delivered to individually targeted left dorsolateral prefrontal cortex (DLPFC) site at 90% resting motor threshold (RMT). The stimulation pattern and total number of pulses: triplet 50 Hz bursts, repeated at 200 ms (5 Hz) intervals, 2 s on, 8 s off; 1800 pulses per session; total duration 10 min. Ten iTBS sessions/day x 5 sequential days. Neuroimaging sessions were conducted prior to iTBS treatment ('pre'), and 24 hours following day 5 ('post').

Sham iTBS: Both active and sham iTBS used a Magventure Cool B65 A/P coil. The sham treatment blocks the magnetic field with an internal spacer, allowing the appropriate coil surface (active vs. sham) to be placed against the scalp. During sham iTBS, brief electric pulses were delivered to the scalp simultaneous to the TMS pulse to mimic the scalp sensation.

Neuroimaging procedures: MRI data was acquired on a 3 Tesla Siemens Prisma scanner using a 64-channel head coil (Erlangen, Germany). Our acquisitions were optimized for Siemens Prisma scanners, rigorously tested as part of the human connectome project (HCP) and consisted of T1, T2 and rs-fMRI (2x 8 min) scans. During the visits, patients were co-registered to the T1 scan using fiducial points at the Nasion, Tragi and nose tip. TMS pulses were delivered to the target at the optimal orientation, and the accuracy of this targeted stimulation was monitored and tracked by the Brainsight software.

Neuronavigation and Targeting: DLPFC TMS targets were individually optimized based on anticorrelated functional connectivity with the subgenual anterior cingulate cortex (SGACC), methods detailed in Cash et al (2021). Coil orientation was defined individually such that the e-field at the cortical target was maximized (Balderston et al). Prior to the iTBS visits, target coordinates and orientation vectors were generated from the fMRI data and the e-field models and loaded into the Brainsight neuronavigation system along with the subject's reconstructed T1 image. Scalp and cortical surfaces were generated from the T1. During the visits, the subject was co-registered to the T1 using fiducial points and the accuracy of targeted stimulation was monitored and tracked by the Brainsight software.

Results: 7 participants meeting inclusion and exclusion criteria were included. Participants had a history of a mean of 5 failed treatment trials with antidepressants, (range 3-8) and a mean of 1 augmentation trial, as well as treatment with mood stabilizers. MADRS scores decreased from baseline (mean 30.8 + /-5.5, range 23-50) to post-treatment (mean 15 + /- 10.4, range 0-26). All participants tolerated the therapy without adverse events. Functional connectivity between SGACC and individually targeted DLPFC stimulation sites decreased (more anti-correlated) from baseline mean z = -0.02 + /- 0.24 to post-treatment mean z = -0.07 + /- 0.19. The decrease in MADRS scores was correlated with decrease in correlation (increased anticorrelation) of SGACC-individually targeted DLPFC pre- to post-treatment calculated as r = 0.79.

Conclusions: We remain blinded to treatment status in this small sample of treatment refractory depressed bipolar patients but the study indicates the potential for being able to discriminate treatment effects. Some patients experienced a large decrease in depressive symptoms while others did not. Overall, the connectivity of SGACC to individually targeted DLPFC decreased (became more anti-correlated) and the decreases in MADRS pre- to post-treatment positively correlated with the changes in functional connectivity, suggesting a possible mechanism for treatment responses. The treatment was well tolerated with no adverse events. We will continue to recruit participants to this study and expect to have a larger sample to report.

Keywords: Bipolar Depression, TMS, Connectivity, Subgenual Anterior Cingulate Cortex, DLPFC

Disclosure: Nothing to disclose.

P234. An Open Label Pilot of The Safety and Efficacy of Comp360 Psilocybin in Patients With Severe Treatment-Resistant Depression

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Background: Psychedelics are rapidly gaining ground as an intervention of interest for severe mood and anxiety disorders. The focus for most of this research is for patients who have failed four or fewer adequate interventions. Few trials of any intervention have looked at patients with depression who have failed more than four treatments in the current episode. This is a pilot study looking at the open label administration of 25 mg of COMP360 (synthetic psilocybin) in twelve patients with documented failures of at least five treatments in the current major depression episode.

Methods: Over 100 patients were screened to identify 12 patients ((n = 12 (6 men and 6 women)) with age a mean of 40.5 + /- SD 9.2 years) with documented major depressive disorder with failures of at least five treatments in the current episode. Failures could include FDA approved medications, neurostimulation and up to one failed trial of psychotherapy. Potential participants with a history of psychosis or of concurrent substance use disorders were excluded. Subjects with active suicidal ideation were excluded. Participants with a history of post-traumatic stress disorder (PTSD) were included provided the primary diagnosis was major depressive disorder and not PTSD. Participants were dosed with a single 25 mg dose of a proprietary synthetic psilocybin (COMP360) after withdrawal from antidepressants and most psychotropic medication (benzodiazepine related hypnotics were allowed) (subjects were drug free for at least two weeks prior to dosing). All subjects had three sessions of psychological preparation followed by a nine-hour dosing session, and then at least three psychological integration sessions. Subjects were followed for 12 weeks post dose. Subjects were monitored throughout the study by regular visits with the treating psychiatrist as well as a therapist. The primary outcome measure

was the MADRS at three weeks post dose. Response was defined as a MADRS score reduction of ≥50% since baseline and remission as a score of ≤10. Subjects were also followed by CSSRS scores to monitor for safety. This trial is registered at clinicaltrials.gov under NCT04433858.

Results: There were no unexpected adverse events. For participants with co-morbid PTSD histories, the dosing sessions were often quite emotional though no interventions with medication or hospitalization were necessary. Nor overall was there evidence of psychological difficulties with the dosing sessions. At one week post dose 9 of 12 (75%) participants met both response and remission criteria. By the primary outcome measure at week 3 eight of 12 (67%) met response and 5 of 12 (41.7%) met remission criteria. At week 12, 7of 12 (58%) were responders and 3 of 12 (25%) were still remitters. There was no evidence of an increase in suicidal ideation or adverse events post dosing. Curiously, the five participants determined to be co-morbid for PTSD at baseline were the five participants who did not meet response criteria at week 12. Those participants were also more likely to report minimal psychedelic or mystical impact or effect during the dosing sessions.

Conclusions: Psilocybin may have efficacy across the spectrum of depression treatment resistance. Presence of PTSD may identify participants less likely to achieve durable response from this single dose strategy. These results are supportive of continued investigation into the use of psychedelic therapy in even the most treatment resistant patients who have not benefitted from even the most aggressive treatment interventions.

Keywords: Depression, Treatment Resistant Depression, Psychedelic Medicine, Psilocybin

Disclosures: Compass Pathways: Grant (Self), Zylorion: Advisory Board (Self), Merck: Contracted Research, Consultant (Self), Sunovion, Servier: Honoraria (Self), Impel, Intracellular Therapies: Consultant (Self)

P235. Improvements in Cognitive and Physical Functioning Outcomes in Depressed Patients Treated With AXS-05 (Dextromethorphan-Bupropion): Results From the EVOLVE Open-Label, Long-Term Study

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Background: Cognitive dysfunction and fatigue are common in major depressive disorder (MDD). Data from STAR*D suggest that sad mood, concentration difficulty, and fatigue are the symptoms of MDD that contribute the most to overall impairment. Treatments that rapidly improve mood and related symptoms may result in improved clinical and functional outcomes.

AXS-05 (dextromethorphan-bupropion) is a novel, oral, investigational, NMDA receptor antagonist with multimodal activity being developed for MDD. The dextromethorphan component of AXS-05 is an antagonist of the NMDA receptor, an ionotropic glutamate receptor, and a sigma-1 receptor agonist. The bupropion component of AXS-05 serves primarily to increase the bioavailability of dextromethorphan, and is a norepinephrine and dopamine reuptake inhibitor.

Here we report the impact of AXS-05 on cognitive and physical functioning outcomes in MDD, as assessed by the Massachusetts General Hospital Cognitive and Physical Functioning Questionnaire (CPFQ). The CPFQ is a 7-item patient-rated scale used to measure cognitive and executive dysfunction in mood and anxiety disorders and includes assessments pertaining to cognition, energy, wakefulness, and motivation.

Methods: EVOLVE (Evaluation of NMDA Modulation for Depressive Episodes) was an open-label, US trial, in which patients were treated with AXS-05 (dextromethorphan HBr 45 mg-bupropion HCl 105 mg) twice daily for up to 15 months. Eligible patients had either rolled in following completion of a prior AXS-05 study or were directly enrolled and had a DSM-5 diagnosis of MDD, a MADRS score of ≥25, and had been treated with at least 1 prior antidepressant in the current major depressive episode. A total of 186 patients were enrolled, consisting of 35 roll-over and 146 directly enrolled. Efficacy endpoints included MADRS, CPFQ, and the Sheehan Disability Scale (SDS). The primary efficacy analysis was the change from baseline to Week 6 (primary timepoint) and Weeks 1 and 2 (key secondary timepoints). Here we present the results for the directly enrolled patients.

Results: Treatment with AXS-05 resulted in meaningful improvement in the CPFQ. Mean baseline CPFQ scores were 28.4. Mean reductions from baseline to Weeks 1, 2, and 6 were 2.0 ± 4.28 ($p < 0.001$), 4.4 ± 5.61 ($p < 0.001$), and 7.5 ± 6.58 ($p < 0.001$), respectively. Improvements on the CPFQ were durable through Month 6 (-9.5 ± 6.48; $p < 0.001$) and Month 12 (-8.5 ± 6.90; $p < 0.001$). A ≥ 1 category improvement in CPFQ was achieved by 40.0%, 58.8%, and 75.8% of patients at Weeks 1, 2, and 6, respectively. At Months 6 and 12, 82.1% and 77.1% of patients had achieved ≥ 1 category improvement in CPFQ.

Rapid, durable, and statistically significant improvements were also seen in the MADRS total score and the SDS, starting at Week 1 and every subsequent timepoint measured.

Long-term treatment with AXS-05 was well tolerated. The most commonly reported adverse events were COVID-19 infection (8.9%), nausea (8.9%), headache (7.5%), dry mouth (6.2%), insomnia (5.5%) and dizziness (5.5%).

Conclusions: Treatment with AXS-05 resulted in rapid and sustained improvement in the CPFQ, depression symptoms, and functioning in patients who failed one prior antidepressant in the current major depressive episode. These data provide additional evidence of the long-term efficacy and safety of AXS-05 in MDD.

This research was supported by Axsome Therapeutics.

Keywords: Major Depressive Disorder (MDD), NMDA Receptor Antagonist, Cognition, Rapid-acting Antidepressant

Disclosure: Axsome Therapeutics: Stock / Equity (Self)

P236. An Open Label Study of COMP360 (Synthetic Psilocybin) in Bipolar Type II Depression

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Background: Treatment options are limited for patients with bipolar depression. With accelerating interest in the use of psychedelics in difficult to treat mood and anxiety disorders and early evidence of efficacy along with safety, the question is raised as to whether some patients with cyclical mood disorders may also benefit from psychedelic therapy. The single biggest concern is whether use of psilocybin could precipitate a manic or hypomanic episode in a vulnerable population and second is whether there is evidence for efficacy. For this pilot study, we chose patients with a history of bipolar type II depression and followed a similar protocol of treatment used in earlier studies in unipolar depression.

Methods: Thirteen adults (5 Males/8 Females) age: (avg 36.1 SD 11.5) with bipolar type II depression who had failed at least two medications in the current episode, without rapid cycling by history, and no history of mania or psychoses were recruited to be dosed with a single 25 mg dose of a proprietary synthetic

psilocybin (COMP360) after withdrawal from psychotropic medication (subjects were drug free for at least two weeks prior to dosing). Subjects with active suicidal ideation were excluded. All subjects had three sessions of psychological preparation followed by a nine-hour dosing session, and then at least three psychological integration sessions. Subjects were followed for 12 weeks post dose. Subjects were monitored throughout the study by regular visits with the treating psychiatrist as well as therapist. The primary outcome measure was the MADRS at three weeks post dose. Response was defined as a MADRS score reduction of ≥50%since baseline and remission as a score of ≤10. Subjects were also followed by CSSRS and YMRS scores to monitor for safety. This trial is registered at clinical trials.gov under NCT04433845.

Results: There were no unexpected adverse events or evidence of psychological difficulties with the dosing sessions. No subjects developed hypomanic or mood instability following the dosing session. At one week after dosing 10 of 13 (76.9%) met remission criteria and 11 of 13 (84.6%) met response criteria. At three weeks, our primary outcome measure, 10 of 13 (76.9%) met both remission and response criteria. At 12 weeks 10 of 12 (83.3%)met both remission and response criteria. One subject was never a responder, though 8 subjects were in remission for all 12 weeks and 2 subjects were in remission from week 2 to week 12. One subject initially remitted but relapsed into depression at week 6, and one responded, relapsed into depression at week 3, resumed antidepressant medication at week 6 which discontinued them from the study as a non-responder at week 6. No subject showed evidence of mania or hypomania by YMRS score at any visit after dosing, and no significant disruption to sleep was reported. No subject reported an increase in suicidal ideation after dosing. One subject was dropped from the study prior to dosing due to worsening bipolar symptoms during withdrawal from mood stabilizing drugs and was not included in this analysis. Most subjects stayed off medication for 12 weeks post dose with three resuming mood stabilizing medication after the three week primary outcome measure for prophylaxis and not due to any symptomatic depression worsening.

Conclusions: In this small, open-label pilot study, most subjects reported significant improvement in chronic depressive symptoms with durability lasting for the three months they were followed post dosing. The relative improvement was notably greater in treatment-resistant depression patients with bipolar II disorder versus patients with major depression. Further exploration into the efficacy of psychedelic therapy within this poorly served psychiatrically ill population is warranted.

Keywords: Psychedelics, Psilocybin Therapy, Bipolar Type II Depression, Clinical Trial

Disclosure: Compass Pathways: Grant (Self), LivaNova, Neuronetics, Genomind, Janssen, Compass Pathways, Sage Therapeutics: Consultant (Self)

P237. Clinical Response to High-Dose Eicosapentaenoic Acid (EPA) Supplementation is Greater in MDD Patients Who Achieve Higher Plasma Concentrations of EPA-Derived Pro-Resolving Lipid Mediators

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Background: Chronic inflammation has been implicated in the pathophysiology of major depressive disorder (MDD). Chronic inflammation is characterized by an impaired resolution of inflammation. We hypothesize that activation of the resolution of inflammation through ω-3 fatty acid supplementation may be a successful therapeutic strategy for the treatment of MDD.

Methods: MDD patients with body mass index >25 kg/m2 and plasma high-sensitivity C-reactive protein ≥3 μg/mL (n = 61) were enrolled in a 12-week randomized trial consisting of 4 parallel arms: eicosapentaenoic acid (EPA) 1 g/d, 2 g/d, and 4 g/d, and placebo. The supplement contained EPA and docosahexaenoic acid (DHA) in a 3.9:1 ratio. Depression symptoms were assessed using the clinician-rated IDS-C30 scale. Plasma fatty acids and pro-resolving lipid mediators (SPMs) were measured in 42 study completers at baseline and at the end of treatment by liquid chromatography/mass spectrometry.

Results: The response rate (defined as ≥50% reduction in IDS-C30 score) was higher in the 4 g/d EPA arm than in the placebo and EPA 1 g/d and 2 g/d arms (64%, 40%, 38% and 36%, respectively). In the 4 g/d EPA arm, responders had significantly greater increases in 18-hydroxyeicosapentaenoic acid (18-HEPE), a direct precursor of the SPM resolvin E1-3, than non-responders. In addition, at week 12, resolvin E2 and E3 were detectable in plasma in a greater number of responders than non-responders. Within the 4 g/d EPA arm, the increase in 18-HEPE was significantly associated with reductions in plasma hs-CRP concentrations and IDS-C30 scores.

Conclusions: Response rates were higher among MDD patients randomized to EPA 4 g/d supplementation and in those who showed a greater ability to activate the synthesis of 18-HEPE and its SPMs. The inverse association of 18-HEPE with both systemic inflammation and symptoms of depression highlights the activation of the resolution of inflammation as a likely mechanism in the treatment of MDD with ω-3 fatty acid supplementation.

Keywords: EPA, Major Depressive Disorder (MDD), Inflammation

Disclosure: Nothing to disclose.

P238. Evaluation of Mania and Hypomania in Late-Phase Clinical Trials of Lumateperone in the Treatment of Major Depressive Episodes Associated With Bipolar I or Bipolar II Disorder

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Background: Although atypical antipsychotics are efficacious in treating bipolar disorder, some have been associated with case reports of switching to mania/hypomania. Thus, identifying antipsychotic treatment options with a low risk for inducing mania/hypomania is important to optimize patient outcomes.

Lumateperone is an FDA-approved antipsychotic to treat schizophrenia and depressive episodes associated with bipolar I or bipolar II disorder as monotherapy and as adjunctive therapy with lithium or valproate. Lumateperone is mechanistically novel, simultaneously modulating serotonin, dopamine, and glutamate neurotransmission. A robust late phase clinical trial program established the safety and efficacy of lumateperone 42 mg in patients with a major depressive episode (MDE) associated with bipolar I or bipolar II disorder. This analysis evaluated measures of mania and hypomania across short- and long-term studies of lumateperone in patients with bipolar depression.

Methods: All trials enrolled men and women (18-75 years) with a clinical diagnosis of bipolar I or II disorder who were experiencing an MDE (Montgomery-Åsberg Depression Rating

Scale [MADRS] Total score ≥20 and Clinical Global Impression Scale-Bipolar Version-Severity [CGI-BP-S] score ≥4 at screening and baseline). Lumateperone 42 mg was administered once daily in the evening.

Mania and hypomania data were reported for 3 trial groups: (1) data pooled from 2 short-term, 6-week, placebo-controlled studies of lumateperone 42-mg monotherapy (Study 401 [NCT02600494]; Study 404 [NCT03249376]); (2) a 6-month open-label extension period (OLE) of Study 401 that evaluated long-term effects of lumateperone 42-mg monotherapy; (3) a Phase 3 placebo-controlled study (Study 402, NCT02600507) that investigated lumateperone 42-mg therapy adjunctive with lithium or valproate in patients with bipolar depression.

Assessments included the incidence and severity of treatment-emergent adverse events (TEAEs) of mania/hypomania in the safety population (all patients who received ≥1 dose of study drug). Young Mania Rating Scale (YMRS) Total score and CGI-BP-S Mania subscore were measured in the intent-to-treat (ITT) population (all randomized patients who received ≥1 dose of study drug and had a valid baseline and ≥1 valid post-baseline MADRS assessment) in placebo-controlled studies. Change from baseline was analyzed using a mixed-effects model for repeated measures in the placebo-controlled studies and using a last observation carried forward approach for the OLE.

Results: The short-term safety population comprised 746 patients in pooled monotherapy trials (placebo, 374; lumateperone 42 mg, 372) and 352 patients in the adjunctive study (adjunctive placebo, 175; adjunctive lumateperone 42 mg, 177). TEAEs of mania/hypomania were reported in 11 patients (placebo, 5 [1.3%]; lumateperone, 6 [1.6%]) in the pooled monotherapy groups and in 2 patients (adjunctive placebo, 1 [0.6%]; adjunctive lumateperone, 1 [0.6%]) who received adjunctive treatment. Mania/hypomania TEAEs were mild or moderate in severity. There was 1 serious TEAE of mania reported in the lumateperone 42 mg monotherapy group and no serious TEAEs of hypomania. The ITT population comprised 719 patients in pooled monotherapy trials (placebo, 365; lumateperone 42 mg, 354) and 348 patients in the adjunctive study (adjunctive placebo, 174; adjunctive lumateperone 42 mg, 174). Mean change in YMRS Total score from baseline to Day 43 was similar with lumateperone and placebo in monotherapy trials (least squares mean difference vs placebo [LSMD], − 0.5; 95% confidence interval [CI] − 1.0, 0.1; P = .10)) and the adjunctive study (LSMD, − 0.2; 95% CI − 0.8, 0.4; P = .56)). Change from baseline to Day 43 in CGI-BP-S Mania subscore with lumateperone was also similar to placebo for monotherapy trials (LSMD, − 0.0; 95% CI − 0.1, 0.1; P = 0.77) and the adjunctive study (LSMD, 0.0; 95% CI − 0.1, 0.1; P = .83).

The long-term OLE safety population comprised 127 patients treated with lumateperone 42 mg. One patient (0.8%) had a TEAE of mild mania which was not serious. No patients had TEAEs of hypomania. There was not a significant mean change from baseline to end of treatment in YMRS score with lumateperone treatment (−0.5; 95% CI − 1.7, 0.6; P = .37). Lumateperone 42 mg significantly improved CGI-BP-S Mania subscore from baseline to end of treatment (mean change from baseline: 0.2; 95% CI 0.0, 0.4; P < 0.05).

Conclusions: In patients with bipolar I or bipolar II disorder experiencing an MDE, lumateperone 42-mg monotherapy or adjunctive therapy was associated with low rates of treatment-emergent mania and hypomania that were similar to those of placebo-treated patients for both acute and long-term treatment.

Keywords: Lumateperone, Bipolar Disorder, Mania, Hypomania

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P239. Sham Response in Transcranial Magnetic Stimulation Depression Trials is Increasing Over Time

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Background: Psychophysiological and clinical studies with transcranial magnetic stimulation (TMS) require a valid control stimulation condition to achieve blinding of experimental conditions, hence allowing for the differentiation between active and placebo effects. A common approach is to use a sham intervention or active stimulation of a control site. The ideal sham condition should reproduce the ancillary aspects of TMS without producing significant direct brain stimulation. Thus, the ideal sham TMS system should be identical in appearance to the active system. In addition, the auditory (coil clicking) and somatic (coil vibration and scalp nerve and muscle activation) sensations should match between active and sham modes. Various coil manipulations and dedicated sham systems have been proposed, including coil tilt techniques, reverse current coils, spacers, field-blocking shields, synchronous electrical stimulation, and multi-coil configurations. These sham strategies differ in their ability to mask TMS operators and subjects. Our objective for this study was twofold, 1) to characterize and compare the physical aspects of different sham strategies, including electrical stimulation, and 2) to evaluate clinical research usage of the various strategies and factors influencing sham response in clinical TMS depression trials.

Methods: To assess the sham TMS effects across clinical trials, studies were aggregated from Mutz, Edgcumbe, Brunoni, and Fu (2018) and updated with an independent search of PubMed up to the year 2021. Inclusion criteria for the review included the following: randomized, sham-controlled trial, primary diagnosis of major depressive disorder, and patients 12¬–70 years old. Two independent raters extracted the data. We calculated the sham and active response as percent change in the primary clinical outcome from baseline. We performed a univariate regression analysis in R. The regression model for the sham response included the following covariates: year of publication, mean sample age, percentage females, baseline depression severity, active percent change in depression score, sham strategy, blinding assessment, number of sessions, targeting method, stimulation site, stimulation protocol, and device manufacturer. We also ran an exploratory linear model for the active response, with the following covariates: year of publication, mean sample age, percentage females, baseline depression severity, active percent change in depression score, coil type, blinding assessment, number of sessions, targeting method, stimulation site, stimulation protocol, and device manufacturer.

Results: We compiled 79 randomized, controlled TMS trials in patients with major depressive disorder, with total n = 1647 patients in the sham control arms and total n = 2128 patients in the active arms. Mean sample size was 21 patients in the sham groups and 19 patients in the active groups. The average age of the sham samples was 46.2 years and the mean percentage of the sample that was female was 56%. For the active samples, the average age was 46.4 years and 58% percent were female. Of the sham strategies used, the 90-degree coil tilt and dedicated sham coil were the most common (35% and 31%, respectively) followed by the 45-degree tilt method (27%). Synchronous electrical stimulation in addition to a dedicated sham coil was only used in 3 out of the 79 trials.

For predictors of sham TMS response, year of publication ($F(1, 76) = 6.18$, $p = 0.015$), sham strategy ($F(6, 71) = 1.806$, $p = 0.11$), number of sessions ($F(1, 76) = 5.45$, $p = 0.022$), and percent

change in depression score in the active arm ($F(1, 71) = 17.22$, $p < 0.001$) significantly influenced the sham percent change in depressions score. Post hoc comparison showed that the 45-degree coil tilt method produced the highest sham response, possibly due to the undesired residual electric field induced in the brain from this technique. For predictors of active TMS response, device manufacturer ($F(5, 96) = 3.895$, $p = 0.003$), targeting method ($F(4, 97) = 3.817$, $p = 0.006$), and sham percent change ($F(1, 72) = 15.84$, $p < 0.001$) are associated with change in depression score in the active arms.

Conclusions: As seen with medications, there is a trend of rising sham response in TMS depression trials over the past 25 years. This time effect is not significant with active TMS. Therefore, the therapeutic effect size of TMS, that is, the difference between active TMS and placebo response, may be shrinking over time, requiring larger sample sizes in clinical trials to detect clinical benefits. It also argues for developing novel forms of TMS with fewer ancillary effects, e.g. controllable pulse TMS and quiet-TMS, which may be easier to blind with sham interventions that may have a lower placebo response.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Depression, Placebo Response, Meta-Analysis

Disclosure: Nothing to disclose.

P240. Predicting Depression Outcomes Through the Influence of Therapeutic Alliance and the Psychedelic Experience Using Path Modelling in a Phase IIb Randomized Controlled Trial of COMP360 Psilocybin Therapy

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Background: A recent phase IIb clinical trial of COMP360 (COMPASS Pathways' proprietary synthetic psilocybin formulation) psilocybin therapy demonstrated efficacy in treatment-resistant depression (TRD), with significant improvements in depressive symptom severity following a single 25mg dose. Evidence suggests that therapeutic alliance and subjective psychedelic experience during COMP360 psilocybin administration are potentially important elements of the treatment (1, 2). Understanding how these components relate to COMP360 psilocybin therapy response is important in order to optimize the treatment paradigm, clarify underlying mechanisms, and help identify patients most likely to benefit.

A previous study in participants with major depressive disorder treated with either COMP360 or escitalopram with psychological support, found that depressive symptom severity reduction was significantly predicted by acute psychedelic effects, measured by the Emotional Breakthrough Inventory (EBI) and Mystical Experience Questionnaire (MEQ) scores (1). Here we aim to compare these findings with data from our larger trial of COMP360 psilocybin therapy.

Methods: This was a phase IIb, international, multicenter, randomized, fixed-dose, parallel group, double-blind trial investigating the safety and efficacy of a single dose of COMP360 psilocybin 25mg or 10mg compared to a 1mg dose in participants with TRD (COMP 001). COMP360 was administered alongside psychological support from trained therapists, delivered pre-, during and post-treatment. The primary efficacy endpoint was change from baseline in Montgomery–Åsberg Depression Rating Scale (MADRS) total score at Week 3. Subjective psychedelic experience was measured using the Five-Dimensional Altered States of Consciousness rating scale (5D-ASC), assessed at the end of the administration day, and EBI total scores, assessed the day following COMP360 psilocybin administration. Therapeutic alliance, measured by the Scale to Assess the Therapeutic Relationship Patient Version (STAR-P), was hypothesized to predict depressive symptom severity at Week 3 (absolute MADRS total score), through its interaction with the psychedelic experience (5D-ASC and EBI).

Path analysis, a type of multiple regression evaluating relationships between variables to assess causality, was conducted on data from the 25mg dose group – the optimal therapeutic dose. Saturated paths were modelled, with each path including three variables: STAR-P total score, either a dimension score from the 5D-ASC or EBI total score, and MADRS total score at Week 3 as the outcome. Due to expected multicollinearity between the psychedelic experience variables, separate paths were fitted for EBI total score and for each of the five dimensions of the 5D-ASC. The path analyses used maximum likelihood estimation as the estimation method.

Results: $N = 233$ participants (mean age 39.8 years; $n = 121$ female; 94% psilocybin-naïve) were randomized to COMP360 25 mg ($n = 79$), 10 mg ($n = 75$), or 1 mg ($n = 79$). The week 3 MADRS total score was significantly predicted by: EBI total score (β [standardized effect]=-0.57, R2[variance explained]=0.35, $p < 0.0001$), and the following 5D-ASC dimensions: visual restructuralization ($\beta = -0.54$, $R2 = 0.30$, $p < 0.0001$), oceanic boundlessness ($\beta = -0.53$, $R2 = 0.29$, $p < 0.0001$), and auditory alterations ($\beta = -0.24$, $R2 = 0.09$, $p = 0.027$). These variables all had a positive effect on the MADRS total score at Week 3 corresponding to a reduction of the total score. The absolute standardized effects and variance explained were largest for EBI total score, indicating it as the most reliable predictor of MADRS total scores at Week 3. The indirect effect of STAR-P on depression outcome was not significant for any path.

Conclusions: Path analyses conducted on data from our phase IIb trial found that EBI total scores, and scores from three dimensions of the 5D-ASC were significant predictors of depressive symptom severity at Week 3 following COMP360 psilocybin therapy. The indirect effect of therapeutic alliance was not significant for any of the psychedelic experience paths. The outcome of this path analysis, using data from a larger, more robust TRD sample than in Murphy et al. (1), confirms previous reports: that subjective psychedelic effects were significant predictors of depressive symptom severity; if, as is very likely, intensity of psychedelic experience reflects drug level, it also echoes the dose related response seen in primary analysis of the COMP 001 trial. In contrast, indirect effects of therapeutic alliance here were non-significant. We cannot rule out the possibility that this may be due to differences in the therapy model, but effects in smaller studies are often not confirmed in larger samples. Further research is warranted to understand how we can use these findings to enhance the COMP360 psilocybin therapy treatment paradigm, and identify not only those who may be likely to benefit from this treatment, but also those who may not, and thus what can be improved to facilitate greater benefit.

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Keywords: Psilocybin Therapy, Major Depressive Disorder, Randomised Controlled Trial, Subjective Effects, Therapeutic Alliance

Disclosure: COMPASS Pathways Ltd: Employee (Self)

P241. Dose-Dependent Acute Subjective Psychedelic Effects Following COMP360 Psilocybin Across Three Clinical Studies and its Relationship to Therapeutic Response

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Background: Dose-dependent subjective psychedelic effects have been reported following psilocybin administration (1) and a positron emission tomography study found correlations between 5HT2a receptor occupancy, subjective psychedelic experience, and plasma levels of psilocin (the active metabolite of psilocybin) (2). In developing COMP360 (COMPASS Pathways' proprietary synthetic psilocybin formulation) therapy for treatment-resistant depression (TRD), a phase IIb study (COMP 001) demonstrated significant efficacy for a 25mg dose as compared to 10mg dose. This dose-response relationship is an important finding in support of a central pharmacological effect of psilocybin, independent of expectation, in reducing depressive symptoms.

Here we describe the Five-Dimensional Altered States of Consciousness (5D-ASC) questionnaire, assessing subjective psychedelic experience, and the Emotional Breakthrough Inventory (EBI), assessing emotional experience, and their relationships to changes in depressive symptom severity for the three doses employed in COMP001 and for comparison, the results in studies COMP 002 and 003. COMP 002 investigated the subjective effects of 25mg, 10mg, and placebo in healthy volunteers and COMP 003 investigated the effects of 25mg in TRD participants who continued to take a selective serotonin reuptake inhibitor (SSRI). Since serotonergic drugs downregulate 5HT2A receptor number and function in rodents (3), it was hypothesized that the action of psilocybin would also be attenuated in these participants.

Methods: In all studies a single dose of COMP360 was given with psychological support from trained therapists pre-, during, and post-study drug administration. Participants completed the 5D-ASC (94 questions organized into five dimensions: oceanic boundlessness, anxious ego dissolution, visual restructuralization, auditory alterations, and reduction of vigilance) at the end of the administration day. In the TRD studies, the EBI was completed one day post-COMP360 administration and the Montgomery-Åsberg Depression Rating Scale (MADRS) was completed at multiple timepoints with the primary efficacy endpoint as the change from baseline in MADRS total score at Week 3.

Results: In COMP 001, 233 participants (n = 121 female) were randomized to COMP360 25mg (n = 79), 10mg (n = 75), or 1mg (n = 79). In COMP 002, 89 participants (n = 41 female) were randomized to COMP360 25mg (n = 30), 10mg (n = 30), or placebo (n = 29). In COMP 003, 19 participants (n = 13 female) received open-label COMP360 25mg adjunct to a SSRI. Clear dose-dependent subjective psychedelic effects were observed in COMP 001 and COMP 002. At the same doses, 5D-ASC dimension scores for COMP360 25mg were generally similar across all three studies. In COMP 003, dimension scores indicated a psychedelic experience did occur, with an intensity between that observed for COMP360 25mg and 10mg in COMP 001.

In COMP 001, moderate correlations were observed between the change from Baseline in MADRS total score at Week 3 and scores on three dimensions of the 5D-ASC; for COMP360 25mg, 10mg and 1mg, respectively; oceanic boundlessness (Pearson correlation coefficient; -0.508, -0.485, -0.477), visual restructuralization (-0.516, -0.431, -0.410), and auditory alterations (-0.293, -0.224, -0.358). Moderate correlations were also seen between the change from Baseline in MADRS total score at Week 3 and EBI total score; for COMP360 25mg, 10mg and 1mg, respectively (-0.614, -0.363, -0.424).

Conclusions: Improvement in depression symptoms correlated with three dimensions of the of the 5D-ASC and the EBI. This would be expected if a given dose produces a range of psilocin levels, receptor effects and subjective experience. It supports the core pharmacological action that underlies the clinical effect of psilocybin. COMP360 adjunct to an SSRI did not appear to block either the psychedelic effect of psilocybin or its clinical benefit.

In COMP 001, the 10mg arm produced a psychedelic experience but it was broadly ineffective in reducing depressive symptom severity, as compared to the 25mg dose. This suggests that simply knowing one has received an active dose (unblinding) and having a psychedelic experience is insufficient in improving symptoms, and thus points towards the validity of a pharmacological mechanism of action.

These dose-dependent results from the 5D-ASC, and the correlations observed between changes in depressive symptom severity and 5D-ASC dimension and EBI total scores, warrant additional research to further understand the role that subjective psychedelic effects play in achieving therapeutic response.

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Keywords: Psilocybin Therapy, Major Depressive Disorder, Subjective Effects, Randomised Controlled Trial

Disclosure: COMPASS Pathways Ltd., MindMed, Relmada, Perception Neuroscience, Atai, Tripp, Neurolief, Brainsway: Contracted Research (Self)

P242. Improvement in Depressive Symptoms With Zuranolone Treatment in Patients With Major Depressive Disorder With or Without Elevated Anxiety: Pooled Analysis From the MDD-201B, MOUNTAIN, and WATERFALL Studies

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Background: Patients having major depressive disorder (MDD) with elevated anxiety often have more severe depression and are less responsive to standard-of-care antidepressants than those without elevated anxiety. Zuranolone, a positive allosteric modulator of both synaptic and extrasynaptic GABA(A) receptors and a neuroactive steroid, is thought to upregulate GABA(A) receptor expression and enhance inhibitory GABAergic signaling. Zuranolone is hypothesized to rapidly restore network balance in brain areas dysregulated in depression. Zuranolone is investigational and in clinical development as an oral, once-daily, 14-day treatment course for adults with MDD. Clinical trials have demonstrated that treatment with zuranolone (30 or 50 mg) vs placebo leads to improvements in depressive symptoms as measured by change from baseline (CFB) in the 17-item Hamilton Rating Scale for Depression total score (HAMD-17). This pooled

analysis of 3 completed studies of zuranolone evaluated the effect of zuranolone on depressive symptoms in patients having MDD with or without elevated anxiety at baseline.

Methods: MDD-201B (NCT03000530; zuranolone 30 mg), MOUNTAIN (NCT03672175; zuranolone 20 or 30 mg [20 mg Cohort not included here]), and WATERFALL (NCT04442490; zuranolone 50 mg) were randomized, double-blind, placebo-controlled trials. MDD with elevated anxiety was defined as having a Hamilton Anxiety Rating Scale (HAM-A) total score ≥20 at baseline. The primary outcome (CFB in HAMD-17 at Day 15) and secondary outcome (CFB in HAM-A at Day 15) in individual studies were analyzed in a pooled analysis using the Mixed Model for Repeated Measures. Only patients with non-missing HAM-A scores at baseline were included. Analyses were not adjusted for multiplicity and p values reported here are nominal. Safety and tolerability were evaluated by incidence of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs).

Results: Of the 1007 patients included in this analysis, 508 (50.4%) met the criterion for MDD with elevated anxiety (zuranolone: n = 260; placebo: n = 248) and 499 did not (zuranolone: n = 245; placebo: n = 254). In the MDD with elevated anxiety subgroup, mean (SD) HAMD-17 (zuranolone vs placebo) was 27.3 (2.8) vs 27.6 (2.9) at baseline and 12.4 (8.0) vs 15.1 (8.2) at Day 15. This represented a least squares mean (LSM) (SE) CFB of −14.8 (0.6) vs −12.5 (0.6) (p = 0.002) at Day 15. In the MDD without elevated anxiety subgroup, mean (SD) HAMD-17 (zuranolone vs placebo) was 25.2 (2.2) vs 25.2 (2.1) at baseline and 12.1 (7.9) vs 13.6 (7.6) at Day 15. This represented a LSM (SE) CFB of −13.0 (0.6) vs −11.4 (0.6) (p = 0.038) at Day 15. In both subgroups, zuranolone separated from placebo at the first measured time point (Day 3; p < 0.0001). In the MDD with elevated anxiety subgroup, LSM (SE) CFB in HAM-A (zuranolone vs placebo) was −13.5 (0.6) vs −11.5 (0.6) at Day 15 (p < 0.0027). The proportions of patients who reported TEAEs were similar in both subgroups (MDD with elevated anxiety: zuranolone, 62.3% vs placebo, 47.6%; MDD without elevated anxiety: zuranolone, 55.5% vs placebo, 48.0%). Most patients reported TEAEs that were mild or moderate in severity, regardless of elevated anxiety status at baseline (MDD with elevated anxiety: zuranolone, 96.3% vs placebo, 97.5%; MDD without elevated anxiety: zuranolone, 96.3% vs placebo, 98.4%). Rates of SAEs were low and consistent across treatment groups, regardless of elevated anxiety status at baseline (MDD with elevated anxiety: zuranolone, 1.5% vs placebo, 0.4%; MDD without elevated anxiety: zuranolone, 0.8% vs placebo, 1.2%).

Conclusions: In this pooled analysis of 3 randomized, double-blind studies—MDD-201B, MOUNTAIN, and WATERFALL—improvement in depressive symptoms with zuranolone was observed in patients with MDD, regardless of elevated anxiety status at baseline. Zuranolone was generally well tolerated and had a consistent safety and tolerability profile in patients having MDD with or without elevated anxiety at baseline. This analysis supports zuranolone as a potential treatment for the difficult-to-treat subpopulation of patients who have MDD with elevated anxiety.

Keywords: Zuranolone, Major Depression Disorder, Neuroactive Steroid, Anxiety, Positive Allosteric Modulators

Disclosures: Sage Therapeutics, Inc.: Employee, Stock/Equity (Self)

P243. Lumateperone in the Treatment of Major Depressive Episodes Associated With Bipolar I or Bipolar II Disorder: Evaluation of Extrapyramidal and Motor Symptoms in Late-Phase Clinical Trials

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Background: Atypical antipsychotics exhibiting high dopamine D2 receptor occupancy (65%-80%) are associated with an increased risk of extrapyramidal symptoms (EPS). Lumateperone is an FDA-approved antipsychotic to treat schizophrenia and depressive episodes associated with bipolar I or bipolar II disorder as monotherapy and as adjunctive therapy with lithium or valproate. Lumateperone is mechanistically novel, simultaneously modulating serotonin, dopamine, and glutamate neurotransmission, with a low D2 receptor occupancy (~39%) at the recommended 42-mg dose.

A robust late-phase clinical trial program established the safety and efficacy of lumateperone 42 mg as monotherapy and as adjunctive therapy with lithium or valproate in patients with a major depressive episode (MDE) associated with bipolar I or bipolar II disorder. This analysis evaluated the incidence of EPS across short- and long-term studies of lumateperone in patients with bipolar depression.

Methods: All trials enrolled men and women (18-75 years) with a clinical diagnosis of bipolar I or II disorder who were experiencing an MDE (Montgomery-Åsberg Depression Rating Scale [MADRS] Total score ≥20 and Clinical Global Impression Scale-Bipolar Version-Severity [CGI-BP-S] score ≥4 at screening and baseline). Lumateperone 42 mg was administered once daily in the evening.

EPS data were reported for 3 trial groups: (1) data pooled from 2 short-term, 6-week, placebo-controlled studies of lumateperone 42-mg monotherapy (Study 401 [NCT02600494]; Study 404 [NCT03249376]); (2) a 6-month open-label extension period (OLE) of Study 401 that evaluated long-term effects of lumateperone 42-mg monotherapy; (3) a Phase 3 placebo-controlled study (Study 402, NCT02600507) that investigated lumateperone 42-mg therapy adjunctive with lithium or valproate in patients with bipolar depression.

Incidence and severity of EPS-related treatment-emergent adverse events (TEAEs) were reported according to narrow standard MedDRA query. EPS assessment scales included the clinician-rated Abnormal Involuntary Movement Scale (AIMS), the Barnes Akathisia Rating Scale (BARS), and the Simpson Angus Scale (SAS). Patients without akathisia (BARS ≤ 2) or parkinsonism (SAS ≤ 3) at baseline were evaluated for the emergence of BARS-confirmed akathisia (BARS > 2) and SAS-confirmed parkinsonism (SAS > 3) during treatment.

Results: The short-term safety population comprised 746 patients in pooled monotherapy trials (placebo, 374; lumateperone, 372) and 352 patients in the adjunctive study (adjunctive placebo, 175; adjunctive lumateperone, 177). In the short-term studies, the only EPS-related TEAEs reported were 1 patient (0.3%) with mild dyskinesia on lumateperone monotherapy, 1 patient (0.6%) with mild akathisia on adjunctive lumateperone, and 1 patient (0.3%) with severe akathisia on placebo in the monotherapy trials. Benztropine and propranolol use was rare, with less than 1% of patients in any group receiving these treatments. Mean change from baseline in AIMS, BARS, and SAS scores were similar across groups for both monotherapy and adjunctive therapy. In patients without SAS-assessed parkinsonism at baseline (SAS ≤ 3), SAS-confirmed incidences of parkinsonism (SAS > 3) were rare in monotherapy (placebo, 1/347 [0.3%]; lumateperone, 2/327 [0.6%]) and adjunctive therapy (placebo, 1/168 [0.6%]; lumateperone, 2/168 [1.2%]). In patients without BARS-assessed akathisia at baseline (BARS ≤ 2), BARS-confirmed akathisia (BARS > 2) was also rare for monotherapy (placebo, 2/353 [0.6%]; lumateperone, 0/334 [0%]) and adjunctive therapy (placebo, 2/170 [1.2%]; lumateperone, 2/168 [1.2%]).

The long-term OLE safety population comprised 127 patients. As observed in short-term trials, long-term treatment with lumateperone was associated with a low incidence of EPS-related TEAEs, with 2 patients (1.6%) experiencing akathisia of mild and moderate severity. Rates of concomitant benztropine

(1.6%) and propranolol (0%) use were low. There were no notable changes in AIMS, BARS, or SAS scores during 6 months of treatment and no SAS-confirmed incidences of parkinsonism (SAS > 3, 0/122 [0%]). BARS-confirmed akathisia (BARS > 2) was reported in 3 of 122 patients (2.5%) without BARS-assessed akathisia at baseline (BARS ≤ 2).

Conclusions: In patients with bipolar I or bipolar II disorder experiencing an MDE, lumateperone 42-mg monotherapy or adjunctive therapy had a favorable EPS profile in both acute and long-term treatment.

Keywords: Lumateperone, Bipolar Disorder, Extrapyramidal Symptoms

Disclosure: Intra-Cellular Therapies, Inc.: Employee, Stock/Equity (Self)

P244. Psilocybin Assisted Therapy for Treatment-Resistant Depression: A Phase II, Randomized, Feasibility Study

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Background: Emerging preliminary evidence suggests that psilocybin-assisted therapy may be associated with rapid, robust and potentially enduring antidepressant effects in individuals with major depressive disorder (MDD). Given several challenging regulatory and logistical barriers, there have been no investigator-initiated psilocybin trials in Canada for several decades. Our study aimed to assess the feasibility, safety, and efficacy of single and repeat doses of psilocybin (25 mg) in persons with treatment-resistant depression (including both MDD and bipolar II disorder).

The primary objective was to evaluate the feasibility of repeated high-doses of psilocybin in adults with treatment-resistant depression. The secondary objectives were to assess the antidepressant efficacy and tolerability of psilocybin-assisted therapy in a real-world, complex patient population with minimal exclusion criteria and no upper limit for degree of treatment resistance. Exploratory objectives of this pilot trial were to determine the effects of psilocybin on quality of life, wellbeing, functioning, disability, cognitive function, anxiety, anhedonia and mystical experiences.

Methods: In this phase 2 randomized, wait-list controlled clinical trial, participants were randomized to immediate (n = 15) or delayed treatment (n = 15). Participants in the delayed treatment group (waitlist control condition) waited two weeks after enrolment before commencing the study interventions.

The study intervention combines up to three high dose of oral synthetic psilocybin (25 mg; supplied by Usona) with psychotherapy over a 6-month period. With considerations for cost-effectiveness and scalability in mind, an ultra-brief four-session therapy model was evaluated (i.e., four sessions total for each psilocybin dose, including preparation, dosing and integration sessions). Within the four-session model, psychotherapy consists of one 2 hour preparatory session (one week before dosing session), one 6-8 hour supportive psilocybin dosing session and two integration therapy sessions (2 hours each session; one day after and one week after the dosing session).

Participants received a single 25 mg dose of psilocybin with accompanying psychotherapy and were assessed weekly for six weeks and then biweekly for 18 weeks. Participants who responded and then relapsed could receive up to two repeated doses (e.g., maximum of three doses total, including first dose) of psilocybin over the course of the 6-month trial.

Eligibility Criteria: Male and female outpatients, between the ages of 18 and 70, who met DSM-5-defined criteria for a current major depressive episode as part of major depressive or bipolar II disorder. Participants were required to have previously failed two or more first-line treatments with no upper limit to failed treatment trials. Personal and family history of psychosis, active suicidality requiring admission and moderate/severe substance use disorders were key exclusion criteria. Personality disorders, passive suicidality and history of hypomania were NOT exclusion criteria.

Primary Outcome: The primary outcome of the study was feasibility. Feasibility was judged based on meeting regulatory requirements, recruitment, retention, safety, tolerability and preliminary efficacy.

This trial received Health Canada and research ethics board approval and was registered with ClinicalTrials.gov: NCT05029466.

Results: At the time of abstract submission, 22/30 participants (100% MDD to date) were enrolled and randomized with the trial to be completed by December 2022 (final trial results to be presented at ACNP). To date, feasibility results have been positive with all federal and local regulatory requirements met, rapid recruitment (159 patients referred for screening in first 6 weeks) and adequate retention (21/22 retained to primary endpoint). The safety and tolerability profile was positive, with zero serious adverse events (SAEs), zero suicide attempts, one dropout due to acute psychedelic effects, no psychedelic effects persisting past 12 hours and no emergency medications required during dosing sessions. 19/22 requested to receive a second dose believing benefits outweighed side effects.

Preliminary efficacy testing was positive with statistically and clinically significant antidepressant effects observed with psilocybin treatment, as measured by the Montgomery–Åsberg Depression Rating Scale (MADRS) ($p < 0.01$ at all time points compared to baseline and wait list control). Significant reductions of suicidal thoughts and anxiety were also observed with subjective improvements in overall function, quality of life and wellbeing.

Mean MADRS scores reduced by 8.9 points (SD = 7) 2-weeks after the first psilocybin dose (primary endpoint), as compared to 3.7 (5.1) in the waitlist control group, with a significant between group difference ($p < 0.01$). Greater reductions were observed 2-weeks after the second dosing session with an overall mean MADRS reduction of 17.5 (7.9), significantly greater than the MADRS reduction with the first dose ($p < 0.01$). Analysis of third dose effects are still pending.

Conclusions: Interim results supported feasibility with adequate recruitment, retention and safety. Preliminary efficacy results suggest that the ultra-brief psilocybin-assisted therapy intervention was beneficial in an ultra-refractory patient population. Two-doses were associated with greater reduction in depressive symptoms than a single dosing session.

Keywords: Psilocybin, Psychedelics, Psychotherapy, Treatment Resistant Depression, Major Depressive Disorder (MDD)

Disclosures: Baxia Scientific Copr: Employee, Consultant (Self)

P245. Adjunctive Simvastatin for Treatment-Resistant Depression: A 12-Week, Multicentre, Randomized Controlled Trial

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Background: Small clinical trials of adjunctive statin use in major depressive disorder (MDD) have reported improvement in

depressive symptoms. However, there are no appropriately powered clinical trials assessing the antidepressant efficacy of these repurposed agents in treatment-resistant depression (TRD).

Methods: This 12-week, randomised, double blind, placebo-controlled trial, was conducted across five centres in Pakistan. It aimed to assess the efficacy of adjunctive simvastatin 20 mg compared to placebo for reduction of depressive symptoms in TRD. The study involved adults (age: 18-65) with a DSM-5 major depressive episode that had failed to respond to at least two adequate trials of antidepressants. Participants were enrolled between March 1, 2019 and Feb 28, 2021; the modified intent-to-treat analysis was performed from February 1 to June 15, 2022 using mixed models. Participants were randomised to receive: (i) simvastatin 20 mg plus standard care or (ii) placebo plus standard care. The primary outcome measure was mean change from baseline to week 12 on the Montgomery-Äsberg Depression Rating Scale (MADRS) scores. Secondary outcomes included changes in scores on the 24-item Hamilton Rating Scale for Depression, the Clinical Global Impression scale, the 7-item Generalized Anxiety Disorder scale and change in body mass index from baseline to week 12. C-reactive protein and plasma lipids measured were measured at baseline and week 12. The trial was registered on Clinicatrials.gov (NCT03435744) on Feb 16, 2018.

Results: A total of 150 participants were randomised to simvastatin (n = 77) or placebo (n = 73) across study sites. Overall baseline-to–end point reduction in MADRS total score was observed in both groups however, the reduction in depressive symptoms did not differ significantly between groups (mean adjusted difference [95% confidence interval] for simvastatin vs. placebo: -0·86 (-3·96, 2·25), t(df) 0·5 (350·3), p = 0·589). There were no significant group differences in changes in any of the secondary outcomes and no evidence for differences in adverse effects between groups. A planned secondary analysis indicated that changes in plasma C-reactive protein and lipids from baseline to end-point did not mediate response to simvastatin.

Conclusions: There was no evidence that adjunctive simvastatin was superior to placebo for reduction of depressive symptoms in TRD. This large RCT casts doubt on the potential therapeutic benefits of adjunctive statins as repurposed augmentation strategies in TRD.

Keywords: Major Depression Disorder, Treatment Resistant Depression, Simvastatin, Statins, Clinical Trial

Disclosure: Mindset Pharma, Wake Network: Advisory Board (Self), Psyched Therapeutics: Consultant (Self), COMPASS Pathways: Contracted Research (Self)

P246. Effects of Acute Perimenstrual Estradiol and Progesterone Administration on Gabaergic Neuroactive Steroids and Cytokines: Preliminary Evidence From a Double-Blind Crossover Randomized Trial in Patients With Suicidal Ideation

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Background: More females than males attempt suicide, and these attempts and resulting deaths are most likely to occur around the onset of menses (perimenstrually), when estradiol (E2) and progesterone (P4) drop rapidly. Our recent crossover double-blind placebo-controlled trial of perimenstrual hormone administration (K99MH109667; CT.gov: NCT03720847) provides the first experimental evidence that administration of E2 and P4 in the weeks surrounding menses can reduce perimenstrual exacerbation of depression and suicidality in females with suicidal ideation (SI). Based on these findings and previous human and animal research, we hypothesized that this therapeutic effect of perimenstrual steroid administration (vs. placebo) may have been due to prevention of perimenstrual withdrawal from GABAergic neuroactive steroids (NAS). We also hypothesized that withdrawal from GABAergic NAS may have prevented depression-inducing perimenstrual increases in cytokine levels. We lastly hypothesized that within-person changes in GABAergic NAS and cytokines would covary with suicidal ideation. We evaluated banked serum samples from the same clinical trial to examine these two candidate biological pathways (reduced GABAergic NAS and increased cytokine levels) that might provide clues as to the mechanisms by which E2 and P4 administration (vs. placebo) reduced perimenstrual suicidal ideation.

Methods: 25 naturally cycling participants with past-month SI completed a counterbalanced crossover double-blind RCT, comparing active E2 + P4 stabilization to placebo in the two weeks before and during menses. Participants wore a transdermal 17β-E2 patch (.1mg/day; or placebo) and took oral micronized progesterone (200mg/day, dosed 100mg BID; or placebo) from days +7 to +21 following a positive LH-surge ovulation test (day 0). Participants completed daily symptom ratings for the duration of study participation, and underwent a blood draw on days +7, +14, and +22 (timed relative to the ovulation test). NAS were quantified with gas chromatography/mass spectrometry, and cytokines were analyzed with high sensitivity multiplex assay kits. We ran three-level multilevel models in R (version 4.0.2), where log-transformed biomarkers and a composite SI score were nested within study interval and participant. Monte Carlo simulations estimating L1-to-L1 effects, assuming moderate outcome clustering (ICC = .3), indicate that our frequentist models achieve 80% power to detect roughly medium-sized effects of the intervention (f~.30, accounting for roughly 9% of the variance in the outcome) and roughly medium-to-large effects when looking at associations of variables in the placebo condition (f~.37, accounting for roughly 13% of the variance in the outcome).

Results: We found a significant interaction between experimental condition and cycle phase predicting NAS, such that in the placebo condition (natural E2 + P4 withdrawal), P4-derived GABAergic NAS dropped from the midluteal to the perimenstrual visit, while the E2 + P4 administration prevented this decline (and in fact caused an increase) for 3α5α-tetrahydroprogesterone (3α5αTHP; β=2.97, $SE = 0.22$, $p < 0.001$), 3α5β-tetrahydroprogesterone (3α5βTHP; β=2.76, $SE = 0.27$, $p < 0.001$), and 3α5α-tetrahydrodeoxycorticosterone (THDOC; β=2.48, $SE = 0.19$, $p < 0.001$). We also found a trending interaction for experimental condition and cycle phase predicting IL-6, such that there was a midluteal-to-perimenstrual increase in IL-6 under placebo that was prevented in the E2 + P4 stabilization (β=-0.28, $SE = 0.15$, $p = 0.07$). Within the placebo condition only, between-person increases in IL-1β predicted higher concurrent SI (β = 2.07, $SE = 0.75$, $p < 0.05$), and within-person increases in IL-6 predicted higher concurrent SI at trend-level (B = 0.28, $SE = 0.14$, $p = 0.06$). However, within-person variance in 3α5αTHP, 3α5βTHP, THDOC, IL-1β, and IL-6 did not significantly predict concurrent SI (all p's > 0.2) after accounting for experimental effects.

Conclusions: In the first experimental RCT showing that perimenstrual administration of E2 + P4 reduces perimenstrual exacerbation of suicidal ideation in female outpatients, we used banked serum samples for preliminary analyses exploring the effects of the intervention on peripheral GABAergic NAS and cytokines as potential biological mechanisms. First, we demonstrated proof-of-concept that most hypothesized outcomes of interest (3α5αTHP, 3α5βTHP, THDOC, and IL-6) do change predictably across the natural menstrual cycle, and these changes can be prevented or reversed with experimental E2 + P4 administration. Second, we found that increased overall levels of IL-1β and natural increases in IL-6 (in the placebo condition)

predicted increased concurrent SI. Third, we found that, after controlling for experimental effects of E2 + P4, neither overall levels nor person-centered increases in NAS or cytokines predicted SI. Given the primary trial's demonstration of a causal relationship between E2 + P4 withdrawal and perimenstrual SI, we recommend future translational studies with larger samples combined with imaging or transcriptomic approaches to further probe inflammatory signaling, sensitivity of the GABAergic system (e.g., through differential gene expression of GABA receptor subunits), or other biological mechanisms that could underlie the effects of steroid withdrawal on depression and SI.

Keywords: Ovarian Hormones, Neuroactive Steroid, Cytokines, Suicidal Ideation

Disclosure: Nothing to disclose.

P247. Chronic Stress Affects Negative Cognitive Bias and Related Inflammation in a Sex-Specific Manner in Rats

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Background: Major depressive disorder (MDD) is more common in women than in men, and women have more severe symptoms of MDD. Cognitive symptoms of MDD include negative cognitive bias. Negative cognitive bias is an increased perception of neutral situations or objects as negative. Elevated inflammation has been linked to MDD and cognitive impairment. MDD can be modelled in rodents using a chronic unpredictable stress (CUS) paradigm. We examined whether there were sex differences in cognitive bias after CUS and in neuroinflammation by examining nine cytokine concentrations in the ventral hippocampus and basolateral amygdala.

Methods: Adult male and female Sprague-Dawley rats (N = 40) underwent either 2 weeks of CUS or no stress followed by an 18-day fear-based cognitive bias task. On the last day of cognitive bias testing, brains were collected and electrochemiluminescence was used to measure cytokine (IFN-γ, IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, TNF-α) and chemokine (C-X-C motif ligand 1; CXCL1) levels in the ventral hippocampus and basolateral amygdala.

Results: On day 1 of cognitive bias training, male and female rats that underwent CUS displayed a more freezing (greater fearful response) than controls. By the end of cognitive bias training (day 16), rats exposed to CUS displayed a potentiated fear response in all contexts compared to non-stressed rats, and males displayed more freezing than did females. CUS increased freezing in the ambiguous context. In addition, CUS increased positive relationships between ambiguous freezing and inflammation in the basolateral amygdala in females, and CUS increased negative relationships between ambiguous freezing and inflammation in the ventral hippocampus of males.

Conclusions: These findings indicate that CUS potentiates the freezing response to a neutral context, increasing negative cognitive bias in both sexes and suggests a sex-specific role of neuroinflammation in negative cognitive bias.

Keywords: Cognitive Imparim ent, Stress, Sex Differences

Disclosure: Nothing to disclose.

P248. Stress-Resilient Mice Optimize Both Subjective Value and Food Security on an Economic Foraging Task

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Background: Economic stress can often serve as a "second-hit" for those who have already accumulated a history of stressful experiences. This can precipitate significant changes in behavior that may be adaptive or maladaptive depending on one's unique stress-response predispositions. How an individual recovers from a setback is a core feature of resilience but is seldom captured in animal studies.

Methods: Here, we challenged mice in a novel two-hit stress model by first exposing animals to chronic social defeat stress (first hit) – a protocol known to separate individual differences in stress-resilient versus stress-susceptible phenotypes. Mice were then tested longitudinally across two months on the neuroeconomic task termed "Restaurant Row." On this task, mice had a limited time period each day to forage for their sole source of food by navigating a maze with four uniquely flavored and contextualized feeding sites, or "restaurants." Flavors were used to modulate subjective value determined by revealed preferences without assuming reward value (as opposed to varying pellet number in each restaurant, as this would come with added complexity of opportunity cost due to increased handling time per pellet). Each restaurant had a separate offer zone (OZ) and wait zone (WZ). Upon entry into the OZ, a tone sounded whose pitch indicated how long of a delay mice would have to wait in a cued countdown should they choose to enter the WZ. Tones were randomly selected from a range offer costs. OZ and WZ choices as a function of both cued offer cost and restaurant flavor demonstrate that animals understand the structure of the task while interacting with individual differences in subjective value. Mice were trained on this task for 60 consecutive days, across which the task grew increasingly difficult as the range of offer delays encountered grew more expensive.

Results: An abrupt transition into a reward-scarce environment on this task 3 weeks into testing elicits an economic crisis (second hit) precipitating a massive drop in food intake that mice must respond to in order to survive. This recovery process occurs across a timescale on the order of weeks as mice learn to develop new decision strategies. We found that stress-resilient mice mounted the most robust behavioral response to this economic challenge and readily renormalized food intake back to baseline levels faster compared to stress-susceptible and non-defeated control mice. This was achieved through an efficient increase in effort expenditure and a redistribution in how time was allocated among competing opportunities. Interestingly, stress-resilient mice learned to accomplish this while simultaneously maximizing subjective value by re-establishing flavor preferences that approximated yield previously obtained in a reward-rich environment.

Conclusions: These findings suggest that a resilient individual's capacity to "bounce back" following economic stress while foraging entails the development of a multi-pronged strategy that not only ensures food security necessary for survival but also prioritizes other aspects of well-being including subjective value, highlighting a motivational balance preserved in stress-resilient individuals that may be impaired in depression.

Keywords: Behavioral Economics, Social Defeat Stress, Stress Resilience and Susceptibility

Disclosure: Nothing to disclose.

P249. Linking Brain-Wide Activity Patterns During Neuroeconomic Decision Making to Aggression

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Background: Aggression is an evolutionarily conserved response to a perceived threat that spans a large range of diverse behaviors and computational processes, including those that may be adaptive and protective as well as those that may be pathological and dangerous to others. It is becoming increasingly clear that the neural circuits recruited during the expression of aggressive behaviors, in addition to circuits known to subserve social interactions themselves, are critical nodes of the brain's reward system. An active area of investigation is exploring to what degree pathological aggression may "hijack" key reward circuits in the brain, contributing to maladaptive reinforcement of aggression. Such studies involve the exploration of circuit engagement during social interactions directly as well as circuit engagement during general reward-related processing even for non-social targets (e.g., natural rewards like food). To date, the majority of aggression studies in rodents generally rely on (i) simple, naturalistic social behavioral assays (e.g., home-cage social behavior or social defeat-based interaction screening), (ii) are slowly extending into different ways to quantify social reward (e.g., social-aggression conditioned place preference or aggression self-administration), and (iii) are beginning to relate individual aggression profiles to more generalized behavioral and physiological signatures involved in the processing of non-social rewards. Because the brain has evolved to use multiple decision-making systems, simple tests of reward value may be unable to access computational subtleties that may be altered in the brains of aggressive individuals.

Methods: Here, we aimed to characterize complex decision making profiles of animals screened for aggression in the home cage and then subsequently tested on a naturalistic, neuroeconomic decision-making task, termed "Restaurant Row." This spatial task has been translated for use across species and is capable of operationalizing reward value along many dimensions and across numerous discrete behavioral computations that can reflect dissociable circuit functions. First, individual differences in aggressive behavior were quantified in 40 male outbred Swiss Webster mice. Next, mice were trained on the Restaurant Row task for 60 consecutive days. On this task, mice had a limited time period each day to forage for their sole source of food by navigating a maze with four uniquely flavored and contextualized feeding sites, or "restaurants." Each restaurant had a separate offer zone (OZ) and wait zone (WZ). Upon entry into the OZ, a tone sounded whose pitch indicated how long of a delay mice would have to wait in a cued countdown should they choose to enter the WZ. This task produces a rich behavioral dataset accessing numerous features of reward cost, subjective value, and choice process. On the final day of testing, mice were allowed to engage the task for a short period of time before being removed and prepared for a time-sensitive tissue extraction and perfusion protocol in preparation for whole brain tissue clearing and staining. This approach, immunolabeling-enabled three-dimensional imaging of solvent-cleared organs, or iDISCO+, allows for brain-wide tissue staining for c-Fos expression, an activity-dependent immediate early gene. The timing of this protocol was intended to capture a snapshot of brain-wide activation patterns reflecting task engagement. Whole-brain imaging of cleared tissue was performed using light-sheet microscopy.

Results: We found that aggression levels were generally stable within an individual measured across a 2-month period. We measured c-Fos expression levels in 275 distinct brain regions. Overall, we found that the majority of brain regions revealed decreased levels of c-Fos expression in highly aggressive animals versus non-aggressive animals. This initial finding is surprising given previous data from our lab and others tend to find overall increased activation in aggressive mice, although, measured following engagement of social interaction tests rather than a reward-based task for non-social targets as measured here. Using an unbiased, open-ended analysis approach, top region hits revealed strong negative correlations between aggression score and c-Fos expression, regions that lie in the medial wall of the prefrontal cortex (mPFC), including the anterior cingulate, prelimbic, and infralimbic cortex and are known to be engaged by the Restaurant Row task. Using this open-ended approach, we also found that several regions across the limbic system covaried with numerous key metrics from the Restaurant Row task, suggesting specific regions may be interacting in order to give rise to complex decision-making profiles. Using a focused analysis, we found that c-Fos expression in the mPFC scaled with individual differences in subjective value.

Conclusions: These data provide fodder for future interrogation of sets of circuits that integrate only certain aspects of reward-related information processing. These data reveal how brain-wide activity signatures of aggression may drastically change, and even reverse direction from hyperactivation to hypoactivation, depending on the nature of the task engaged (e.g., social vs food reward) and the type of decision being processed. Lastly, these findings set the stage for exploring a deeper understanding of circuit-computation-specific functions of multiple valuation algorithms and aggression in a brain-wide manner.

Keywords: Neuroeconomics, Whole-Brain Rodent Imaging, Decision Making, Reward, Aggression

Disclosure: Nothing to disclose.

P250. A Synaptic Substrate for Ketamine-Mediated Amelioration of Stress-Induced Anhedonia

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Background: There is a major unmet need for the treatment of depression because current pharmacotherapies for depression require prolonged administration for clinical improvement and they are often associated with high non-response rate. In contrast, recent clinical evidence has shown that a single sub-anesthetic dose of ketamine (K), a noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, induces a robust antidepressant effect within hours of treatment in 70% of treatment-resistant patients. Particularly, such behavioral amelioration occurs within the context of anhedonia. Anhedonia –defined as diminished pleasure from, or interest in, previously rewarding activities– is a core symptom of depression which is commonly precipitated by exposure to chronic stress and is well-suited to study in laboratory animals. Specifically, hedonic deficits can be induced in rodents by chronic stress, which is able to affect the two main components of anhedonia: consummatory (subjective pleasure) and motivational (anticipation of and drive towards rewarding stimuli). The current study investigates the effect of K on both subtypes with a special focus on the Nucleus Accumbens (NAc). Indeed, the NAc plays a major role in the generation of motivated behaviors and stress determines opposing physiological changes within the two main populations of GABAergic medium spiny neurons (MSNs) residing within the NAc, D1 and D2 dopamine receptor expressing-MSNs. Accordingly, in this work we identify specific cellular elements of, and inputs to, the NAc recruited by in vivo K administration and responsible for its anti-anhedonic effects.

Methods: Male and female mice were implanted with corticosterone (CORT) pellets (40 mg/kg). Behavioral and electrophysiological experiments were performed 21 days following implantation of the pellets. We assessed hedonic deficits with three-chamber sociability test, sucrose preference test and sucrose self-administration. Specifically, to investigate K's anti-anhedonic effects, half of the mice received a single injection of K (10 mg/kg, IP) 24 hours before each test, while the other half received saline ($n = 10$-12 mice/test/treatment). To characterize α-amino-3-

hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-mediated synaptic transmission from molecular specified-MSNs in CORT-treated mice exposed to K, we performed in vitro whole cell recordings in transgenic mice expressing fluorescent proteins under the control of the D1 or D2 promoters (D1-TdTomato and A2A-TdTomato, respectively) (n = 10-15 cells from 8 mice/treatment). To test necessity of AMPAR-mediated synaptic plasticity on molecular specified-MSNs for the K's anti-anhedonic effects, we employed DART technology in D1cre or A2Acre mice (n = 10-12 mice/test/condition). To identify specific inputs to the NAc recruited by K, we performed in vitro whole cell recordings in D1-TdTomato and A2A-TdTomato mice while optogenetically interrogating each monosynaptic input to the NAc (n = 10-15 cells from 6-8 mice/treatment/input). To test necessity of monosynaptic inputs to the NAc for K-mediated amelioration of stress-induced anhedonia, we used inhibitory DREADDs to inactivate each specific monosynaptic input to the NAc (n = 10-12 mice/test/condition/input). All experimental protocols were approved by the Washington University Institutional Animal Care and Use Committee with compliance to the National Institutes of Health guidelines.

Results: Chronic CORT treated-mice displayed a reduction in sucrose preference ratio, time spent with a social target and breaking point compared to placebo-treated mice measured in the sucrose preference, social interaction and sucrose self-administration tests, respectively. K was able to rescue such hedonic deficits. Moreover, recordings from NAc slices obtained from CORT-exposed mice 24 h after a systemic saline injection showed decreased amplitude and frequency of spontaneous excitatory post-synaptic currents and reduced AMPAR to NMDAR ratio in accumbal D1-MSNs compared to placebo-treated mice. A single injection of K was able to rescue such reduced excitatory transmission within the NAc. Importantly, specific AMPAR blockade within D1-MSNs prevented K-operated amelioration of stress-induced anhedonia. Finally, we found that information flow from prefrontal cortex and ventral hippocampus to the NAc is enhanced by K exposure, however each input plays a unique role in K-induced anti-anhedonic effects.

Conclusions: These results show circuit, cellular and synaptic mechanisms by which K triggers long-lasting amelioration in stress-induced anhedonia. These results have major implications for the rational design of more targeted, effective and safer medications for the treatment of anhedonia.

Keywords: Ketamine, Anhedonia, Synaptic Plasticity, Nucleus Accumbens

Disclosure: Nothing to disclose.

P251. Sidekick-1, A Novel Regulator of Stress Resilience in the Prefrontal Cortex

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Background: Stress is a major risk factor for depression, yet some individuals remain resilient. Both environmental and genetic factors contribute to the dysregulation of neural circuits in depression and genes that regulate the formation, maintenance, and plasticity of synapses are of particular interest as molecular hubs for regulation of circuit function.

We previously identified Sidekick-1 (Sdk1) as an affective circuit hub gene in a network regulating stress resilience and overexpression of Sdk1 in the prefrontal cortex (PFC) increased resilience to stress in male mice. Separately, several genome-wide association studies in humans found SDK1 variants associated with depression, suggesting an evolutionarily conserved role in regulation of emotional behavior.

Sdk1 is a cell surface molecule implicated in circuit formation in the developing retina. However, little is known about cell-specific expression patterns of Sdk1 in the adult brain and its function within affective circuits. Here we probe Sdk1 cell-type and layer-specific expression in the PFC, conservation between mice and humans, its modulation by stress, and effects on reward learning in both male and female mice.

Methods: We used chronic social defeat stress to generate susceptible and resilient male mice, and chronic witness defeat stress or subchronic variable stress in female mice. Quantitative real-time PCR determined bulk Sdk1 expression levels in the PFC, nucleus accumbens and ventral hippocampus of control and stressed mice (male control n = 13, stress n = 17; female control n = 8, stress n = 10). We assessed expression pattern of Sdk1 mRNA in the PFC control and stressed mice with multiplex RNAScope fluorescent in situ hybridization using probes for Slc17a7 (vGlut1) and Slc32a1 (vGAT) to identify excitatory and inhibitory neurons, respectively (male control n = 9, stress n = 15; female control n = 7, stress n = 7). We used publicly available scRNAseq datasets (Allen Brain Map) from mouse and human whole cortex to identify cell-type specific expression of Sdk1. Reward learning was assessed in a probabilistic reversal learning task.

Results: We found that Sdk1 is expressed in the PFC, nucleus accumbens and ventral hippocampus of adult male and female mice, with expression highest in the PFC ($p < 0.05$ male; $p < 0.0001$ female). RNAScope in situ hybridization in the PFC showed that Sdk1 was expressed in subpopulations of vGlut1-positive neurons and vGAT-positive neurons. The highest level of Sdk1 expression was observed in deep layer 6 ($p < 0.0001$) with moderate expression in layers 2/3 and 5. Patterns of cell-type and layer-specific Sdk1 expression were conserved in scRNAseq analysis of human PFC. De novo clustering of scRNAseq data from mouse PFC showed that Sdk1-expressing cells were found across clusters, with several clusters of excitatory or inhibitory neurons marked by high numbers of Sdk1-expressing cells. Stress modulated Sdk1 expression in the PFC in a sex-specific manner. Cell-type specific overexpression of Sdk1 in the PFC modulated reward learning in the probabilistic reversal learning task with sex-specific effects.

Conclusions: In this study we characterized the prefrontal expression of Sdk1, a novel regulator of stress resilience. We found that Sdk1 is modulated by stress and is expressed in subsets of both excitatory and inhibitory neurons, with a significant enrichment in deep layers of the PFC. Cell-type and cortical layer specificity was conserved between mice and humans suggesting an evolutionarily conserved function. The probabilistic reversal learning task allows us to probe reward learning in animals where prefrontal Sdk1 expression is altered. Future work will address the circuit-specific effects of Sdk1 in behavioral adaptation to stress. As a cell surface receptor, Sdk1 is a potential target for pharmacological intervention. Understanding how Sdk1 confers resilience may lead to development of new mechanistically-informed treatments for depression.

Keywords: PFC, Depression, Stress

Disclosure: Nothing to disclose.

P252. Behind the Veil of Magic Mushrooms: Exploring the Correlation Between Subjective Experiences With 10 Psychedelic Mushrooms and Tryptamine Content

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Background: Psilocybin containing "magic" or psychedelic mushrooms (PM) affect perception, mood, behavior, and consciousness. The neuroplastic, immune modulation, and anti-inflammatory effects of psilocybin are currently being explored for a range of conditions including depression, PTSD, anxiety, and substance abuse. There is anecdotal evidence of distinctive, subjective experiences with different PM and this may be due to variations in psilocybin content and effects of non-psychoactive tryptamines. This phenomenon, the entourage effect, may be the result of the pharmacological interaction between the tryptamines. This study aimed to establish the concentration of these tryptamines in a variety of popular PM that are associated with distinct subjective experiences in the non-clinical setting, and to explore whether there is a correlation between the type of subjective experience and the tryptamine composition of the mushrooms.

Methods: A cross-sectional on-line survey was fielded July 22-July 25, 2022. Participants were recruited from online psychedelic user groups and a general national sample of adults in the US reporting PM experience. The national survey included a fictional PM for quality control. Participants were asked to rate their general overall experience, intensity of visual and physical effects by PM on a 0-to-10-point scale. Endpoints are assessed descriptively for participants in psychedelic user groups, the general national sample and both groups combined.

For analysis, Psilocybe subtropicalis, Panaeolus bisporus, Psilocybe tampanensis, Psilocybe cubensis - "Enigma", Psilocybe natalensis, Panaeolus cyanescens, Psilocybe cubensis - American Mystic (PE) were cultivated from spores. Psilocybe stunzii, Psilocybe ovoideocystidiata, and Psilocybe azurescens were obtained from field collections. Samples of dried ground mushrooms were extracted via methanol. Psilocybin, psilocin, norpsilocin, baeocystin, norbaeocystin and aeruginascin were quantified using a Waters Acquity H-Class UPLC and Xevo TQ-S Micro MS (Waters Corporation, Milford, MA, USA).

Results: The national survey screened out 840 (88.4%) potential participants for selecting a fictional PM (86.5%) and other quality control screens (1.8%). A total of 149 individuals completed the survey with 37 (22%) individuals from psychedelic user groups and 112 (78%) individuals from the general national sample. Participants in the PM user groups, relative to those in the national sample were more likely to be male (77.4% vs. 45.5%) and older with a greater percentage between 43-52 years old (32.3% vs 16.0%). They tended to give lower ratings to their mushroom experiences. Overall, Psilocybe cubensis (75; 50.3%), Psilocybe subtropicalis (60; 40.3%) and Panaeolus cyanescens (60; 40.3%) were the 3 most common PM. Participants rated Psilocybe azurescens and Psilocybe ovoideocystidiata with the highest overall effect with an average score of 7.4; Psilocybe natalensis and Psilocybe ovoideocystidiata had the highest impact on visual effects with an average score of 6.8; and Psilocybe azurescens had the highest average physical effect of 6.7. For the majority of PM there was a wide range of ratings from 0 to 10.

Psilocybin, the most abundant component, ranged from 0.03% (Psilocybe ovoideocystidiata) to 1.79% (Psilocybe subtropicalis) dry wt. %. The total content of other tryptamines varied proportionally to the combined psilocybin and psilocin content. Of note is that strong body and weak visual effects seem to be recorded for mushrooms with a relatively high baeocystin content while mushrooms with an equal ratio of psilocybin versus the other tryptamines seem to produce more visual effects.

Conclusions: Despite interobserver variation in the assessment of experiences with 10 different PM, a pattern emerged pointing towards distinct visual and physical experiences with different mushrooms. This may be explained by the ratio of non-psychedelic tryptamines to psilocybin and the amount of baeocystin. The large number of participants screened out by control measures highlights the challenges in conducting such surveys.

Keywords: Psychedelics, Psilocybin, Baeocystin
Disclosure: BYAS-PEB: Consultant (Self)

P253. Instrumental Control Over Stress Recruits Distinct Circuits in Female Rats

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Background: Exposure to adverse life events is strongly linked to negative mental health outcomes, in which prevalence is often higher in women than men. Thus, investigating sex differences in experiential factors that promote stress susceptibility/resilience, and their circuit implementation, is of clinical interest. In male rats, instrumental control over stressor recruits a corticostriatal system involving the prelimbic cortex (PL) and dorsomedial striatum (DMS) that potently blunts a number of stress outcomes. In contrast, the stress-buffering effects of control are completely absent in females, and the mechanisms underlying this absence are not understood.

Methods: For manipulation of controllability, Sprague-Dawley rats were run in a triadic design. One subject of each triad received escapable shock (ES), a second received yoked-inescapable shock (IS), and a third received no shock (HC). Following stress treatment, the number of Fos-immunoreactive neurons was determined for the DMS and dorsolateral striatum (DLS), and subsequently converted into the number of counts per unit area (mm2) for each region of interest (EXP1: $n = 7$-8/group/female only). In EXP2, stress-induced dopamine (DA) levels in the medial prefrontal cortex (mPFC) were assessed with in vivo microdialysis ($n = 5$-7/group/male and female). Multiplex-fluorescence in situ hybridization was used to quantify expression of the following mRNA transcripts in the ventral tegmental area (VTA) to PL pathway: tyrosine hydroxylase (TH), c-fos, and viral-encoded GFP (EXP3: $n = 3$-5/group/male and female). Intra-PL microinfusion of SCH 23390 or saline was delivered 30 min prior to ES/IS/HC (EXP4: $n = 9$-11/group/female only).

Results: EXP1: At the level of the DMS, both female ES and IS led to a similar level of Fos expression ($p = 0.291$). In contrast, female ES robustly increased Fos immunoreactivity in the DLS ($p < 0.001$) compared to IS. EXP2: To examine whether the impact of PFC DA efflux differs in male and female ES subjects, a microdialysis probe was targeted to the mPFC. Male ES led to only a transient increase in DA, with DA returning to baseline levels by the second sample collection. ES females showed a different pattern - DA efflux was potentiated throughout the entire stress session (stress x sex x time interaction: $F4,80 = 3.070$, $p = 0.021$; Repeated Measures ANOVA). EXP3: Fluorescent in situ hybridization revealed that female ES led to a greater activation of PL-projecting VTA DA neurons compared to male ES (sex x stress interaction: $F1,12 = 6.138$, $p = 0.0291$; 2-way ANOVA). EXP4: Blockade of PL D1 receptors with SCH 23390 shifted Fos expression from the DLS to the DMS ($t11 = 2.28$, $p = 0.043$; independent t-test), thereby enabling behavioral protection in females with behavioral control.

Conclusions: Behavioral control over adverse events, a key aspect of coping, recruits an instrumental learning system in females that differs from males. Our data provide novel insight into the influence of mPFC DA over striatal activity during instrumental learning involving aversive stimuli, and its subsequent impact on behavioral outcome. The implication is that the exercise of behavioral control at a procedural level is not the critical factor in determining its impact, rather the impact depends on the circuitry that is recruited during the acquisition of the controlling response.

Keywords: Medial Prefrontal Cortex, Dopamine, Stress Resilience, Dorsal Striatum, Stress Coping

Disclosure: Nothing to disclose.

P254. Gm-2505 is a Novel 5-Ht2a Receptor Agonist and 5-Ht Releaser That Induces Rapid, Robust, and Durable Antidepressant Effects at Doses Associated With Decreased Power in Low Frequency EEG Bands in Rats

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Background: Prototypical psychedelic agents such as psilocybin, LSD, and DMT continue to show promise as rapid acting antidepressants in clinical trials. These agents, which are 5-HT2A receptor agonists, produce profound alterations in human consciousness, with variable durations of action ranging from very long (8-12 h for LSD) to very short (~10 min for DMT). The antidepressant efficacy of this class of agents is durable and continues long past their clearance, making weekly, or even monthly dosing the preferred treatment paradigm. Here we describe GM-2505, a novel 5-HT2A receptor agonist with 5-HT releasing activity. It was designed to have an intermediate half-life in humans, allowing for sufficient target engagement to provide antidepressant effects without requiring an extended-duration in-clinic experience.

Methods: The in vitro profile of GM-2505 was determined using competition binding and calcium flux (FLIPR) assays in cells expressing heterologous human 5-HT2A, 5-HT1A, 5-HT2C and 5-HT2B receptors. The effect of GM-2505 on 5-HT release from rat brain synaptosomes was also assessed.

The brain and plasma PK of GM-2505 (1 mg/kg, sc and iv) were determined in Sprague Dawley (SD) rats. 5-HT2A receptor agonist activity in vivo was measured by counting wet dog shakes (WDS) and head twitches (HT) in the first 20 min after dosing rats ($n = 6$/group) with GM-2505 (0.03-10 mg/kg, sc). A study in mice ($n = 6$/group) determined the effect of pretreatment with the 5-HT2A antagonist, MDL100,907 (0.1 mg/kg, sc) on HT caused by GM-2505 (0.1–10 mg/kg, sc).

GM-2505 was assessed in a paradigm of Chronic Mild Stress (CMS), whereby Wistar Kyoto rats ($n = 8$/group) were subjected to stressors (e.g., wet bedding, tilted cage) for a period of 10 weeks. After 2 weeks of CMS, animals were dosed once per week with GM-2505 (0.3 or 1 mg/kg, sc) or vehicle for 5 weeks. Rats continued to be exposed to stress for an additional 3 weeks after the final dose to test the durability of the antidepressant effect. Each week, anhedonia was assessed by measuring sucrose intake (24 h after dosing, weeks 3-7). Anxiety levels in the elevated plus maze (EPM) and memory impairment in the novel object recognition (NOR) test were assessed in the 3rd week of treatment, 48 and 72 h after dosing, respectively.

The effects of GM-2505 (0.3-3 mg/kg, sc) on quantitative EEG (qEEG) were measured in a cohort of 8 freely moving SD rats implanted with 2 cortical electrodes connected to a DSI transmitter. Rats were tested once per week using a within-subjects Latin square design.

Results: At human 5-HT2A receptors, GM-2505 bound with high affinity (Ki 4.9 nM and 140 nM, for DOI and ketanserin, respectively) and potent and efficacious agonist activity (EC50 15.0 nM, EMAX 80.6%). GM-2505 was also a potent agonist of 5-HT2C (EC50 9.5 nM), much less potent at 5-HT1A (EC50 16918 nM) while an antagonist at 5-HT2B (IC50 5.8 nM). GM-2505 activated the release of 5-HT from rat synaptosomes with efficacy of 66.8% and potency of 15.7 nM.

GM-2505 (1 mg/kg, sc) achieved a Cmax of 41.3 ng/mL in rat plasma and 188 ng/g in rat brain at Tmax of 30 min and 15 min, respectively. After iv administration (1 mg/kg), the C0 was 159.9 ng/mL in plasma and 1507 ng/g in brain with a T1/2 of ~25 min. This profile was longer than DMT-like and shorter than psilocin-like comparator compounds.

GM-2505 produced HT and WDS in rats with an ED50 of 0.07 mg/kg and peak effect at 1 mg/kg. In mice, the GM-2505 ED50 was 0.32 mg/kg for HT, with the peak response at 3.2 mg/kg. HT was absent in mice pretreated with MDL100,907. GM-2505 showed the expected inverted U-shaped dose response for HT in both rats and mice.

Exposure of rats to the chronic mild stress paradigm caused a phenotype of anhedonia, anxiety and cognitive impairment, which was reversed by GM-2505 (0.3 and 1 mg/kg). 24 h after the first dose, GM-2505 fully reversed the stress-induced deficit in sucrose intake ($P < 0.05$ GM-2505 Stress vs Vehicle Stress group); this effect was maintained through 5 weeks of once weekly dosing and did not fully dissipate until 22 days after the final dose. CMS-induced anxiety and cognitive impairment were apparent in vehicle treated rats (EPM and NOR: $P < 0.05$ Vehicle Ctrl vs Vehicle Stress). Both deficits were reversed by GM-2505 (0.3 and 1 mg/kg): increase in % time in the open ($P < 0.05$ GM-2505 Ctrl vs GM-2505 Stress) and an increase in NOR index ($P < 0.05$ GM-2505 Ctrl vs GM-2505 Stress).

In qEEG, GM-2505 (0.3-3 mg/kg) produced dose-dependent reductions in power in the lower frequency bands (delta, theta, alpha and beta). There was a strong relationship between the time course of this effect and the PK of GM-2505.

Conclusions: Consistent with its in vitro activity as a potent 5-HT2A receptor agonist and 5-HT releaser, GM-2505 produced a robust 5-HT2A-mediated behavioral response in vivo. The PK of GM-2505 in rats was intermediate between DMT and psilocin analogs. In the CMS paradigm, doses of GM-2505 (at and below peak HT/WDS response) had robust antidepressant-like effects 24 h after the initial dose, which were sustained with once weekly dosing for 5 weeks. The durability of this effect was evident in the levels of sucrose intake in GM-2505-treated CMS rats not reverting to vehicle levels until 3 weeks after the final dose. Antidepressant doses of GM-2505 were associated with acute reductions in qEEG power in low frequency bands, an effect reported for psilocybin and DMT at clinically efficacious doses. Together, these data indicate that GM-2505 is a novel 5-HT2A receptor agonist and 5-HT releaser that holds promise as a rapid-acting, robust antidepressant.

Keywords: Fast-acting Antidepressant, 5-HT2A Receptors, Psychedelic Medicine

Disclosure: Gilgamesh Pharmaceuticals: Employee (Self)

P255. Functional Connectivity Differences Related to Age of Mood Disorder Onset

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Background: Mood disorders often begin in adolescence, with this earlier onset being associated with increased likelihood of negative psychosocial outcomes. While studies have demonstrated the presence of neurobiological anomalies in adolescents with mood disorders, limited research has evaluated how these effects present in young adulthood. The cognitive control, default mode, and salience and emotion networks (CCN, DMN, and SEN respectively) have all been implicated in both adults and

adolescents with mood disorders. These networks may be particularly impacted by early onset mood disorders as the presence of the disease may hinder development. Such disruptions may then contribute to the effective management of symptoms that may otherwise lead to functional impairment.

Methods: Adults ages 18-30 with remitted mood disorder completed a resting-state fMRI scan. We examined functional connectivity of the cognitive control network associated with age of mood disorder onset. For the CCN, we used the dorsolateral prefrontal cortex, inferior frontal gyrus, and dorsal anterior cingulate cortex as seeds. For the DMN, we used the posterior cingulate cortex, dorsal medial prefrontal cortex, and temporoparietal junction. For the SEN, we used the amygdala, subgenual anterior cingulate, and anterior insula. We included age at time of scanning, sex, and movement parameters as covariates in all analyses. We reported results that exceeded an uncorrected $p < .001$ and voxel size of 75.

Results: A total of 138 participants (97 female) were included in analyses. Average age of participants at the time of the scan was 22.54 (SD = 3.10), with an average age of mood disorder onset of 15.99 (SD = 3.93) (range = 5-26 years). Younger age at mood disorder onset was negatively associated with connectivity of CCN regions with right superior and medial temporal gyrus, right primary motor and visual association cortices, left secondary visual cortex, and bilateral fusiform. Younger mood disorder onset was also negatively associated with DMN connectivity with the right temporal pole and primary sensory cortex. There were no significant relations between age of onset and SEN connectivity.

Conclusions: Individuals experiencing remission from mood disorders who have an earlier age of onset demonstrate decreased CCN and DMN involvement with regions implicated in social and emotional cognition and perception. As adolescence is a key period of rapid neurodevelopment, conditions such as depression can have significant effects that may disrupt typical neurobiological trajectories. By evaluating the effect of when mood disorders develop, longitudinal data that incorporates degree of functional impairment may aid in our understanding of what neurobiological changes have deleterious effects. Furthermore, we can also better identify which neurobiological processes may contribute to effective adaptation to the illness. These processes may aid in reducing illness recurrence and/or serve as a buffer against negative outcomes.

Keywords: Mood Disorders, Connectivity, Onset

Disclosure: Nothing to disclose.

P256. Dynamic Resting State Default Mode and Frontoparietal Network Alterations in Youth at High Familial Risk for Major Depressive Disorder

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Background: Major Depressive Disorder (MDD) is characterized by altered functioning of the default mode network (DMN) and the frontoparietal network (FPN). Although most of this work has focused on examining "static" functional network properties, the assessment of "dynamic" network properties may be particularly valuable in furthering our understanding of DMN-FPN imbalances in MDD. Co-activation pattern (CAP) analyses allows for the examination of brain dynamics by clustering the data into distinct co-activation patterns of brain activity at each time point. Prior studies have found that adults with MDD spend more time in a CAP consisting of DMN and FPN regions as well as transition more frequently between a DMN-FPN CAP with other DMN CAPs relative to healthy controls. However, it remains unclear whether dynamic functional DMN-FPN alterations are a scar of prior episodes or rather, reflect a premorbid risk factor present in healthy youth at familial risk for MDD. Further, it remains unclear whether DMN-FPN alterations predict future depressive symptoms and/or increased stress sensitivity.

Methods: Participants included youth with (High-Risk Group, n = 27) and without (Low-Risk Group, n = 62) a maternal history of MDD. Youth and their mothers completed structured clinical interviews. All youth and Low-Risk mothers were free of lifetime psychiatric disorders. Mothers of High-Risk youth had a history of MDD. Youth participants also completed self-report questionnaires on depressive symptoms (Mood and Feelings Questionnaire, MAFQ), anxiety symptoms (Multidimensional Anxiety Scale for Children, MASC), and perceived stress (Perceived Stress Scale, PSS) at baseline and follow-up assessments every 3 months for 2 years. Additionally, resting state fMRI data were acquired at the baseline assessment for all youth.

A CAP analysis was conducted on the resting state fMRI data. The optimal number of CAPs was determined using consensus clustering, which provides an index of clustering quality. Consensus clustering results indicated an optimal k of 9 and the 9 CAPs included a: 1) CAP with visual system activation, DMN deactivation, 2) CAP with dorsal medial prefrontal cortex activation, visual system deactivation, 3) CAP with DMN activation, FPN and dorsal attention network deactivation, 4)CAP with DMN activation, somatosensory network deactivation, 5) CAP with visual system activation, somatosensory network deactivation, 6) CAP with salience and dorsal attention networks, DMN and visual system deactivation 7) CAP with salience network activation, FPN deactivation 8) CAP with posterior DMN and FPN activation and 9) CAP with salience network activation, DMN deactivation. Given prior CAP studies on MDD, our analyses centered on CAP 8, the DMN-FPN CAP as well as transitions between CAP 8 with other DMN CAPs (CAPs 3 and 4). Both "Time in CAP 8" (i.e., the total number of volumes the participant spent in CAP 8) and "CAP 8 Persistence" (i.e., number of times each participant remained in CAP 8 from t to t + 1) was computed. Additionally, the number of transitions between CAP 8 and CAP 3 as well as CAP 8 and CAP 4 were calculated.

Four ANCOVAs controlling for MAFQ and MASC scores were conducted to examine group differences in Time in CAP 8, CAP 8 Persistence, CAP 8 óCAP3 transition frequencies, and CAP 8ó CAP 4 transition frequencies. CAP metrics showing differences between the High-Risk and Low-Risk group served as predictors in multilevel models (MLM) predicting depression symptoms or perceived stress across the follow-up assessments. Restricted maximum likelihood estimation was used, and random intercepts and slopes were specified for each of the models.

Results: The High-Risk group spent more time in CAP 8 (DMN-FPN) compared to the Low-Risk group, $F(1,85) = 4.53$, $p = 0.036$, $\eta p2 = 0.05$. Additionally, the High-Risk group showed higher persistence in CAP 8 compared to the Low-Risk group, $F(1,85) = 5.22$, $p = 0.025$, $\eta p2 = 0.06$. However, the groups did not significantly differ with respect to CAP 8 óCAP3 and CAP 8 óCAP4 transition frequencies, ps >0.3. Given group differences in Time in CAP 8 and CAP 8 Persistence, we conducted two MLMs predicting depressive symptoms, with each including Group, Time in CAP 8/ CAP 8 Persistence, and study visit as predictors. We conducted two additional MLMs using the same set of variables predicting PSS scores. To correct for multiple comparisons, the p threshold was set to $p < 0.025$ for each set of omnibus MLMs. A significant Group x Visit x CAP 8 Persistence interaction predicting PSS scores emerged, $p = 0.013$. Follow-up MLMs within each group separately showed a Visit x CAP 8 Persistence interaction amongst the High-Risk Group, $p = 0.042$, but not the Low-Risk Group, $p = 0.570$. Those in the High-Risk group exhibiting high CAP 8 Persistence,

reported increasing levels of perceived stress over time, whereas those exhibiting low CAP 8 Persistence had persistently lower PSS scores across time. No other effects were significant.

Conclusions: Dynamic DMN and FPN alterations are present in healthy youth at high familial risk for MDD, potentially reflecting a premorbid vulnerability factor versus a scar of MDD. Additionally, persisting in a DMN-FPN CAP predicted stress sensitivity over time amongst the high-risk youth. High-Risk youth showing high levels of DMN-FPN CAP persistence show increasing levels of stress sensitivity over time, which may enhance future vulnerability to MDD. Dynamic dysfunction in the DMN and FPN may serve as a biomarker of risk for MDD.

Keywords: Adolescent Depression, Resting-State fMRI, Co-Activation Pattern Analysis, Familial Risk for MDD, Stress

Disclosure: Nothing to disclose.

P257. The Effect of Selective Slow Wave Sleep Deprivation on Mood, Rumination, and Default Mode Network Connectivity in Adolescent Depression

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Background: Safe, rapid-acting treatment strategies are urgently needed for depressed youth. In depressed adults, selective slow wave sleep deprivation (SW-SD) rapidly improved depression severity without disrupting sleep duration, but the effects of SW-SD on mood and the underlying neuro-affective mechanisms remain untested in youth. Sleep deprivation reduces default mode network (DMN) connectivity and heightened anterior-posterior DMN connectivity is associated with depression and rumination. In a pilot study, we tested the hypothesis that SW-SD would improve depression, reduce rumination, and stabilize DMN connectivity in adolescent depression.

Methods: Eighteen adolescent participants in a depressive episode (Ages 13-19y, Mean Age = 17.5 ± 1.5yr; 14 Females) completed 3 consecutive nights of overnight polysomnographic sleep monitoring: Baseline (Night 1), SW-SD (Night 2), and Recovery (Night 3). On the SW-SD night, acoustic stimulation suppressed slow wave sleep (SWS) without impacting habitual sleep duration. After each night, clinician-rated depression severity (modified Child Depression Rating Scale) and self-rated rumination severity (modified Ruminative Response Scale) were assessed. Ten participants completed resting-state fMRI scans the mornings after Baseline and SW-SD; connectivity between anterior (medial prefrontal cortex) and posterior (posterior cingulate cortex) DMN nodes was estimated. Linear mixed-effects models adjusted for age, sex, SSRI medication, and baseline depression severity.

Results: There was a significant effect of night on percent slow wave sleep (SWS%; $F[1,13]=71.4$, $p<.0001$) and rumination ($F[1,13]=3.73$, $p=0.0048$) but not clinician-rated depression severity ($F[1,13]=0.9$, $p=0.91$). SWS% was lower on the SW-SD night relative to Baseline ($p<.0001$) and Recovery ($p<.0001$) but did not differ between Baseline and Recovery ($p=0.778$). SW-SD reduced rumination relative to Baseline ($p=0.027$) and the effect was sustained through Recovery sleep ($p=0.017$). Anterior-posterior DMN connectivity also declined after SW-SD relative to Baseline ($F[1,5]=6.13$, $p=0.0351$). In exploratory analyses, reduction in %SWS (SW-SD minus Baseline) was associated with trend-level reductions in rumination ($B=0.47$, $p=0.104$) and Anterior-Posterior DMN connectivity ($B=0.72$, $p=0.072$), and there was a significant relationship between changes in rumination and DMN connectivity ($B=0.74$, $p=0.049$).

Conclusions: SW-SD acutely reduced next-day rumination in depressed adolescents, but not overall depressed mood. Rumination improvement following SW-SD may be supported by stabilization of DMN connectivity. These preliminary data implicate SWS manipulation as a therapeutic approach for modifying DMN-mediated psychological processes, such as rumination, in adolescents.

Keywords: Depression, Slow Wave Sleep, Adolescent, Default Mode Network, Rumination

Disclosure: Nothing to disclose.

P258. Ventral Visual Stream Functional Coupling During Implicit Emotional Face Perception is Associated With Internalizing Symptoms: A Double Dissociation by Face Valence at Baseline and Six Months Post-Scan

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Background: Internalizing symptoms are characterized by alterations in emotional processing, including perceptual biases for negative face stimuli. Both depressive and anxiety disorders are associated with biases for negative stimuli. However, depressive symptoms are also associated with impaired emotional processing that is not specific to negative stimuli, including reduced cortical response to positive stimuli. Given shared perceptual biases for negative emotional faces, and distinct perceptual biases for positive affective faces, there are likely shared and distinct neural circuits associated with depression and anxiety symptoms.

The anterior lateral fusiform gyrus is associated with face perception and its response to emotional faces is modulated by bottom-up signals from more posterior portions of the ventral visual stream, as well as more anterior temporal, limbic, and prefrontal regions. The fusiform gyrus offers a potential novel target for intervention in internalizing disorders given its key role in emotional face perception. Furthermore, it remains unclear how fusiform functional coupling is related to symptom improvement over time. In this emotional face functional MRI study, we hypothesized that fusiform coupling during happy face perception would be associated with depression symptoms, accounting for anxiety symptoms, while fusiform coupling during sad face perception would be associated with internalizing symptoms generally.

Methods: Sixty-one adults (mean age 23.13 ± 2.86, 36 female, 3 non-binary) seeking treatment for psychological distress underwent 3T MRI scanning and completed an implicit emotional face processing task in the scanner. Participants were administered the Hamilton Depression and Anxiety Rating Scales. Clinical scores were transformed and summed for a general internalizing symptom score. The transformed anxiety scores were also subtracted from the depression scores to create a metric of the relative contributions of depressive and anxiety symptoms (i.e., HAMD-HAMA). We modeled functional coupling during implicit perception of sad and happy emotional faces. Using an anterior lateral fusiform gyrus seed consistent with the fusiform face area, generalized psychophysiological interaction analyses were performed at the whole brain level ($p<0.001$, uncorrected, $k=20$) with summed internalizing symptom scores, HAMD-HAMA scores, gender, and age included in all models.

A subsample of 36 participants (mean age 23.56 ± 3.11, 23 female, 1 non-binary) returned 6 months post-scan and were administered the HAMD and HAMA once more. We modeled anterior lateral fusiform gyrus coupling during sad and happy face

perception with summed HAMD and HAMA scores at follow-up, controlling for baseline internalizing symptoms and demographic characteristics ($p < 0.001$, uncorrected, $k = 20$).

Results: During perception of sad faces, there was a positive association between internalizing symptom severity and coupling of the fusiform gyrus with the temporal pole ($T = 6.79$, $k = 32$), right inferior temporal gyrus ($T = 6.47$, $k = 27$), and orbitofrontal cortex ($T = 5.59$, $k = 77$). In contrast, for the happy face condition, there was a negative association between internalizing symptoms and fusiform coupling with the temporal pole ($T = 5.68$, $k = 48$) and left superior parietal cortex ($T = 4.20$, $k = 117$). There were no significant associations for the HAMA-HAMD variable in either task condition.

Internalizing symptom severity six months post scan was negatively associated with coupling of the fusiform gyrus and medial prefrontal cortex (mPFC, $T = 4.74$, $k = 109$) during happy face perception. The relationship was such that greater coupling was associated with more internalizing symptom improvement at follow up. There were no significant associations during sad face perception and follow up scores.

Conclusions: These findings indicate a potential double dissociation between internalizing psychopathology and fusiform-temporal cortex coupling during sad versus happy emotional face perception. The positive association during sad face perception and the negative correlation during happy face perception could be related to the relative salience of negative and positive emotional stimuli in internalizing disorders. These findings lend support to the importance of perceptual processes and the ventral visual stream in internalizing disorders. Furthermore, greater fusiform-mPFC coupling during happy face perception predicted more improvement six months post scan, highlighting the importance of neural response to positive affective stimuli to symptom change over time.

Keywords: Internalizing Disorders, Ventral Visual Stream, Longitudinal Imaging

Disclosure: Nothing to disclose.

P259. Approach-Avoidance Behavior and Conflict in Remitted Depression: Neural Correlates and Latent Decision-Making Processes

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Background: Abnormalities in approach-avoidance (Ap-Av) behaviors are implicated in a host of psychiatric disorders. Conflict between approach and avoidance typically arises when a decision has both rewarding and aversive consequences and is particularly central for major depressive disorder (MDD) and anxiety disorders. Rodent and human studies have implicated the amygdala, hippocampus, striatum, orbitofrontal cortex (OFC), and medial prefrontal cortex (mPFC) in Ap-Av behavior and conflict. Moreover, recent work has provided converging evidence for mPFC recruitment during Ap-Av conflict and aversive responsiveness across non-human primates and humans (Ironside et al., 2020). Importantly, in our recent neuroimaging study, individuals with current MDD showed reduced approach-related dorsolateral prefrontal cortex (dlPFC) activation as well as reduced avoidance-related pregenual anterior cingulate cortex (pgACC) activation. Whether aberrant Ap-Av-related neural responses persist after recovery from MDD remains unclear; understanding such mechanisms may provide important insights into relapse risk for individuals with a history of depression.

Methods: Preliminary analyses from this ongoing study were based on 51 symptom-free and unmedicated individuals, including 17 remitted MDD (rMDD, age mean=25.2, m/f: 0/17, BDI mean= 0.55) and 34 healthy controls (HC, age mean=25.9, m/f: 10/24, BDI mean=0.59). Participants performed a novel adaptive Ap-Av conflict (aAAC) task during functional magnetic resonance imaging (fMRI). In this task, participants were offered a combination of monetary reward and aversive electrical stimulation parametrically spanning a continuum of reward and aversion across ten levels. Participants decided via joystick movements to either receive the reward and aversive stimulation (approach) or to forgo the offer (avoid), modeling Ap-Av behavior. For each trial, prior Ap-Av decisions were used to determine the next trial's offer in order to adaptively sample closely around individuals' decision boundaries; this strategy allowed to induce variable amounts of decision conflict while sampling the individual's reward-aversion space. Measures of Ap-Av behavior and conflict were assessed in a multimodal and integrative approach utilizing skin conductance response (SCR) measurements, fMRI, and computational modeling (i.e., drift-diffusion modeling, DDM) to probe neural and latent decision-making processes.

Results: General successful task performance and conflict induction in the aAAC task emerged in the form of a sigmoidal take probability and higher reaction times around the individual decision boundaries. Similarly, SCRs were higher in approach compared to avoidance trials [$t(44) = 4{,}14$, $p < 0.001$] and parametrically followed reaction times, with higher SCRs in slow response trials which is indicative of high conflict [$t(44) = 3.57$, $p < 0.001$]. fMRI analyses revealed expected Ap-Av-related neural patterns such as higher dorsal anterior cingulate (dACC) and striatal activation during approach trials and higher mPFC and OFC activation during avoid trials (all $FWESVC < 0.001$). Further analyses showed that dACC and dlPFC recruitment was strongly related to the degree of conflict as parametrically defined by distance to the decision boundary and increased reaction times, suggesting successful conflict induction (all $FWESVC < 0.001$). Group comparisons revealed that rMDD participants reported higher general trait avoidance scores [$t(48) = 2.43$, $p = 0.02$] as assessed by the Cognitive and Behavioral Avoidance Scale. On a neural level, decreased avoidance-related pgACC activation [$t(49) = 2.29$, $p = 0.027$] emerged exclusively in rMDD participants paralleling observations in individuals with current MDD (Ironside et al., 2020). In addition, rMDD participants more strongly recruited the mPFC with increasing conflict than HCs [$t(49) = 3.01$, $p = 0.002$]. These observations were complemented by posterior distributions on DDM model parameters showing that rMDD participants were more sensitive to the impact of aversiveness on evidence accumulation toward avoidance (rMDD<HC, 98% posterior samples). Additionally, whereas HCs showed an overall starting point bias toward approach (>99% posterior samples; independent of reward and aversiveness), rMDD participants showed no such bias. Finally, replicating previous findings in MDD patients (Pedersen et al., 2021), rMDD and HC showed opposite influences of accumbens activity on this starting point bias (HC > rMDD, 92% posterior samples). This was reflected in HC greater accumbens activity on a trial-to-trial basis related to an increased bias to approach, whereas increased accumbens activity in rMDD was related to an increased bias to avoid.

Conclusions: Persistent aberrant approach-avoidance behavior and conflict processing may be important vulnerability factors for asymptomatic individuals with a history of depression and may index a heightened risk of relapse. Using a novel adaptive Ap-Av conflict task, we provide preliminary support for residual abnormalities in Ap-Av-related neural and latent decision-making processes in rMDD, which parallel alterations previously

observed in patients with MDD. Elucidating neural and latent mechanisms linked to persistent abnormal Ap-Av decision-making promises to advance the development of new prevention and treatment strategies in remitted and acute depression.

Keywords: Approach-Avoidance Conflict, Major Depression, Decision-Making, Functional MRI (fMRI), Remitted Depression

Disclosure: Nothing to disclose.

P260. Contributions of Prenatal Exposure to Air Pollution and Childhood Housing Insecurity to Adolescent Hippocampal Volumes and Depression

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Background: Housing insecurity and exposure to air pollution are common, modifiable threats to child development that often disproportionately affect low-income communities of color. Some studies have demonstrated that material hardship and prenatal air pollution exposure have compounding impacts on child neurodevelopment. Separately, housing stress and air pollution have been linked with risk for depression. Here, we sought to parse the shared and unique contributions of lifetime housing insecurity and prenatal air pollution exposure on adolescent depression and hippocampal volume as a potential mechanism.

Methods: The Mothers and Newborns longitudinal birth cohort recruited largely low-income Black and/or Latinx women in northern Manhattan between 1998 and 2006. Exclusion criteria included maternal smoking during pregnancy, gestational diabetes, hypertension, or HIV. Beginning in the third trimester of pregnancy, mothers completed questionnaires assessing their environmental exposures, housing status, and other characteristics every two years. At birth, maternal blood was assessed for the presence of benzo[a]pyrene (a polycyclic aromatic hydrocarbon (PAH)) DNA adducts, which are DNA lesions due to air pollution exposure. Children underwent structural MRI at one timepoint between age 14 and 21 years, and the Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS) was conducted. Multiple regression ($n = 197$) examined the associations between hippocampal volumes or depressed mood during adolescence and either the presence of PAH DNA adducts at birth, cumulative housing insecurity, or their interaction.

Results: Housing insecurity over the first 14 years of life ($\beta = 0.68$, $p = .004$) and the presence of PAH DNA adducts at birth were associated with increased risk for depressed mood ($\beta = 1.54$, $p = .009$) independently, but did not interact. This interaction was significant on left hippocampal body volume ($\beta = .30$, $p = .008$).

Conclusions: The current findings provide evidence that childhood housing insecurity and prenatal air pollution exposure, two ubiquitous environmental insults, have unique and convergent effects on adolescent depression and hippocampal structure. These findings are consistent with previous studies establishing associations between depression and both housing insecurity and air pollution exposure.

Keywords: Housing, Hippocampus, Depression

Disclosure: Nothing to disclose.

P261. Cognitive Effort Preference in Veterans With Suicide Attempt Histories

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Background: Suicide attempts (SA) are increasing in the United States, especially in veterans. Critical to suicide prevention is understanding individual differences that demarcate the subset of suicide ideators who attempt suicide. Cognitive theories attribute SA to schema (gist)-based negative interpretations of environmental events. In light of over-general autobiographical memory and facile solutions in problem-solving tasks in SA survivors, aversion to expending cognitive effort itself may be a neurobehavioral marker of SA risk.

Methods: In veterans receiving care for mood disorder, we compared choices in a cognitive effort discounting task and evidence-gathering in a beads task between veterans with (SAHx+; $n = 26$) versus without (SAHx-; $n = 22$) a history of SA. Groups did not differ in current depressed mood or in a proxy metric of premorbid intelligence.

Results: Compared to SAHx- participants, SAHx+ participants self-reported significantly more severe cognitive problems in most domains, and also eschewed choice to earn higher monetary reward if earning it required a slightly (but not greatly) increased working memory (WM) demand relative to an easy WM task. There was no group difference, however, in extent of evidence-gathering before declaring a conclusion in a beads task.

Conclusions: These preliminary data suggest that aversion to expenditure of cognitive effort, potentially as a component of cognitive difficulties, may be a marker for SA risk.

Keywords: Suicide Risk Factors, Cognitive Effort, Veterans, Mood Disorder

Disclosure: Nothing to disclose.

P262. Oscillatory Dynamics of Frontal-Striatal Circuits Underlie Symptom Dimensions Within Major Depressive Disorder

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Background: Patients with major depressive disorder (MDD) present with a heterogenous collection of symptoms. Factor analysis of depressive symptoms has consistently found two prominent dimensions within depression: anhedonia and anxiety. However, the neural oscillatory basis for how these symptom dimensions of depression arise from specific circuits in the brain is unknown. Previous research suggests that reward-based decision-making may elicit systematic differences in neural activity as a function of depressive symptoms.

Methods: In our first experiment (sub-study of NCT03449979), 42 participants in a major depressive episode and 41 age and sex-matched euthymic control participants ($N = 83$; 66 women) performed a streamlined version of the expenditure of effort for reward task (S-EEfRT) with EEG. Behavior was quantified along two dimensions: goal-directed behavior was defined as willingness to exert effort for an uncertain reward and reward-evaluation was the degree to which effort was strategically exerted for higher incentives. We analyzed interregional phase-amplitude coupling between prefrontal cortex (PFC) and posterior regions, and related coupling strength to behavior and symptom dimensions. In our follow-up experiment (NCT05084924), 48 participants with MDD performed the S-EEfRT during EEG and functional MRI. This dataset was used to replicate the findings from the first experiment and to further investigate the contributions of frontal-striatal circuitry to behavior in the S-EEfRT. Task-evoked functional connectivity in fMRI was quantified between the striatum, dorsal caudate versus

nucleus accumbens, and the PFC, dorsolateral versus ventromedial.

Results: In experiment 1 (N = 79 analyzed), anhedonia displayed a significant negative relationship with goal-directed behavior (t = -2.19, p = 0.03) and with delta-beta coupling from PFC to left motor cortex (t = -2.24, p = 0.03). Similarly, anxiety was positively related to theta-gamma coupling between PFC and posterior visual areas ($p < 0.05$, k = 11) and with anxiety symptoms (t = 2.15, p = 0.04). We did not find a significant effect of biological sex in our analyses. In experiment 2, we replicated the presence of delta-beta coupling from PFC to motor cortex (t = 2.242, p = 0.047) and its relation to goal-directed behavior (r = 0.265) and symptoms of anhedonia (r = -0.373). Our task-evoked functional connectivity analysis recapitulated canonical circuitry between lateral PFC and dorsal caudate (t = 3.234, p = 0.008) that was positively correlated with delta-beta coupling (r = 0.718, p = 0.029), but not theta-gamma coupling (r = -0.358, p = 0.344). In addition, we found evidence for the canonical circuitry between medial PFC and nucleus accumbens (t = 5.494, $p < 0.001$), although the strength of this circuit was not correlated with the strength of theta-gamma coupling (r = -0.489, p = 0.182). While the strength of functional connectivity in both circuits was predictive of goal-directed behavior ($r > 0.48$), only medial PFC to nucleus accumbens was predictive of reward-evaluation (r = 0.362).

Conclusions: These experiments provide a novel neurobehavioral model for the biological basis of symptoms of depression. The underlying cause of anhedonia may be reduced integrity of circuitry for goal-directed behavior, wherein lateral PFC displays reduced connectivity to the dorsal caudate nucleus and decreased delta-beta coupling to the motor cortex. Additionally, symptoms of anxiety might drive increased engagement of reward-evaluation circuitry, such that medial PFC displays increased connectivity with nucleus accumbens and elevated theta-gamma coupling to perceptual regions. The discovery that frontal-striatal circuitry exhibits distinct oscillatory control signals that are altered by specific symptoms of depression will provide novel targets for individually precise interventions using non-invasive brain stimulation.

Keywords: Research Domain Criteria (RDoC), Major Depression Disorder, Cognitive Neuroscience, Functional MRI (fMRI), EEG

Disclosure: Nothing to disclose.

P263. Sex Differences in Sleep Slow-Wave Activity in Major Depression May Reflect Fundamental Differences in Regulation of Neuroplasticity and Disease Pathophysiology

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Background: Males with major depressive disorder (MDD) have been shown to exhibit less slow-wave activity (SWA) than females with MDD or their healthy counterparts. SWA has been implicated in the homeostatic regulation of neuroplasticity; thus it is possible that due to these SWA deficiencies, males with MDD may have impairments in the modulation of cortical excitability, and by extension learning and memory. This could also suggest that manipulating SWA in males with MDD could serve to reduce these deficiencies and improve performance. Therefore, the aim of the present study was to examine sex differences in short-term memory performance and cortical excitability at baseline and in response to selective slow-wave disruption.

Methods: 17 individuals (7 M) with MDD were recruited for an ongoing clinical trial. Auditory stimulation was utilized to disrupt slow-wave sleep on one of two overnight sleep laboratory visits (Baseline, SWD) separated by one week. Following each overnight visit, a computer-based N-back task was administered to assess short-term memory, and motor evoked potentials (MEP) generated from transcranial magnetic stimulation (TMS) were measured as a proxy measure of cortical excitability. Repeated measures ANOVA was used to evaluate sex differences in memory performance at baseline and change in MEP amplitude following SWD with condition (Baseline, SWD) as the within-subject factor and sex (F, M) as the between-subjects factor.

Results: Results from repeated measures ANOVA revealed a significant main effect of sex for accuracy on the N-Back, (F(1,16) =7.42, p = .02). At baseline, males with MDD showed significantly worse accuracy on the N-Back than females (F(1,16) =11.49, $p < .01$). However, following SWD, this discrepancy was reduced, and males showed significantly improved performance, t(6)=-2.10, p = .04, that was no longer significantly different from females. With regard to cortical excitability, results from repeated measures ANOVA revealed a significant Condition x Sex interaction, (F(1,15) =4.42, p = .05) indicating that while males with MDD exhibited a trend towards increased MEP following SWD, t(6)=-1.85, p = .057), females demonstrated no change.

Conclusions: Our results demonstrate that SWD may differentially impact males and females with MDD. While females did not exhibit any modulation of cortical excitability following SWD, males with MDD showed evidence of increasing cortical excitability, providing preliminary evidence that SWA is directly associated with the regulation of neuroplasticity in males with MDD. Relatedly, this increase in cortical excitability was also paralleled by an improvement in memory performance on the N-back, suggesting that SWA manipulation can potentially be used as a future intervention target. These results may also suggest that the observed sex differences in SWA in those with MDD reflect a fundamental neurobiological difference in disease mechanisms in males and females.

Keywords: Neuroplasticity, Slow-Wave Activity, Sleep, Major Depressive Disorder

Disclosure: Nothing to disclose.

P264. Stress Levels, Psychological Symptoms, and C-Reactive Protein Levels in COVID-19: A Cross-Sectional Study

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Background: Although many studies have pointed out a possible relationship between COVID-19 and the presence of psychiatric and neurological disorders, the majority of the studies have important limitations, such as not having a control group of individuals without COVID-19 or reporting the presence of psychiatric conditions through self-reported tools. This study investigates the role of COVID-19 infection on stress, anxiety, depression, insomnia, and C-reactive protein (PCR) levels.

Methods: This cross-sectional study included a matched sample of adult individuals with COVID-19 (cases; n = 114) or without COVID-19 (controls; n = 236). We used Mini International Neuropsychiatric Interview – Plus for the diagnosis of psychiatric disorders. The severity of depressive or insomnia and anxiety symptoms were assessed using the Hamilton Rating Scale for Depression and for Anxiety, respectively. The stress level was

evaluated by an inventory of stress symptoms adapted from Checklist-90-R. Blood samples were used to assess the CRP levels. The comparison between groups was tested using Man-Whitney U test. The data showed as absolute and relative frequency were compared using the chi-squared test. Spearman correlation was used to test the association between two continuous variables.

Results: Findings showed greater severity of depressive symptoms ($p = 0.034$), higher levels of stress ($p = 0.020$), and greater CRP levels ($p = 0.014$) in individuals with COVID-19 when compared to controls. The severity of depressive ($p = 0.005$) and insomnia ($p = 0.006$) symptoms, as well as the CRP levels ($p = 0.013$) were more remarkable in individuals with moderate/severe COVID-19 symptoms than in asymptomatic or individuals presenting mild symptoms. We found a positive correlation between stress and severity of anxiety, depression, or insomnia in individuals with or without COVID-19 ($p < 0.001$). There was a positive correlation between PCR levels and severity of anxiety, depression, or levels of stress in individuals with COVID-19. Interestingly, we found that individuals with COVID-19 and depression had greater CRP levels as compared to individuals with COVID-19 without current MDD ($p = 0.023$).

Conclusions: Individuals with COVID-19 showed greater severity of psychological symptoms, which may impact the development of psychiatric disorders in the future. CPR levels seem to be a promising biomarker to help on the earlier detection of the post-COVID depression. Longitudinal studies are needed to understand the clinical trajectories of these individuals.

Keywords: Emotional Stress, Depression and Anxiety, Inflammation, c-Reactive Protein, COVID-19

Disclosure: Nothing to disclose.

P265. The Relationship Between Free Triiodothyronine (FT3) and Performance on the Iowa Gambling Task in Bipolar Disorder: Sex Differences Explored

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Background: Thyroid dysfunction has been implicated in the pathophysiology of bipolar disorder (BD) because of the known effects on cognition and mood. Here we aimed to examine the effect of thyroid hormones and behavior on a reward learning paradigm, the Iowa Gambling Task (IGT).

Methods: The study included 101 males and 94 females with a diagnosis of BD. BD diagnosis was confirmed by the structured clinical interview based on the DSM-IV (SCID-IV). For both males and females, we performed a repeated-measures analysis of covariance (RM ANCOVA) with five blocks of the IGT as the within-subjects factor, free triiodothyronine (FT3) high-versus-low median split as the between-subjects factor, and age in years as a covariate. To assess the associations between three expectancy valence (EV) measures (consistency, attention to losses, and recency) and FT3, we performed partial Pearson correlations controlled for age.

Results: We observed a significant interaction between FT3 and IGT blocks in males with BD ($F(4,95)=2.9$, $p = 0.02$), but not in females with BD ($F(4,88)=0.2$, $p = 0.9$). For males, those in the low FT3 group appeared to have a more gradual and normal learning curve compared to those in the high FT3 group. In terms of learning strategies, we observed a significant negative correlation between FT3 and consistency in males ($R = -0.3$, $p = 0.001$), indicating that they employ a more exploratory approach in association with higher FT3. We did not observe this relationship in the females ($R = -0.03$, $p = 0.7$). We did not find significant associations in any of the other EV measures.

Conclusions: We observed differential reward learning styles in males with high versus low FT3 in the IGT. These results suggest a role of the thyroid in emotional learning and exploratory behaviors in males with BD.

Keywords: Bipolar Disorder, Monetary Reward, Thyroid Hormone

Disclosure: Nothing to disclose.

P266. Different Brain Functional Connectome Changes Following 12-Week Treatment With Mixed Amphetamine Salts in ADHD Youth With and Without Familial Risk for Bipolar I Disorder

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Background: Prevalence rates of attention deficit/hyperactivity disorder (ADHD) in youth with bipolar I disorder (BD) are substantially higher than the general population, and prospective studies have found that prodromal ADHD commonly precedes the onset of BD and significantly increases risk for developing BD. Additionally, having a first-degree relative with BD robustly increases the risk of developing BD, and youth with a first-degree BD relative exhibit higher rates of ADHD and more severe ADHD symptoms. Although the pathoetiological mechanisms associated with the risk of developing BD in youth with ADHD remain poorly understood, retrospective studies suggest that antecedent psychostimulant exposure for the treatment of ADHD may precipitate or exacerbate manic symptoms in a subset of vulnerable individuals. The initial onset of BD frequently occurs during the peripubertal period which is associated with dynamic changes in frontal cortex connectivity with limbic regions implicated in emotional regulation. Although cross-sectional resting-state MRI studies indicate that both BD and ADHD are associated with hypo- and hyper-connectivity in different cortical networks, the majority of these studies did not control for BD familial risk, ADHD comorbidity, and/or psychostimulant exposure. In the present study, we used graph theoretical analysis to investigate the effects of 12-week psychostimulant treatment on brain network organization and connectivity in ADHD youth with ('high-risk', HR) and without ('low-risk', LR) a first-degree relative with BD.

Methods: ADHD youth (ages 10-18 years) with ('high-risk' for BD, HR) and without ('low-risk', LR) a first-degree relative with BD and typically developing healthy controls (HC) were enrolled. LR youth received 12-week open-label mixed amphetamine salts-extended release (MAS-XR), and HR youth were randomized to MAS-XR or placebo (PBO). All ADHD patients met DSM-5 criteria for ADHD (any type), had no exposure to psychostimulants for at least 3 months prior to enrollment, and had no comorbid mood, conduct, eating, or psychotic disorders. Resting-state fMRI scans were acquired on a Philips 3.0 T MR scanner at baseline and week 12. The Automated Anatomical Labeling (AAL90) template included 90 regions/nodes, some of which are implicated in attention and emotion regulation. Whole-brain global- and nodal-level network topological properties were evaluated using graph theoretical analysis. Global topological metrics included global efficiency (Eglob), local efficiency (Eloc), clustering coefficient (Cp), characteristic path length (Lp), normalized clustering coefficient (γ), normalized characteristic path length (λ) and small-worldness

scalar (σ). Nodal topological metrics reflecting centrality include degree, betweenness, and efficiency. We first compared the three patient groups and the HC group at baseline and endpoint using MANCOVA. Group x time interaction effects were then investigated using linear mixed models with age and sex as covariates. FDR correction was applied to control for multiple comparisons.

Results: A total of n = 135 youth were included in the analysis (HC, n = 45; LR-MAS, n = 46; HR-MAS, n = 28; HR-PBO, n = 16). No significant group differences were observed for age, sex, handedness, or prior psychostimulant exposure in youth with ADHD. At baseline, LR-MAS youth exhibited reduced global efficiency Eglob and increased characteristic path length Lp, as well as topological abnormalities in the superior parietal cortex and orbital and medial prefrontal cortex (mPFC) compared with HC. Both HR-MAS and HR-PBO exhibited reduced nodal efficiency in the mPFC, and HR-MAS additionally exhibited reduced degree in the cingulum and increased caudate efficiency, compared with HC. Following 12-week MAS-XR treatment, significant group x time interactions were observed between LR-MAS and HR-MAS for global efficiency ($p = 0.012$), which increased in LR-MAS but not HR-MAS, and characteristic path length ($p = 0.0045$), which decreased in LR-MAS and increased in HR-MAS. Additionally, group x time interactions were observed for nodal efficiency of the right amygdala ($p = 0.0001$), which decreased in HR-MAS but not LR-MAS, and nodal degree in the left superior parietal gyrus ($p = 0.0002$), which increased in LR-MAS but not HR-MAS. For HR-PBO vs. HR-MAS, a significant group x time interaction was observed for betweenness in the right inferior frontal gyrus ($p = 0.0005$), which increased in HR-MAS but not HR-PBO. Consistent with a normalizing effect, LR-MAS did not exhibit any global or nodal differences compared with HC at week 12, whereas HR-MAS exhibited significant residual reductions in left mPFC and right caudate nodal efficiency and new reductions in the right amygdala nodal efficiency.

Conclusions: Psychostimulant-free ADHD youth with and without a BD family history exhibit different global and nodal network connectivity abnormalities compared with healthy youth at baseline, and their functional brain connectomes change differently following 12-week MAS-XR treatment. These differences in network connectivity and plasticity suggest that ADHD in conjunction with BD family history is associated with different neuropathophysiologic processes that are less amenable to modification with psychostimulant treatment.

Keywords: Bipolar Disorder, ADHD, Resting State Brain Imaging, Prodrome

Disclosure: Nothing to disclose.

P267. Differential Changes in Left Uncinate Fasciculus Microstructure following 12-Week Mixed Amphetamine Salts in ADHD Youth With and Without Familial Risk for Bipolar I Disorder: A Prospective DTI Study

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Background: Prevalence rates of attention deficit/hyperactivity disorder (ADHD) in youth with bipolar I disorder (BD) are substantially higher than the general population, and prospective studies have found that prodromal ADHD commonly precedes the onset of BD and significantly increases risk for developing BD. Additionally, having a first-degree relative with BD robustly increases the risk of developing BD, and youth with a first-degree BD relative exhibit higher rates of ADHD and more severe ADHD symptoms compared with ADHD youth with healthy parents. Although the pathoetiological mechanisms associated with the risk of developing BD in youth with ADHD remain poorly understood, retrospective studies suggest that antecedent psychostimulant exposure for the treatment of ADHD may precipitate or exacerbate manic symptoms in a subset of vulnerable individuals. Furthermore, the initial onset of BD frequently occurs during the peripubertal period which is associated with dynamic changes in frontal cortex connectivity with limbic regions implicated in emotional regulation. The uncinate fasciculus (UF) is the main fiber tract connecting the ventral prefrontal cortex with the amygdala, and meta-analyses of cross-sectional diffusion tensor imaging (DTI) studies indicate that BD patients exhibit lower bilateral UF fractional anisotropy (FA). Although prior evidence suggests that ADHD is not associated with alterations in UF FA, the majority of these studies did not control for BD familial risk and/or psychostimulant exposure. The present DTI study investigated the effects of 12-week psychostimulant treatment on bilateral UF FA in ADHD youth with ('high-risk', HR) and without ('low-risk', LR) a first-degree relative with BD. It was hypothesized that HR ADHD youth would exhibit lower bilateral UF FA at baseline, and greater reductions in bilateral UF FA following 12-week psychostimulant treatment, compared with LR ADHD youth and healthy controls. Exploratory analyses investigated associations between UF FA and relevant symptoms including mania and dysregulation.

Methods: ADHD youth (ages 10-18 years) with ('high-risk', HR) and without ('low-risk', LR) a first-degree relative with BD and typically developing healthy controls (HC) were enrolled. LR youth received 12-week open-label mixed amphetamine salts-extended release (MAS-XR), and HR youth were randomized to MAS-XR or placebo (PBO). All ADHD patients met DSM-5 criteria for ADHD (any type), had no exposure to psychostimulants for at least 3 months prior to enrollment, and had no comorbid mood, conduct, eating, or psychotic disorders. Clinician ratings of ADHD (ADHD-RS), mania (YMRS), depression (CDRS-R), global functioning (CGAS), and global symptom severity (CGI-S), and parent-rated Child Behavior Checklist (CBCL), were obtained at baseline and week 12. DTI scans were acquired on a Philips 3.0 T MR scanner at baseline and week 12. Tract-based analysis (Tract-Based Spatial Statistic, TBSS) were applied to evaluate large fiber tracts, and FA and mean diffusivity (MD) maps were calculated. FA and MD were extracted from the bilateral UF, and group x time interaction effects were assessed using linear mixed effect models with age and sex as covariates. Linear correlation analyses evaluated associations between baseline-endpoint changes in DTI metrics and clinical ratings.

Results: A total of n = 145 (HR, n = 45; LR, n = 51; HC, n = 49) youth (mean age: 14.0 ± 2.5 years) were enrolled. There were no significant baseline group differences in demographics including sex, race, pubertal status, hand dominance, body mass index, and in the ADHD groups prior exposure to psychostimulants and ADHD age at onset. At baseline, there were no significant main effects of group (4) for left ($p = 0.09$) or right ($p = 0.85$) UF FA or left ($p = 0.23$) and right ($p = 0.16$) UF MD, and neither UF FA nor UF MD correlated with symptom ratings. Following 12-week MAS-XR treatment (median MAS-XR dose: LR-MAS: 17.7 ± 5.2 vs HR-MAS: 15.8 ± 5.7, $p = 0.12$), the LR-MAS group exhibited significantly greater reductions in ADHD-RS total score ($p = 0.015$) and inattentive subscale scores ($p = 0.0001$) compared with HR-MAS, and a larger percentage of LR-MAS youth achieved remission (ADHD-RS endpoint score of <18) compared with HR-MAS (92% vs. 61%, $p = 0.0001$). For all ratings, there were no significant baseline-endpoint change differences between HR-MAS and HR-PBO. For left UF FA, a significant group by time interaction was found ($p = 0.005$), and the baseline-endpoint change in the HR-MAS group differed significantly from HR-PBO ($p = 0.003$), LR-MAS

($p = 0.015$) and HC ($p = 0.007$). Specifically, left UF FA decreased significantly in HR-MAS (-5.3%, $p = 0.029$) but not HR-PBO (+3.4%, $p = 0.37$), LR-MAS (+1.7%, $p = 0.41$), or HC (+2.6%, $p = 0.27$). Changes in left UF FA did not correlate significantly with changes in any symptom rating in either HR-MAS or LR-MAS.

Conclusions: Contrary to our first hypothesis, psychostimulant-free ADHD youth with a BD family history did not exhibit lower bilateral UF FA compared with ADHD youth without a BD family history or healthy controls. In support of our second hypothesis, ADHD youth with, but not without, a BD family history exhibited reductions in left UF FA following 12-week MAS-XR treatment. This differential response to MAS-XR in ADHD youth with a BD family history may be relevant to lower UF FA observed in youth with BD, and additional studies are warranted to evaluate the long-term impact of psychostimulant exposure.

Keywords: Bipolar Disorder, ADHD, Diffusion Tensor Imaging (DTI), Prodrome

Disclosure: Nothing to disclose.

P268. A Neural Signature for Emotion Regulation in Depressed Adults: Associations With Real-World Management of Stress and Suicidal Ideation

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Background: Emotion regulation (ER), the ability to modulate emotional responses to meet situational demands and personal goals, is essential to psychological wellbeing. Across age groups, individuals who report ER difficulties also experience higher levels of suicidal ideation (SI). ER is often assessed by explicitly prompting participants to regulate; however, this may not approximate how people regulate in their daily lives. In the current study, we aimed to clarify how directed and non-directed ER in the context of an fMRI task relates to management of stress and SI in real-world settings, in a sample of unmedicated depressed adults. Based on prior work linking mood- and anxiety-related psychopathology to non-directed ER, we hypothesized that only non-directed ER would predict lower stress-responsive increases in SI.

Methods: To quantify ER, we applied a two-step multivariate pattern analysis (MVPA) to train and test a signature that identifies ER-related neural activity. The training sample consisted of 77 unmedicated adults, including depressed and healthy individuals, who completed two fMRI tasks. In Task 1, participants were prompted to simply view negative images ("look) or use an ER strategy of reappraisal ("reappraise") while viewing. In Task 2, participants were cued to recall negative autobiographical memories while using either an "immersed" (i.e., first-person) or "distanced" (i.e., third person) perspective, for 12s. The distanced perspective represents a regulated approach to recall. All memory trials began with a 10s non-directed recall period in which participants were not instructed to immerse or distance. A binary classifier was first trained on Task 1 data to identify a spatially distributed neural pattern (i.e., signature) associated with reappraisal trials (AUC = 0.63, 1,000-permutation p-value<0.001). In a subset of 33 depressed adults, the signature was used to track non-directed ER expressed during the Task 2 recall period and directed ER during the "distanced" period. Neural signature output in Task 2 reflects the degree to which participants' neural activity resembles the putative neural systems underlying reappraisal in Task 1. Participants also completed a 7-day ecological momentary assessment (EMA) period during which they reported on SI and stressors six times daily, within 2-hour epochs. Stress-responsive increases in SI were used as an indication of difficulty regulating negative mood states.

Results: Signature output during Task 2 was higher for distance trials compared to immerse trials (b[5925]=-0.030, SE = 0.070, $p < .001$). We examined whether signature output for non-directed ER (recall period) and directed ER ("distanced" period) predicted changes in SI following negative stressors. A time-varying stress indicator was used to denote EMA epochs with and without stressors. Separate mixed effects regression models were fit with stress-reactive SI change as the outcome variable; signature output, the time-varying stress indicator (stressor vs. no stressor), and their interaction, as the predictors; subject-specific random intercepts, and an AR1 covariance matrix. There was a significant interaction between the stress indicator and signature output for the recall period, with higher signature output predicting greater SI increases after a stressor (b[920]=5.70; SE = 2.73; t = 2.08; $p = 0.037$). The interaction for signature output during the "distanced" period (i.e., directed ER) and stress-responsive SI was not significant (b(857)<0.00; SE < 0.00; t = 1.07; $p = 0.284$).

Conclusions: In validation of the neural signature, Task 2 distance trials were associated with the same neural systems underlying Task 1 reappraisal trials. Signature output for the directed ER period was not associated with stress-responsive SI, as hypothesized. However, contrary to our expectation, signature output for the non-directed ER period predicted greater (rather than lower) stress-responsive SI. While it may appear paradoxical that greater engagement of neural systems supporting ER is related to increased stress-reactive SI, in the context of depression, engagement of a neural pattern associated with dampening initial affective responses may be a compensatory response in anticipation of difficulty managing negative affective states. These findings have important implications for mood- and suicide-related clinical interventions in depressed populations.

Keywords: Emotion Regulation, Functional MRI (fMRI), Ecological Momentary Assessment, Suicidal Ideation, Depression

Disclosure: Nothing to disclose.

P269. Hopelessness as a Key Indicator of Suicide Risk: Clinical and Magnetoencephalographic Results

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Background: A suicide crisis is a medical emergency. Clinical and neurobiological targets for the acute suicide risk state are critically needed for better identification and treatment. However, patients often minimize suicidal ideation (SI) due to concerns of hospitalization and stigma; additionally, SI may be particularly reactive to dramatic fluctuations over time, necessitating the identification of non-SI indicators of active suicide risk. There is a longstanding research literature linking hopelessness, or pessimism about the future, with risk for suicidal thoughts, behavior and death. However, less is known about whether hopelessness can be a proximal sign for imminent suicide risk as distinct from a chronic risk factor which places an individual for increased suicide risk over their lifetime. Additionally, understanding the neural correlates of hopelessness in the context of suicide risk could be useful for future intervention development and evaluation of response to treatment in at-risk individuals. In this analysis, we examined hopelessness as a potential indicator of active suicide risk by comparing patients who had experienced a suicide crisis in

the last two weeks to those who had experienced a suicide crisis more than a year previously. Additionally, in a sample of participants across the suicide risk spectrum, we examined electrophysiological correlates of hopelessness using magnetoencephalography (MEG) to evaluate hopelessness as a potential biomarker for acute suicide risk.

Methods: Participants: Participants were recruited as members on one of five groups across the continuum of suicide risk, ranging from individuals who recently attempted or seriously considered suicide in the last two weeks ("acute crisis", n = 14), individuals who had a history of suicide attempt, but no suicidal ideation in the last year ("chronic suicide risk", n = 39), patients with chronic suicidal ideation (n = 12), patient controls (n = 38), and healthy volunteers (n = 27).

Clinical Measures: Participants were assessed for hopelessness (Beck Hopelessness Scale (BHS)), suicidal ideation (Scale for Suicidal Ideation (SSI) and Columbia Suicide Severity Rating Scale (C-SSRS)), depression (Montgomery-Asberg Rating Scale (MADRS)) and psychological pain (Mee-Bunney Psychological Pain Scale (MBP)). Effect sizes were used to quantify differences between active crisis and chronic suicide risk groups.

MEG: Participants across all groups underwent two resting state MEG scans with their eyes closed within two days of clinical ratings. MEG power was source-localized in the theta (4-8 Hz), alpha (9-14 Hz), beta (15-29 Hz), gamma (30-58 Hz), and high gamma (62-118 Hz) frequencies using synthetic aperture magnetometry. Linear mixed effects models examined effects of hopelessness, group, and group-by-hopelessness interactions on whole-brain estimates of power.

Results: Clinical: When comparing the "active crisis" and "chronic suicide risk" groups, hopelessness was greater in the "active crisis" group (Effect Size= 1.26 95%CI: 0.58-1.90), similar to depression ratings (MADRS total score: 1.49, 95%CI: 0.79-2.18) and psychological pain (MBP: 1.22 95%CI: 0.56-1.87). In contrast, effect sizes for SI ranged from 0.93 (SSI, 95%CI: 0.22-1.63) to 0.26 (C-SSRS, -.37-0.89).

MEG: There were no significant group or group-by-hopelessness effects for any of the MEG measures. However, there was a significant main effect of hopelessness ($p < 0.05$ cluster-level correction) across all frequency bands (theta, alpha, beta, gamma, and high gamma), where negative associations were found between total BHS score and source-localized power in a right frontal region centered in the anterior insula, a key node of the salience network.

Conclusions: Hopelessness may be a key indicator of acute suicide risk and is linked with brain areas implicated in the salience network, which has been linked to the transition from suicidal thoughts to suicidal behaviors. Future evaluations of hopelessness as a potential indicator of suicide risk and biomarker of response to suicide-focused treatments are indicated. Limitations include the small sample size of recently suicidal individuals; further analyses are needed to disentangle the relationship between hopelessness, SI and depression at both the clinical and electrophysiological levels.

Keywords: Suicide, Hopelessness, MEG

Disclosure: Nothing to disclose.

P270. Chronic Escitalopram in Healthy Volunteers Has Specific Effects on Reinforcement Sensitivity

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Background: There have been several studies of the effects of acute or sub-chronic escitalopram on cognition in healthy volunteers, which have found changes in learning and reinforcement outcomes (Kanen et al., 2021; Seymour et al., 2012; Skandali et al., 2018). In contrast, to our knowledge, there have been no studies of the chronic effects of escitalopram on cognition in healthy volunteers. The interpretation of acute effects is complicated by the possible pre- and post-synaptic actions of the drug. Therefore, it is important both to understand the steady state effects of escitalopram in healthy people, but also because it is given chronically to patients with Major Depressive Disorder (MDD), as well as other psychiatric conditions, such as Obsessive-Compulsive Disorder (OCD). We aimed to investigate the effect of the chronic selective serotonin reuptake inhibitors (SSRI), escitalopram, on 'hot' and 'cold' cognition, and particularly on reinforcement learning.

Methods: The study was a double-blind placebo controlled trial of 66 participants of which 32 received 20mg of escitalopram (mean age (s.d.) 24.25 (5.56)) and 34 received placebo for 21 days (mean age (s.d.) 25.76 (6.64)). Participants were semi-randomised into the two groups and matched for age, sex and IQ. The study was pre-registered on clinicaltrials.gov (NCT04239339). Comprehensive questionnaires and neuropsychological tests were conducted and serum escitalopram measures were taken. Questionnaire measures included demographics (e.g. ethnicity, education, and marital status), personality, life experiences, medical history, mood, stress and sleep quality. Neuropsychological tests included both 'hot' and 'cold' cognition, including domains such as learning, memory, attention, executive functioning, emotion recognition and reinforcement learning. Statistical analyses were conducted in R, version 4.1.1 (R Foundation for Statistical Computing). All questionnaire group comparisons were conducted using two-tailed t-tests in R. The Benjamini-Hochberg (Benjamini and Hochberg, 1995) false discovery rate (FDR) with q = .05 correction was used for the questionnaires. Group comparisons for cognitive measures were conducted using linear models allowing for unequal variances with the gls function from the R package nlme. The Sequential Model-Based/Model-Free task analysis was conducted according to Decker et al. (2016), where a generalized linear mixed-effects regression analysis of group behaviour data was performed using the lme4 R package. Age, sex, IQ and reaction time were included as covariates in the analyses. The corrections for multiple comparisons were conducted according to the pre-registered cognitive domains using the Benjamini-Hochberg FDR with q = .05. In addition the Probabilistic Reversal Learning task was analysed using hierarchical Bayesian methods, incorporating parameters that have been studied previously in the reinforcement learning literature(Kanen et al., 2019; Marzuki et al., 2021).

Results: The analysis revealed that the escitalopram group had lower reinforcement sensitivity compared to placebo controls on both the Sequential Model-based/Model-Free (estimated coefficient = -.34, p = .004, 95% CI[-.575 to -.105]) and the Probabilistic Reversal Learning tasks (MD = -2.676 [90% HDI, -5.285 to -.396]). There were no significant differences between the two groups for any of the other cognitive measures.

There were no significant group differences at baseline on any of the questionnaires. Examining the change scores between the baseline visit and the 3 week visit there was a significant difference between the two groups on 2 dimensions of the CSFQ-14 Sexual Functioning Questionnaire (Orgasm/Ejaculation ($t(42.25)=2.68$, $p = .01$) and Orgasm/Completion ($t(42.25)=2.68$, $p = .01$)), where the escitalopram group had significantly lower responses.

Using the serum escitalopram levels we confirmed that all participants in the escitalopram group complied with the drug administration.

Conclusions: Unlike previous reports on the acute effects of serotonin3, we did not find any significant effects on 'cold' cognitive measures after 3 week SSRI administration. In addition, there were no effects on emotion recognition or moral judgements. Importantly, we did find significant effects specific to reinforcement learning, where the escitalopram group had lower reinforcement sensitivity. These findings have key implications for the role of serotonin in reinforcement learning. In addition, the results have clinical implications as they may reflect the 'blunting' effect often reported by MDD patients receiving chronic SSRI treatments (Marazziti et al., 2019).

Keywords: Cognition, Reward Sensitivity, Selective Serotonin Reuptake Inhibitors (SSRIs)

Disclosure: Nothing to disclose.